UNIVERSITY OF CAMBRIDGE

# Diet and risk of acute myocardial infarction in Bangladesh: the Bangladesh Risk of Acute Vascular Events (BRAVE) study

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This dissertation is submitted for the Degree of Doctor of Philosophy

## DECLARATION

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University of similar institution except as declared in the Preface and specified in the text.

This dissertation is 53,889 words long, excluding Summary, Preface, Acknowledgements, Contents, Figures, Tables, Appendices and References. It does not exceed the word limit set by the Degree Committee for Department of Public Health.

## SUMMARY

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**Title: Diet and risk of acute myocardial infarction in Bangladesh: the Bangladesh Risk of Acute Vascular Events (BRAVE) study**

**Background** Coronary Heart Disease (CHD), with myocardial infarction (MI) as its main manifestation, is increasing at an alarming rate in South Asian countries, however evidence on its determinants is sparse. Dietary risk explains about one-third of global mortality and is a most important modifiable risk factor for CHD. Although there is extensive evidence on diet and risk of CHD from western populations, this cannot be generalised to South Asian populations where the dietary habits are very diverse.

**Objectives:** The main aims of this thesis are to (1) summarise existing epidemiological evidence on diet and risk of CHD in South Asians; (2) characterise in detail the lifestyle socio-demographic and other correlates of dietary factors in a South Asian population; (3) investigate the association of dietary food groups, patterns and nutrients with the risk of MI and (4) discuss public health implications of the findings.

**Methods:** BRAVE is a hospital-based case-control study from Dhaka, Bangladesh which has about 8000 cases and 8000 controls frequency matched by age and sex. This study has overlapping data looking at lifestyle (including dietary determinants), biochemical, genetic and environmental risk factors for acute MI (AMI). Using data from this study dietary determinants of AMI were investigated through (1) cross-sectional analyses of the association of diet with various correlates and (2) case-control analyses with risk of MI.

**Results:** The systematic review demonstrated that there was scarce evidence on diet and risk of CHD from South Asia.

Cross-sectional analyses from BRAVE study demonstrated that dietary food groups, patterns and nutrients had different associations with the various characteristics showing the role of modest confounding. There were few strong correlations between food groups, nutrients and dietary patterns.

Findings from food group analyses showed an inverse association between fruits, vegetables, yoghurt, certain spices and risk of AMI. In contrast, higher consumption of biryani and fish was associated with higher risk of AMI.

Three distinct dietary patterns were identified using principal component analysis; the “energy dense pattern”, the “vegetable pattern” and the “fruits and dairy pattern”. The vegetable pattern and fruit and dairy pattern had an inverse association with the risk of AMI. In contrast, “energy dense pattern” had no significant association with the risk of AMI.

As for the analyses on dietary nutrients, higher intake of refined carbohydrates was not associated with the risk of AMI, while non-refined carbohydrates were associated with lower risk of AMI. Animal protein showed a higher risk of AMI, whereas plant protein showed a weak inverse association. As for specific fatty acids, modest intakes of saturated fatty acid from dairy sources and polyunsaturated fatty acids were associated with a slightly lower risk of AMI. In contrast monounsaturated fatty acids showed an increased association only in highest quintile.

**Conclusions:** The present analyses are the largest detailed study on diet and CHD solely based on a South Asian population. It confirms previous observed association of some food groups with CHD in western populations and has also yielded some novel insights on the association of diet with CHD specific to Bangladesh. However, owing to an observational nature of the study a causal assessment could not be done, findings of this study stimulates further detailed work including prospective cohort studies which may have important potentials for the local dietary guidelines in Bangladesh and in similar settings to help reduce the rising burden of CHD.

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# PREFACE

A number of people have contributed to aspects of the work in this thesis, which I would like to acknowledge here. Details of other individuals are specified below:

**Chapter 1.** Dr Rajiv Chowdhury, Dr Marleen Lenties and Dr Angela Wood commented on the search strategy and inclusion and exclusion criteria used for the literature review. Dr Rajiv Chowdhury and Shazeen Bokhari commented on the draft.

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# List of commonly used ABBREVIATIONS

**AMI** Acute Myocardial Infarction

**BMI** Body Mass Index

**BRAVE** Bangladesh Risk of Acute Vascular Events

**CHD** Coronary Heart Disease

**CVD** Cardiovascular Disease

**FAO** Food and Agriculture Organisation

**FFQ** Food Frequency Questionnaire

**HEALS** Health Effects of Arsenic Longitudinal Study

**HIES** Household Expenditure and Income Survey

**HDL-C** High Density Lipoprotein

**HIC** High Income Country

**LDL-C** Low Density Lipoprotein

**LMIC** Low-and Middle-Income Country

**MI** Myocardial Infarction

**MUFA** Monounsaturated Fatty Acids

**Odds Ratios** ORs

**PCA** Principal Component Analysis

**PUFA** Polyunsaturated Fatty Acids

**PURE** Prospective Urban Rural Epidemiology

**RCTs** Randomised Controlled Trials

**SFA** Saturated Fatty Acids

**SSB** Sugar Sweetened Beverages

**WHO** World Health Organisation

# CHAPTER 1. Introduction

## 1.1 Chapter summary

Cardiovascular disease (CVD) is the leading cause of death and morbidity in the world, with 80% of the disease burden of CVD occurring in developing countries. Coronary heart disease (CHD) is the main clinical manifestation of CVD. Among low- and middle-income countries (LMICs), South Asian countries have a higher burden of CHD characterised by earlier age of onset and high severity. However, direct evidence on the determinants of the burgeoning burden of CHD is sparse in this population. Diet has long been considered as a major risk factor for CHD. Most research examining the relationship between diet and CHD has been conducted among western populations, with little information on diet-based disease risk available from other parts of the world, where the majority of CHD occurs. In addition, the dietary habits and patterns in South Asia are very different from the rest of the world. Therefore, research from western countries may not be generalizable to this population. In this respect, study of dietary determinants in a South Asian population can be useful to (i) highlight the potential association of diet and CHD; (ii) observe if the associations of diet and CHD in western populations is similar in this population and (iii) help inform local policy and guidelines specific to this area and to broadly similar settings to reduce the increasing burden of CHD. This thesis aims to characterize, reliably and in detail, the association of diet and risk of CHD using a large pioneering case-control study from an understudied population of Bangladesh.

## 1.2 BACKGROUND

### 1.2.1 CHD definition and aetiology

CHD is the most common form of heart disease. CHD is caused by the accumulation of cholesterol in the coronary arteries which supply blood to the heart muscle. These deposits cause the lumen of the arteries to get narrow and walls of the arteries to thicken, which leads to the process of atherosclerosis.[1–3] In addition, risk factors, such as smoking, diet, diabetes or high blood pressure can lead to endothelial dysfunction, facilitating the entry of low density lipoproteins (LDL-C) into the arterial wall, where LDL-C particles get oxidised causing further damage to the arterial wall.[1] Myocardial infarction (MI) (myocardial necrosis) is the main clinical manifestation of CHD.[4]

As shown in **Figure 1.1** monocytes then reach the area of damage wall and become macrophages. These macrophages engulf the LDL-C particles to form "foam cells".[3,5] When foam cells die they release their contents causing inflammation. This leads to vascular smooth muscle proliferation, forming a fibrous cap that results in the formation of a stable atherosclerotic plaque.[2,6] The build-up of this atheroma (cholesterol deposits) or plaque results in restriction of the oxygen and nutrients supply to the heart muscle, and may lead to chest pain or angina pectoris.[7] More general symptoms include fatigue, nausea, vomiting and back pain.[8] If the plaque ruptures it can lead to thrombosis. Moreover, if this ruptured thrombotic plaque occludes the coronary artery, it can cause severe ischaemia and myocardial cell necrosis leading to MI (death of heart muscle). Ischemia induces profound metabolic and ionic changes in the affected heart muscle and causes rapid depression of systolic function and then eventually death of cardiac muscle.[9]

Although it is postulated that biological mechanisms such as lipid metabolism, systematic inflammation, metabolic functions and haemostatic play a role in atherosclerosis leading to CHD, the exact causal mechanisms are not fully understood. According to the World Health Organisation (WHO) updated definition of MI and its diagnosis, MI is diagnosed by looking at electrocardiogram (ECG) scans and elevation of cardiac biomarkers such as troponins, which can measure tissue necrosis.[7] However, these biomarkers cannot be widely used in resource constrained health systems of LMICs. Hence, in such cases a diagnosis may be made using clinical presentation and signs (in ECGs) of the patients or by looking at results from autopsy.[7]

### 1.2.2 Coronary heart disease: a multifactorial disease

As described above, the complex disease process of CHD demonstrates that it is a multifactorial disease. Evidence from numerous epidemiological studies have indicated that CHD is determined by interplay of lifestyle, environmental and genetic risk factors.[10–12] Over the years studies have not only identified non-modifiable risk factors (age, male sex, family history of and pre-existing CVD) of CHD but also many modifiable risk factors such as elevated levels of cholesterol, high blood pressure, diabetes, smoking, physical inactivity, high alcohol consumption and unhealthy diets.[13–18] Although these studies have provided important insights in identifying potential targets for prevention and treatment of CHD, there is still a lot to be understood. **Figure 1.2** shows the proposed determinants and risk factors of CHD, highlighting the multifactorial disease aetiology. Below is the summary of some modifiable risk factors of CHD.

*Lipids*

The earliest evidence from animal studies and various epidemiological studies suggested an association of total cholesterol with atherosclerosis and CVD risk.[19–21] Once total cholesterol was identified as an important risk factor for CHD, findings from animal and clinical studies also confirmed that LDL-C, a transporter on cholesterol in blood was also an important marker for CVD.[22] Then came the diet- heart hypothesis, which postulated that LDL-C increased the risk of CHD, whereas high density lipoprotein (HDL-C) decreased the risk of CHD. Consequently lowering LDL-C and raising HDL-C has become important therapeutic targets to reduce the incidence of CHD.[20]

*Hypertension, diabetes mellitus and adiposity*

Framingham and other epidemiological studies have reported that blood pressure has an increased association with CVD.[23,24] In addition, anti-hypertensive drugs that target lowering blood pressure may reduce CHD events by estimated 22%.[25] Diabetes mellitus is also known to be associated with 2-3 fold higher risk of CVD;[26] high levels of glucose in blood (insulin resistance) can damage the walls of the arteries and accelerate the process of atherosclerosis. Adiposity is associated with a 27% higher CHD risk per 5 $kg/m^2$ increase in body mass index (BMI).[27]

*Non-dietary behavioural risk factors of CHD*

Tobacco smoking and physical inactivity are non-dietary behavioural risk factors for CHD. Smoking is suggested to cause about 10% of CVD deaths.[28] Chemicals in smoke have oxidising radicals that can affect potential mechanisms of CHD such as inflammation, endothelial dysfunction, oxidation of LDL-C, and platelet activation.[29] Reported studies of

physical activity and CHD show considerable protection for those who are physically active. One such study of 7735 men with pre-existing CHD demonstrated that moderate or moderately vigorous physical activities reduced the risk of CHD by 50%, compared to those who were inactive.[30]

*Genetics*

There is evidence that hereditary influence accounts for between 40% and 60% of CHD cases based on family and twin studies.[31] In the Framingham Offspring Study, the age-specific incidence of CHD increased by approximately 2-fold in participants with a family history after adjustment for conventional CHD risk factors. In addition, genome wide association studies (GWAS) have identified chromosomal loci as underlying genetic predisposition to CHD.[32] The first GWAS for CHD were published in 2007, when three independent groups reported common variants at the 9p21 locus associated with approximately 30% increased risk of CHD per copy of the risk allele.[33] Preliminary evidence has highlighted that 9p21 affects cell proliferation and inflammatory signalling.[33]

*Air pollution*

Epidemiological studies have reported that air pollution increases the risk of morbidity and mortality due to respiratory illnesses and CVD. A meta-analysis of 35 studies reported that heart failure hospitalisation or death was associated with increases in carbon monoxide, sulphur dioxide and nitrogen dioxide. In addition, increases in particulate matter concentration were also associated with heart failure hospitalisation or death.[34]

*Environmental toxic metals*

In recent decades, exposures to environmental toxic metals such as arsenic, lead, cadmium, mercury, and copper have become a global public health concern owing to their potential deleterious health effects in humans. A recent metanalysis of 37 unique studies postulated that exposure to arsenic, lead, cadmium and copper was associated with increased risk of CVD and CHD.[35] These effects may be exerted through oxidative stress, inflammation, endothelial dysfunction and vascular injury.[35]

*Sleep*

Abnormal sleep duration is also postulated to be a risk factor for CHD. A Finnish cross-sectional study reported that both sleep lengths of 6 or less hours/night and 9 or more hours/night were associated with higher odds of risk of history of MI.[36] In addition, a meta-analysis of 13 prospective studies with 6332 CVD events reported that insomnia increased the risk of developing or dying from CVD by 45%.[37] The mechanisms that underlie these associations are not fully understood. Some studies have postulated that short duration of

sleep may change in circulating levels of leptin and ghrelin that would increase appetite and reduce energy expenditure facilitating the development of obesity and impaired glucose levels. Another study has reported that short sleep may activate low grade inflammation.[38]

### 1.2.3 Coronary Heart Disease burden: a major public health challenge globally and among South Asians

CVD is the leading cause of death and morbidity in the world causing about 280 deaths per 100,000 worldwide.[39] It is projected that the burden of CHD; the key clinical manifestation of CVD, will rise from 47 million disability-adjusted life years (DALYs: 'healthy years of life lost') in 1990 to 82 million in 2020.[40] The burgeoning burden of CHD is not only increasing in the developed countries but also in the developing countries, with 80% of the disease burden occurring in LMICs.[7]

In particular, South Asia, which makes up more than 40% of the developing world, with three countries; India, Pakistan and Bangladesh among the 10 most populous countries in the world, is projected to experience a greater burden of CVD in the next 10 years than any other region worldwide.[41] Furthermore, while age-standardised CHD death rates have decreased during recent decades in many high-income countries (HIC), these rates continue to rise in South Asia, it is reported that the age standardised death rate for CHD in South Asia was 212 deaths per 100,000 in 2015.[39]

There is evidence that CHD in South Asian countries is characterised by premature onset (5-10 years earlier onset of first MI) and higher severity as compared to other countries.[42,43] It is also postulated that coronary arteries in South Asians are narrower than in Europeans, facilitating the build-up of atheroma leading to CHD.[44] In addition, prevalence of risk factors of CHD are also high in South Asian countries. In particular, the National Health and Nutritional Health Examination Survey (NHANES III) reported that prevalence of diabetes in South Asians living in USA was 18%, which was 4 times more than that of Whites.[45] Another study of South Asians living in the UK reported that they had higher systolic and diastolic blood pressures than the Europeans.[46] Furthermore, the INTERHEART case-control study of 52 countries reported 50% lower levels of HDL-C in healthy South Asians as compared to western populations.[47] Recently upper cut-points of BMI for South Asians have been proposed by the American Diabetes Association (ADA) since South Asians are also known to have higher BMI and tend to gain more weight.[48] As for lifestyle factors, indigenous forms of tobacco use (e.g. beedi- hand rolled cigarettes, jarda or gul) are highly prevalent in South Asian countries. Due to this excessive use of tobacco, approximately 1.2 million people die every year in South Asia.[49] On the other hand, consumption of fruits and vegetables is low in South Asian countries and as a result, more than half the population does not meet the dietary recommendations.[50]

However, due to limited resources, inadequate health systems, paucity of high-quality large-scale research data and lack of awareness about the determinants of CHD, low priority is given to the research on determinants of CHD. Therefore, it is extremely

important to understand the potential determinants of CHD in the South Asian population in order to come up with health promotion strategies and interventions specific to this area.[51]

### 1.2.4 Bangladesh a country with high burden of CHD and its risk factors

Bangladesh is a riverine country with about 165 million population as of 2017.[52] The rural areas are based on agrarian economy whereas in urban areas the economic growth is driven by high remittance and the garment industry.[53] Over the years poverty has declined and life expectancy of people has increased. Bangladesh has experienced an epidemiological transition leading to a shift from communicable to non-communicable diseases in the last decades. Although this country remains one of the few low-income nations to achieve the Millennium Development Goals–a rapid increase in CHD appears to undermine major health and economic gains in this country.[54] CVD is the most common cause of death in Bangladesh with 277,942 deaths (33% of total deaths) annually in 2016 as shown in **Figure 1.3**.[55] CVD forms a major proportion of disability, lost earnings and social dislocation – all of which have a substantial burden on the country.[56] In addition, there has been an increase in DALYs for ischemic heart disease/ CHD by 237% in Bangladesh from 1990 to 2013 as shown in **Figure 1.4**.[57]

As aforementioned ethnicity has been postulated to play a role in the greater susceptibility of South Asian populations to CHD.[58] Studies from immigrant populations have reported that among South Asians, Bangladeshis are even more prone to develop CHD.[59] Bangladeshi South Asians living in New York, US had more severe heart disease than the Caucasions.[60] In the UK, Bangladeshi men have 112% higher CHD mortality estimates than people of European origin. In addition, among all South Asians living in the UK, Bangladeshis appear to have the highest prevalence of most of the CHD risk factors including smoking and diabetes.[61]

According to the Global Burden of Disease (GBD) study, the major risk factors contributing to overall mortality in Bangladesh are high blood pressure, smoking, air pollution, high blood sugar and an unhealthy diet as shown in **Figure 1.5**.[55] Furthermore, evidence from the INTERHEART study stated that among five South Asian countries, Bangladesh had the highest prevalence for the most risk factors among the controls: current and former smoking (59.9%), elevated ApoB100 / Apo-I ratio (59.7%), abdominal obesity (43.3%) and history of hypertension (14.3%).[47] In contrast, Bangladesh had the lowest prevalence for regular physical activity (1.3%) and daily intake of fruits and vegetables (8.6%).[47] In addition, a national representative survey on risk factors of non-communicable diseases in Bangladesh stated that about half used tobacco in some form, <1% consumed alcohol within the past 30 days, 92% did not consume adequate fruit and vegetables (five servings or more), and 35% had low physical activity levels.[62] In Bangladesh the consumption of alcohol is prohibited by the law due to religious reasons. Another study reported that about 95% of the people in Bangladesh were lifetime abstainers of alcohol.[63] A survey in

Bangladesh reported that 21.5% adults (male 21%, female 22%) have BMI ≥ 25 kg/m2 and increased waist circumference is alarming, especially in women (33.7%).[64] Bangladesh is one of the top ten countries in the world with high prevalence of current smoking among men (44.7%).[65]

In addition, air and water pollution are a major public health concern in Bangladesh. An earlier study found that Dhaka had the poorest air quality with respect to total suspended particles, sulphur dioxide, and nitrogen dioxide, with the pollutant levels far higher than the WHO thresholds.[66] Furthermore, an estimated 85 million people are at risk of arsenic-contaminated drinking water and foods.[59] The Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh had also reported an increased association between arsenic exposure from drinking water and plasma levels of markers of systemic inflammation and endothelial dysfunction.[67]

However, despite the accelerating burden of CHD in Bangladesh and high prevalence of risk factors of CHD, there remains a paucity of reliable evidence on its potential determinants: 1) what determines the levels of various behaviour risk factors in Bangladesh (socio-demographic basis of diet); 2) what is the distribution and pattern of these risk factors and 3) what is the relative contribution of these factors to CHD risk. Therefore, it is crucial to comprehensively study the determinants in this population to help develop preventive strategies specific to this region.

### 1.2.5 Diet in Bangladesh

A typical Bangladeshi diet is characterised by nearly two-thirds of rice, some vegetables, pulses and fish. **Table 1.1** and **Figure 1.6** show the dietary guidelines set by Food and Agriculture Organisation (FAO) specific to Bangladesh.[68] Bangladeshi diet incorporates use of various spices such as dried red chilli, bay leaf, garlic and coriander. Bananas and papayas are among the most commonly consumed fruits. The dietary intake of fat is somewhat meagre.[69]

According to the National Nutrition Survey, 70% of the calories consumed by Bangladeshis come from cereal and grains.[53] In addition, the per capita consumption of rice is the highest for Bangladesh as compared to other parts of the world, making it the main cereal crop consumed in Bangladesh.[70] Being a riverine country, there is high consumption of fish in Bangladesh. Concerning types of fish, common carp and pangas (or river fish) are the commonly consumed types. As for other types of animal protein, consumption of red and white meat is relatively low in Bangladesh, especially in low and middle-income groups therefore fish remains the most common source of animal protein. In this regard, it has been postulated that the low consumption of other types of meat, is the major contributor of poor diet and undernutrition, particularly among mothers and young children.[53] As for fruits and vegetables, the national statistics suggest that their consumption is below the dietary recommendations.[53] In Bangladesh, consumption of alcohol is prohibited by law due to religious reasons. A study reported that about 95% of the people in Bangladesh were lifetime abstainers of alcohol.[63]

Food intake patterns over the years assessed by nutrition surveys from 1991 to 2005, show high intakes of rice and low intakes of wheat in Bangladesh.[71] In addition, over the years there has been increased intakes of non-cereal food items, particularly meat, egg, potato, fruits and vegetables, indicating positive transition towards dietary diversification.[71]

### 1.2.6 Diet and CHD: a potentially intriguing link

Dietary risk explains about one-third of global mortality, and it is the most important risk factor for CVD as shown in **Figure 1.7**.[72] Therefore, diet appears to be a priority to target for CHD prevention, the main clinical manifestation of CVD, and reducing the burden of this disease.[39] It is postulated that diet affects many pathways leading to CHD such as levels of LDL-C, blood pressure, glucose-insulin homeostasis, inflammation, endothelial health, hepatic function, adipocyte metabolism, cardiac function and metabolic expenditure as shown in **Figure 1.8**.[73] Consumption of foods are thought to act on multiple risk factors or inter-mediatory pathways through the myriad of nutrients they constitute. For example, fruits and vegetables have vitamins, fibre, potassium and folate that may have antioxidant, anti-inflammatory and blood pressure lowering effects.[74] A recent meta-analysis of 123 studies collating the evidence of food groups and their association with CHD reported an inverse association of whole grains, legumes, fruits, vegetables, nuts and fish consumption on the risk of CHD and an increased association with eggs, red meat, processed meat and sugar-sweetened beverages (SSB).[75] In addition, meta-analyses with evidence from epidemiological studies investigating nutrients have reported inverse associations with dietary fibre[76], vitamin E[77], vitamin C[77] with CHD risk, and reported higher risk with trans-fat.[78]

Alcohol consumption is also considered as a risk factor for CHD. A recent study performed an individual-participant based meta-analysis involving data from 3 large studies across 19 high-income countries (the Emerging Risk Factors Collaboration, EPIC-CVD, and the UK Biobank),[79] and found that alcohol consumption was roughly linearly associated with a higher risk of stroke, and CHD excluding MI and heart failure. By contrast, increased alcohol consumption was log-linearly associated with a lower risk of MI. Furthermore, studies have shown that elevated systolic blood pressure could mediate the association of alcohol consumption with stroke and excluding MI. On the other hand, pathways related to lowering HDL-C levels may mediate the inverse association shown with MI.[79]

Moreover, previous epidemiological research from western populations has investigated the association of diet and CHD extensively. However, the majority of the research from west has focused on the effect of nutrients, especially fatty acids on CHD.[80,81] Ever since the first evidence of the diet-heart hypothesis, it was postulated that dietary fat intake has implications for CHD aetiology, as atherosclerosis is caused by accumulation of lipids in the arterial wall.[82] Evidence from earlier observational studies indicated that saturated fatty acid (SFA) increases the circulating levels of total cholesterol and LDL-C.[83] Accumulation of these studies led to the establishment of the pioneering Seven countries study in 1950s that suggested that SFA had an increased association with CHD and

unsaturated fats are protective against CHD.[84] The evidence on diet and fats led to dietary guidelines recommending to reduce SFA, replace it with polyunsaturated fatty acids (PUFA) and to avoid trans fats.[85] Over the years, however, the evidence base on nutrients and risk of CHD has evolved. There has been a shift from the focus on reducing SFA for CHD prevention to the understanding the importance of other types of fats and the relevance of the substitution nutrients (carbohydrates or unsaturated fats) with SFA when determining the risk of CHD.[86] However, despite extensive research from western countries, the association of nutrients with CHD is highly debated, leaving us unclear about fat versus carbohydrate- which one is the bad?[87] Studies have also challenged the reductionist approach of investigating isolated nutrients by highlighting the importance of studying food groups as the food we consume is made of a combination of many different types of nutrients that may counterbalance each other effects.[88,89]

Moreover, in the last 15 years, advances in nutritional epidemiology have highlighted the relevance of dietary patterns than food groups and nutrients in relation to disease risk. Dietary pattern analysis highlights the importance of studying food groups collectively to see how they interact with each other to effect the association with chronic diseases like CHD.[88,89] This method investigates the synergistic effects of food items and the inter-collinearity of food groups, providing a more holistic approach to study the potential dietary determinants of CHD.[90] Randomised controlled trials (RCTs) have reported that dietary patterns, including the Mediterranean Diet[91] and the Dietary Approaches to Stop Hypertension (DASH) diet[92], are associated with lower CVD risk. Furthermore, it is postulated that health policies, dietary guidelines and health promotion interventions aimed to follow certain food groups and dietary patterns will be easier to follow by the general public rather than guidelines on isolated nutrients.[89] However, the global relevance of dietary research and guidelines from North American and European countries is unclear.[86]

### 1.2.7 Dietary assessment methods

It is important to measure dietary intake as reliably as possible to describe the trends of consumption, differences between individuals, the dietary changes over time and to investigate diet-disease associations. Methods of self-reported dietary assessment can be divided broadly into two groups: methods of recalling diet and real-time dietary records. Methods of recalling diet include Food Frequency Questionnaires (FFQ) and 24-hour recalls.[93] These two methods are widely used in epidemiological studies due to their low costs and less responder burden. Real-time recording methods include food diaries and duplicated weighted record.[93] In addition real time diet can be recorded using technology such as use of E-button[94] and mobile apps. However, due to their high costs they are not widely used in large epidemiological studies.

Observational studies that use self-reported measure to assess dietary intake are limited by misclassification and recall bias and inability to get complete information on dietary intake, failing to reflect the actual intake of the participants. Investigating nutritional biomarkers in blood can reflect dietary intake more objectively. Previous studies have reported that these markers are highly correlated with dietary intake levels and are free of a social desirability bias.[95] However, data on biomarkers is often sparse due to costs and studies lack power. Moreover, the results based on biomarkers cannot provide information for the subject to change their dietary habits. Thus, direct assessment of dietary intake may be more informative for dietary recommendations than biomarkers are.

*Strengths and limitation of dietary assessment methods:*

**Table 1.2** summarises commonly used dietary assessment methods with their strengths and limitations.

Food frequency questionnaires:

FFQ is a checklist of food items and beverages which includes frequency responses to report the consumption over a specified period.[96] Semi-quantitative FFQs also have information on portion sizes of food items and beverages. Nutrient intakes of the food groups reported in FFQ, can be estimated using food composition databases. FFQs have strengths of capturing the habitual intake, low costs, less burden on the participants and is suitable for large studies.[97] In addition, FFQs are easy to administer and analyse and are useful for repeated dietary assessment over years (such as in prospective cohort studies).[98] However, as FFQs rely on respondent memory to recall the diet retrospectively there may be possibility of recall bias. In addition, there can be systematic underreporting (such as snacks), overreporting (of healthy foods such as fruits and vegetables), or

omission of foods by an individual. FFQs provide little information on food preparation methods specially for mixed dishes.[98] In addition, there may be an observation effect which means that there may be a change in eating behaviours when individuals are asked to record their intake leading to misreporting. To complete self-administered FFQs, high literacy is required. In addition, estimating portion sizes may be difficult in FFQs for the respondents. This can be overcome by showing pictures of different portion sizes to the respondents. Furthermore, it is important to note that as different populations have different dietary patterns therefore FFQs need to be country specific. The FFQs may miss out foods such ready meals or take-away foods if the food list is based on more basic food categories.[98]

24-hour recalls:

24-hour recalls are an open-ended questionnaires in which participants are asked to recall their diets for the day before.[93] Key strengths of 24- hour recalls include low respondent burden, low administrative costs, sensitive to culture specific differences and theoretically unlimited level of specificity for the foods consumed. However, 24-hour recalls are also prone to recall bias as with all self-reported dietary assessment methods.[99] 24-hour recalls can also be affected by misreporting of food groups by individuals. As with FFQs, if the misreporting is associated with their personal characteristics (e.g. age, gender, overweight), this can result in a differential misclassification, which may affect the observed associations in either direction.[93] It can be expensive to enter data if a paper-based 24-hour recall is implemented because the process is labour-intensive. In addition, repeated 24-hour recalls increase time and cost of analysis.[99]

Food diaries:

In food diaries, individuals are asked to record their consumption in real time usually between 3-7 days.[100] Food diaries have less reliance on memory as record is made at the time of consumption, portion sizes are well defined, detailed description of foods is given, eating occasions are recorded and foods consumed outside of the home in restaurants can easily be captured.[100] However, food diaries have high respondent burden as they have to be filled every time they consume food items and require literacy, they are time consuming and costly. There is the risk it may be completed from memory, after the food is consumed instead of at the time of intake or forget to record consumption. There can be misreporting if the individual may alter their diet to make it easier to record, or to not share poor eating habits. The portion sizes of some foods may be difficult to estimate if the description given by the individual is inadequate and therefore high literacy and numeracy skills are

required. Additionally, the assessment of foods that are consumed less often may not be accurate.[100]

### 1.2.8 Literature review

*Epidemiological evidence on the association of diet with CHD in South Asians.*

To review the previously published evidence on the association of diet and CHD among South Asians a systematic search was conducted in PubMed, Web of Science and Embase. Efforts were made to search for the grey literature (such as government publications, reports and abstracts of conferences) and reference list of the included papers. Cross-sectional studies and surveys that did not include clinical CHD endpoints but included only risk factors (such as lipids) of CHD, were excluded. The detailed inclusion and exclusion criteria, and search strategy of the review have been presented in **Table 1.3**.

Overall, seven unique studies were identified from India, Pakistan and Bangladesh **(Tables 1.4-6 and Figure 1.9)**. Six studies reported the association of food groups, four on dietary patterns and one on nutrients with the risk of CHD **(Figure 1.9)**.

*Food groups and risk of CHD in South Asians*

Overall, three unique case-control studies and two prospective cohort studies reported the association of food groups with CHD. In a matched case-control study of 350 cases with acute MI (AMI) and 700 controls in urban hospitals in India, it was reported that the persons consuming a median intake of 3.5 servings/week of green leafy vegetables had a 67% lower relative risk of CHD (OR 0.33; 95% CI: 0.17, 0.64; P for trend = 0.0001).[101] In addition, cereal intake, meat and mustard oil (as compared to sunflower oil) were also associated with lower risk of AMI. In contrast, dairy products, beans, rice and fruits had no association with the risk of AMI. The study adjusted for all the major confounders. However, the possibility of potential sources of bias such as selection bias and a differential recall among cases and controls cannot be ruled out. It could be argued that the controls that gave the consent to participate in the study were living a healthy lifestyle leading to reverse causation bias. Measurement errors are inherent in the use of FFQ while doing dietary assessment, leading to possible under-reporting or over-reporting of intakes of food groups. In addition, the small size of the study questions the reliability of the results. Another small case-control study of 190 cases and 380 controls from five major hospital in Lahore, Pakistan reported that eggs, sweets, butter, desi ghee, desserts and beef were significant risk factors for CHD, and fish and fruit were significant protective dietary predictors of CHD.[102] However, this study did not adjust for all the potential confounders.

A small prospective observational study of 100 patients (50 patients less than 40 years old and 50 more than 40 years old) with AMI based in Bangladesh reported that intakes of rice ≥2 times daily (OR 3.5, 95 % CI 1.15 ,10.6) and beef (OR 4.5, 95 % CI 1.83, 11.3) were significant risk factors for development of AMI in the younger group compared to the

older group.[103] Whereas, vegetable intake of ≥2 times daily was inversely associated with AMI. Limitations include the observational nature of the study on limited number of patients in a single hospital which may not be sufficient to elucidate diet-CHD associations. In addition, the study only adjusted for education and socio-economic status, therefore this incomplete adjustment of confounders may have affected the results.

The Prospective Urban Rural Epidemiology study (PURE) investigated the association of fruits, vegetables and legumes and risk of CVD in 135335 individuals from 18 countries.[50] This study had about 1500 participants from Pakistan, 2700 participants from Bangladesh and 25,000 from India. This study observed an inverse association of fruits, vegetables and legumes with the risk of MI in the age and sex adjusted models. However, after multivariate adjustment for confounders (adjusted for age, sex, centre (random effect), energy intake, current smoker, diabetes, urban or rural location, physical activity, education level, and tertiles of white meat, red meat, breads, and cereals intake) these associations became non-significant. However, the study did not report separate estimates for the association of fruits, vegetables and legumes with MI in South Asians. Strengths of this study include the prospective design, the large sample size, the use of validated country-specific FFQs, the broad range of intake of fruits, vegetables, and legumes (0 to >1000 g/day), and standardised methods to collect and adjudicate events. Limitations include measurement error in the FFQ, and the investigators did not investigate the association of different types of fruits and vegetables with MI. In addition, pooling the results of countries with diverse dietary habits poses challenges in interpreting the results.

INTERHEART case-control study from 52 countries also reported the association of food groups with the risk of MI.[89] This study had about 4000 participants from South Asia, however it also did not report separate estimates for the association of all food groups with MI in South Asians. Overall results of the study reported that the highest quintile vs lowest quintile of green leafy vegetables, other raw vegetables, cooked vegetables, fruits had a significant inverse association with the risk of MI, whereas highest intake of meat and grains were not associated with the risk of MI. In contrast, highest quintile of fried foods and salty foods had a higher risk of MI. Results from the South Asian region reported that intakes of fruits and vegetables >1 portion a day had an inverse association with the risk of MI.[47] However, as aforementioned separate estimates for other food groups were not reported for the South Asian region.

*Dietary patterns and risk of CHD in South Asians*

The evidence on the association of dietary patterns with CHD in the South Asian population was also very limited. A study in India, investigated the association of vegetarian and non-

vegetarian diet with AMI. This study was a matched hospital-based case-control study of 200 cases with AMI and 200 controls which was limited by selection bias as the hospital was not representative of the whole population and had a small sample size.[104] In this study the dietary patterns were defined prior to the analysis and reflected hypothesis oriented combinations of foods rather than dietary patterns derived through exploratory statistical methods. Thus, this may not be the most effective method to study dietary patterns and risk of CHD.

In a hospital based case-control study of about 200 cases and 200 controls in Karachi, Pakistan, three dietary patterns were identified using factor analysis.[105] These dietary patterns were (i)" prudent dietary pattern", characterised by high loading of legumes, cooked and uncooked vegetables, wheat, chicken and fruits; (ii) "combination dietary pattern", which was characterised by high loading of eggs, fish, fruits, juices and coffee; and "western dietary pattern", which was characterised by high loading of meat, fish and tea with milk. This study reported an inverse association of prudent and combination dietary pattern with MI. In contrast, no association was observed between western diet and risk of MI. However, this study was limited by sample size, recall bias and inadequate adjustment of potential confounders (no adjustment for energy intake).

In a prospective cohort study (Health Effects of Arsenic Longitudinal Study- HEALS) of 11,116 participants in Araihazaar, Bangladesh, principal component analysis (PCA) was carried out to derive three dietary patterns: (i) a "balanced" pattern, comprised of steamed rice, red meat, fish, fruit and vegetables; (ii) an "animal protein" diet, which was more heavily weighted towards eggs, milk, red meat, poultry, bread, and vegetables; and (iii) a "gourd and root vegetable" diet that heavily relied on a variety of gourds, radishes, pumpkin, sweet potato, and spinach.[106] No significant association of CHD with animal protein diet was observed, hazard ratio 1.17 (95% CI 0.99-1.38, p = 0.07) after adjusting for age, sex, BMI, smoking status, and energy intake. Limitations include the derivation of food patterns itself. Though pattern analysis can be argued to be a more effective, realistic method of assessing food consumption and risk of CHD, there is still a measure of subjectivity in selection of food groups for PCA. In addition, the study investigated only 64 deaths due to CHD and these outcomes were reported through verbal autopsy. Therefore, the results should be inferred keeping these limitations in mind.

INTERHEART case-control study of 52 countries with about 4000 participants from South Asia used factor analysis to drive three dietary patterns: (i) Oriental (high intake of tofu and soy and other sauces), (ii) Western (high in fried foods, salty snacks, eggs, and meat), and (iii) prudent (high in fruit and vegetables).[89] It was reported that prudent diet had an inverse association with AMI, with higher levels being protective. The Western pattern showed a U-shaped association with AMI (compared with the first quartile, the adjusted

OR for the second quartile was 0.87 (95% CI 0.78, 0.98), whereas it was 1.12 (95% CI 1.00, 1.25) for the third quartile and 1.35 (95% CI 1.21, 1.51) for the fourth quartile; P for trend < 0.001), in contrast the Oriental pattern demonstrated no association with AMI. When stratified by South Asian region, however, the western pattern showed no significant association AMI.

*Nutrients and risk of CHD in South Asians*

There is only one study so far that has investigated the association of nutrients with CHD in South Asians, however it did not report separate estimates. The PURE study reported that in South Asian countries, about 65% of the energy consumed is from carbohydrates.[107] Whereas fat consumption is about 23% and protein consumption is 12% of total energy. Pooled estimates from 18 countries reported that total carbohydrates, fats and proteins were not associated with the risk of MI. In addition, specific types of fatty acids were also not associated with the risk of MI. Nutrient replacement of carbohydrates with SFA, PUFA, monounsaturated fatty acids (MUFA) or proteins had no association with the risk of MI. However, as this study was based on studying single nutrients, the effect of other nutrients on the observed associations cannot be ignored. Furthermore, the study did not have clear definition of the source of total carbohydrates (refined or non-refined) making it difficult to interpret.

### 1.2.9 Importance of scientific inquiry to assess diet-CHD link among South Asians

As summarised above there is scarce evidence on the association of diet and CHD in South Asians (and especially in Bangladeshis). Based on the literature review there were no studies investigating the association of all major food groups (rice, dairy, white meat, red meat, fish, pulses, spices etc.) with CHD in South Asia. Overall there were seven unique studies that reported the association of diet with CHD. The HEALS, PURE, INTERHEART and one hospital based prospective study were the only studies that reported data from Bangladesh with about 11,000, 2800, 500, 100 participants each respectively. Out of these studies, the HEALS and one hospital based prospective study from Dhaka were the only studies that reported separate estimates of associations of diet with CHD in Bangladesh, whereas, the PURE and INTERHEART studies reported pooled estimates. As the dietary habits are diverse in different populations, pooled estimates are difficult to interpret, making conclusions misleading. The previous evidence also did not report the association of different food sources of nutrients with the risk of CHD. For example, carbohydrates from fruits and vegetables may have different associations with CHD than carbohydrates from sugars and refined sources. In addition, most studies were limited by study design, sample size and adjustment of potential risk factors and confounders of CHD.

Overall, an important gap identified by this review is that although the burden of CHD is rising in the South Asian region, there is paucity of evidence about its potential dietary determinants. Since, the dietary patterns are different in South Asia, the numerous studies on diet-CHD associations from western populations cannot be generalised to South Asian populations. Therefore, there is a need to conduct dietary research in the South Asian region.

## 1.3 Aims and structure of the Thesis

In this context, the aim of this thesis is to investigate the association between diet and risk of CHD using epidemiological data from the Bangladesh Risk of Acute Vascular Events (BRAVE) study. The specific aims are to:

(1) Summarise the existing evidence on diet and risk of CHD in South Asia;

(2) Describe the typical diet in a Bangladeshi population;

(3) Characterise in detail the lifestyle, socio-demographic and other correlates of dietary factors;

(4) Investigate the association of food groups, dietary patterns and nutrients with the risk of CHD, adjusting for potential confounders and mediators.

(5) Discuss public health implications of the research.

**Chapter 1:**

- Discusses the burden of CHD in South Asia
- Presents findings from the systematic review on diet and risk of CVD in South Asia.
- Discusses the rationale for the research on diet and AMI in Bangladesh

**Chapter 2:**

- Describes the BRAVE case-control study including collection and harmonization of the data.
- Describes the FFQ and the diet in cases and controls
- Reports the association of conventional risk factors of CHD with AMI in Bangladesh

**Chapter 3:**

- Describes the diet in BRAVE controls
- Reports the lifestyle and other correlates of food groups

**Chapter 4:**

- Investigates the association of various food groups with the risk of AMI, adjusting for potential confounders and mediators.

**Chapter 5:**

- Describes the derivation of dietary patterns
- Reports the lifestyle and biological correlates of dietary patterns
- Investigates the association of dietary patterns with the risk of AMI, adjusting for potential confounders and mediators.

**Chapter 6:**

- Describes the nutrient intake in BRAVE study
- Reports the lifestyle and biological correlates of dietary nutrients
- Investigates the association of nutrients with the risk of AMI, adjusting for potential confounders and mediators.

**Chapter 7:**

- Summarises the findings of the study
- Suggests potential public health implications.

**Appendix 1** provides the list of publications I have authored.

**Appendix 2** provides the Food Frequency Questionnaire of BRAVE study and shows the distribution of food groups used in the analyses.

**Appendix 3** provides the recipes of foods for calculation of energy intake.

**Appendix 4** provides the association of conventional risk factors with AMI using multiple imputation.

**Appendix 5** provides the cross-sectional associates of food groups with waist-to-hip ratio and lipids.

**Appendix 6** provides the scree plot of Eigen values for dietary pattern analysis.

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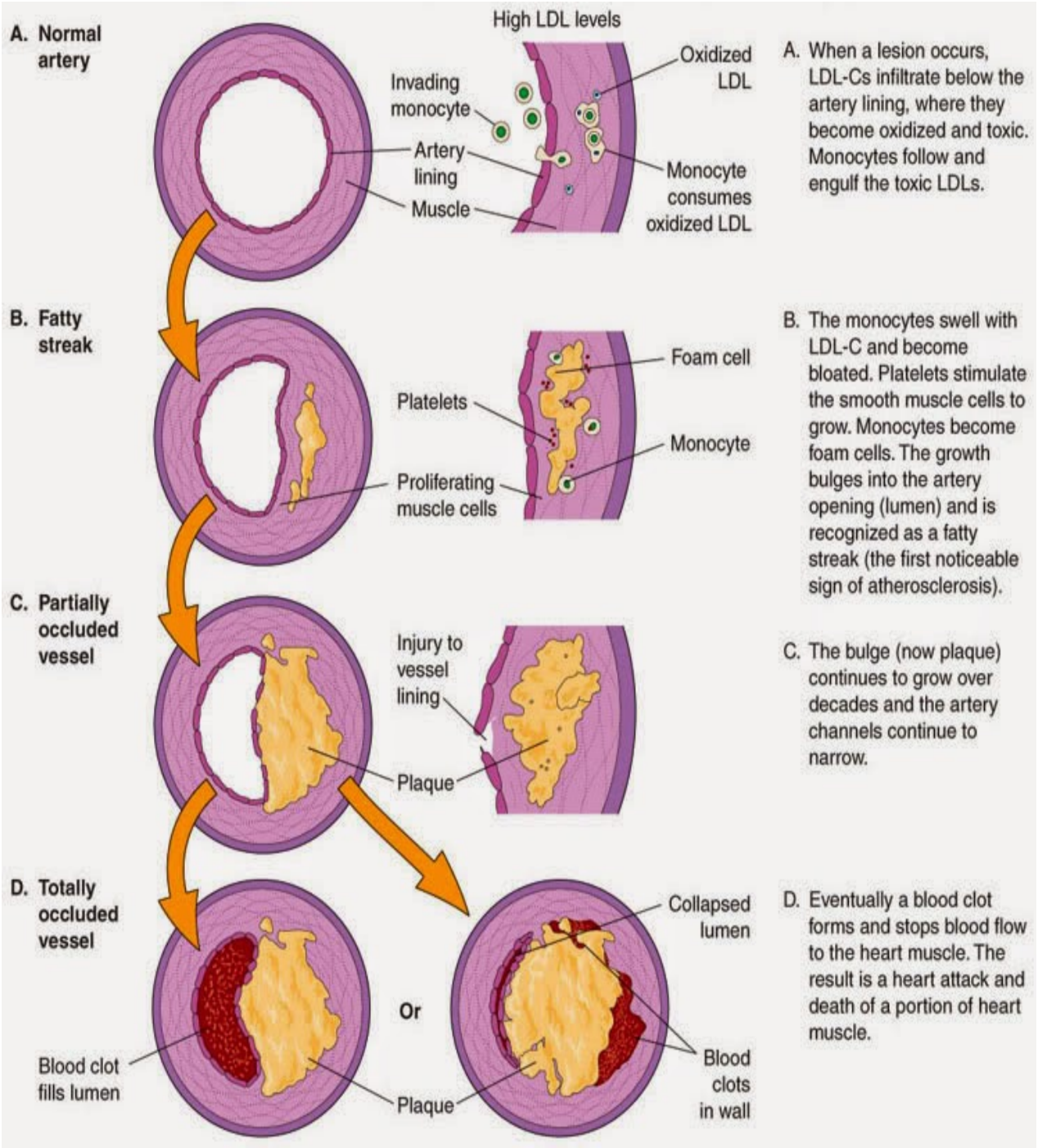
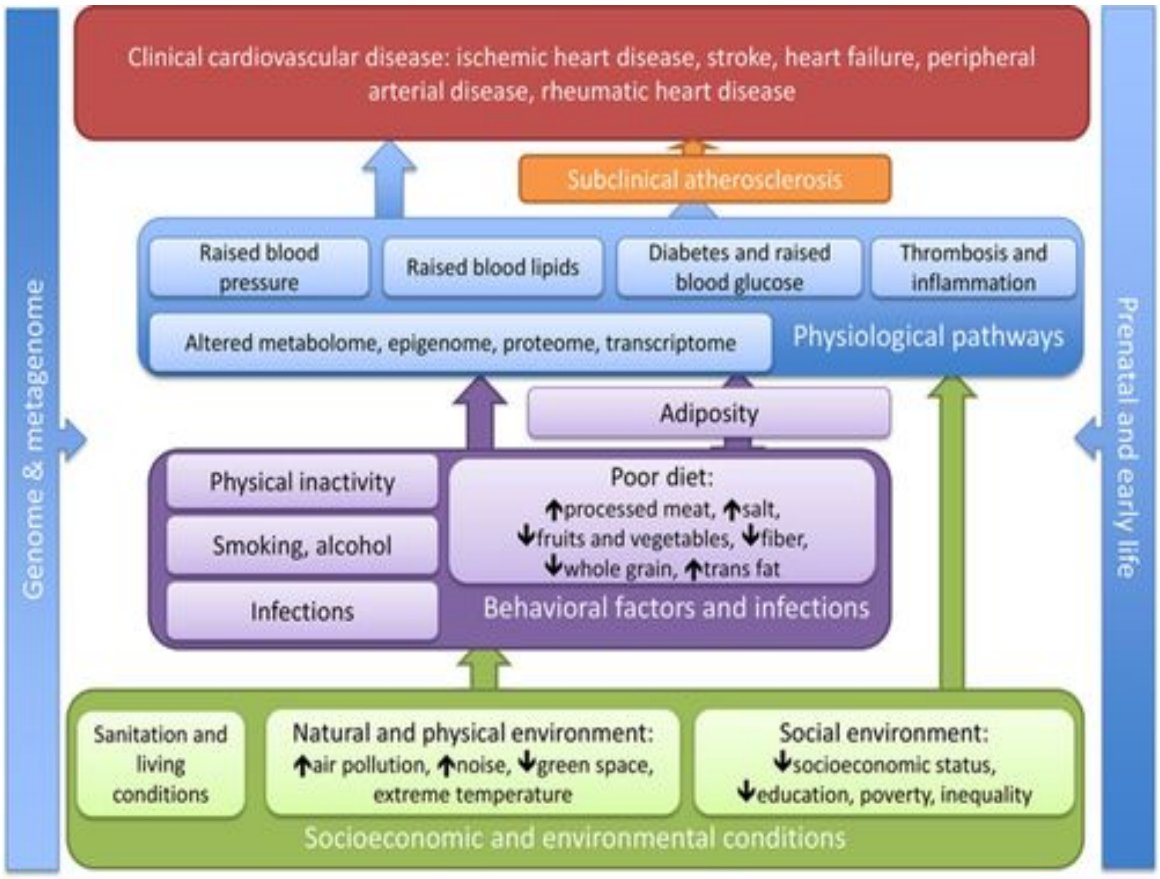
**Figure 1.1**: Schematic diagram of the pathogenesis of coronary heart disease



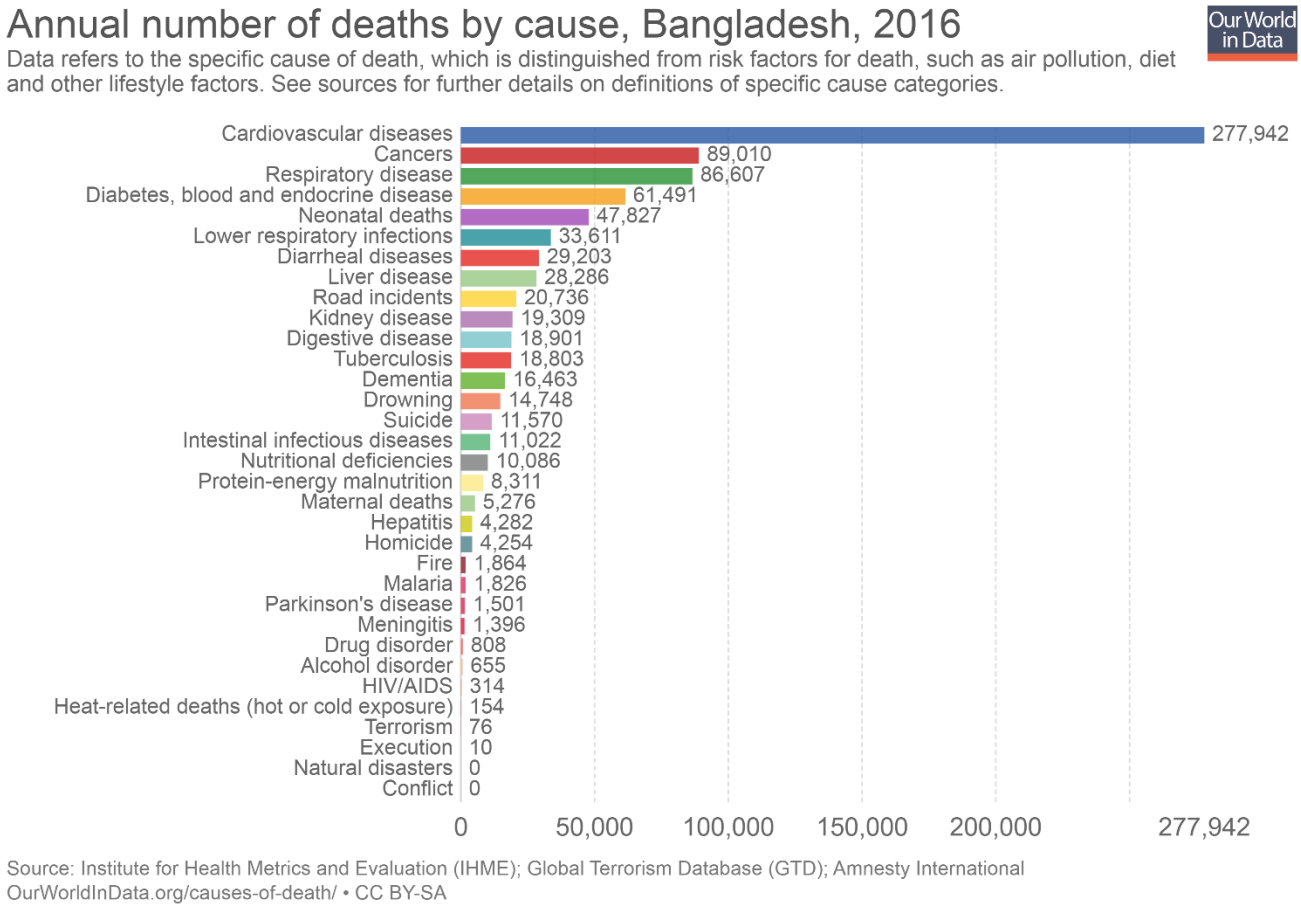

Source:www.homeopathichub.blogspot.com/search/label/atherosclerosis%20cause

**Figure 1.2:** Schematic diagram of the proposed determinants and risk factors for coronary heart disease



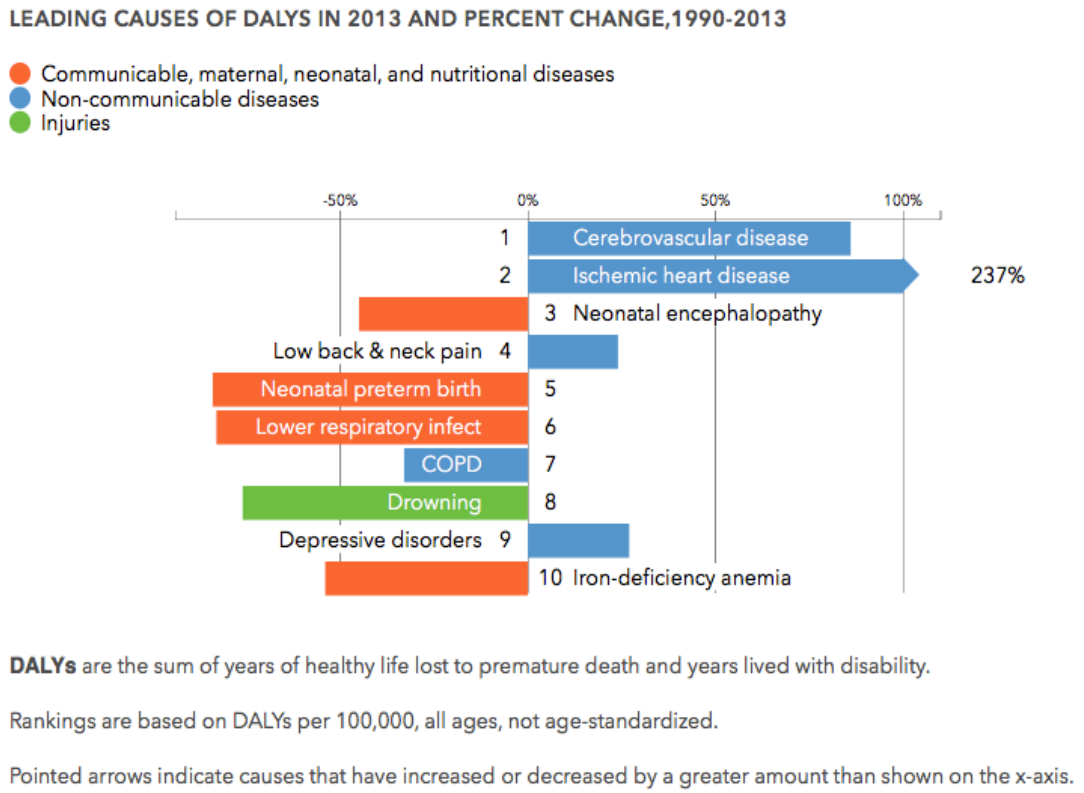

Source: Tzoulaki I et al, 2016[18]

**Figure 1.3:** Showing annual number of deaths by cause in Bangladesh.



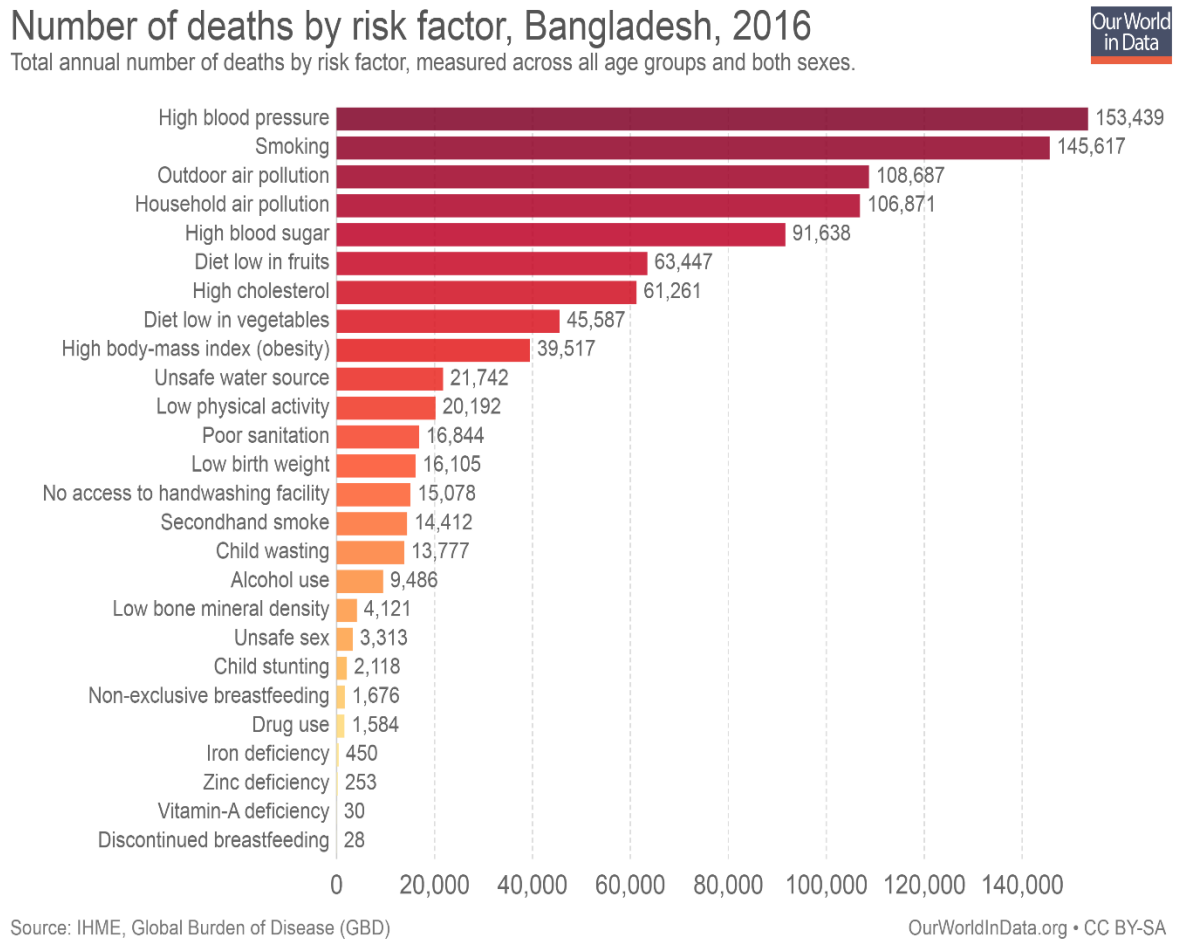

Source: OurWorldInData.org

**Figure 1.4:** Showing the leading causes of DALYS in 2013 and the percentage change from 1990-2003 of the burden of disease in Bangladesh.



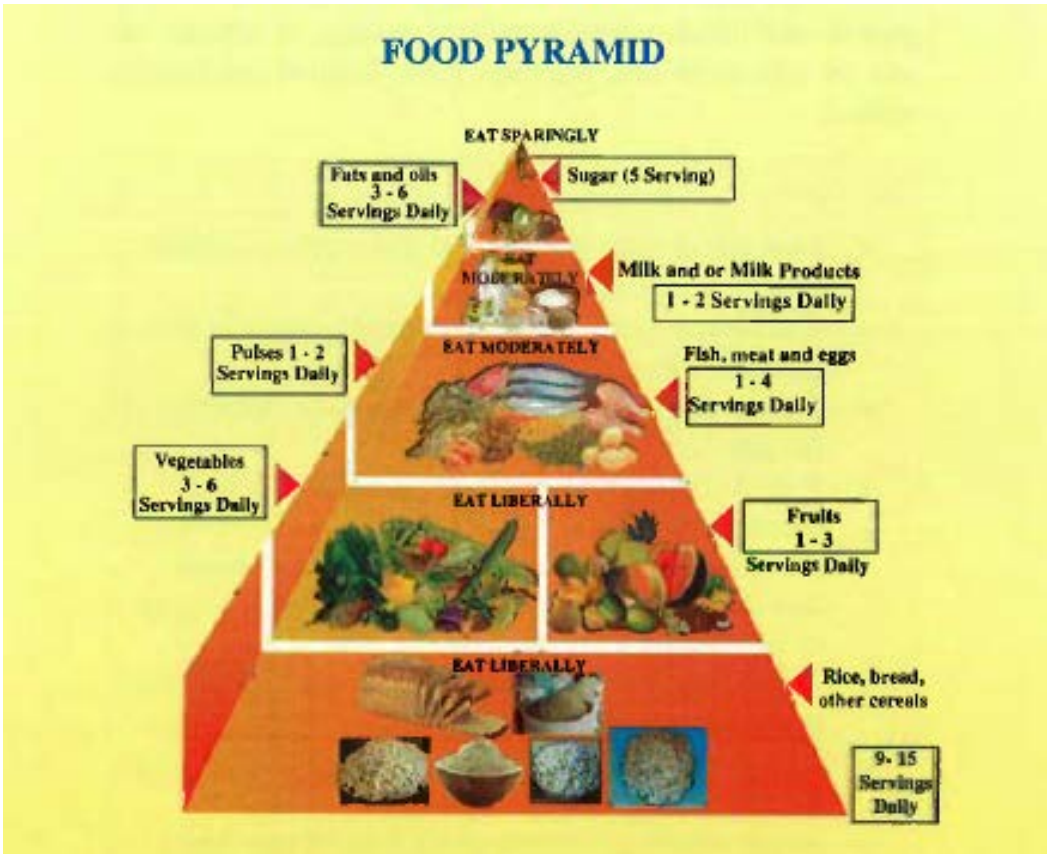

Source: Institute of health metrics and evaluation

**Figure 1.5:** Showing number of deaths by risk factors in Bangladesh.



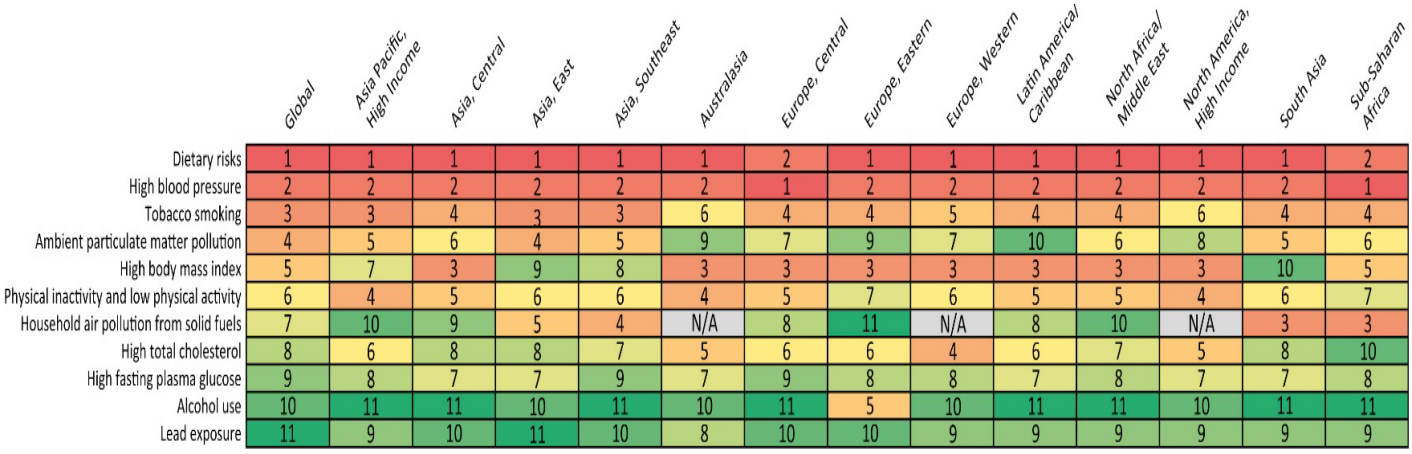

Source: OurWorldInData.org

**Figure 1.6:** Food guide pyramid for Bangladeshi population




Source: http://www.fao.org/3/a-as880e.p

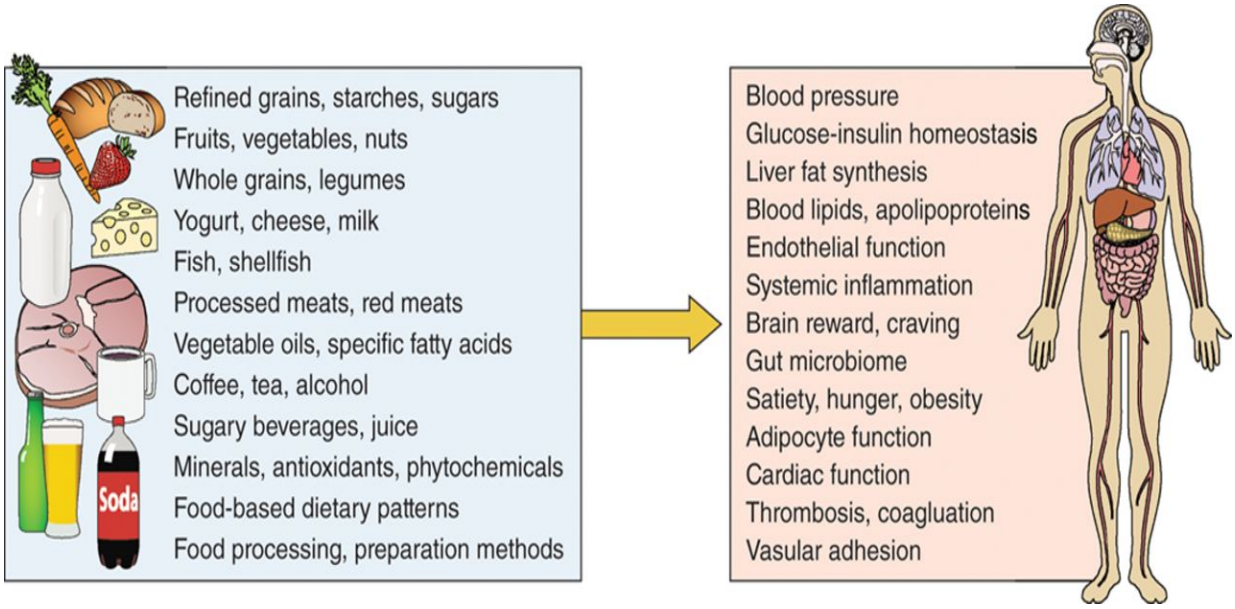
**Figure 1.7:** Risk factors for cardiovascular and circulatory diseases, ranked by disability-adjusted life years (DALYs) attributed to each risk factor.



| | Global | Asia Pacific, High Income | Asia, Central | Asia, East | Asia, Southeast | Australasia | Europe, Central | Europe, Eastern | Europe, Western | Latin America/ Caribbean | North Africa/ Middle East | North America, High Income | South Asia | Sub-Saharan Africa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dietary risks | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| High blood pressure | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 |
| Tobacco smoking | 3 | 3 | 4 | 3 | 3 | 6 | 4 | 4 | 5 | 4 | 4 | 6 | 4 | 4 |
| Ambient particulate matter pollution | 4 | 5 | 6 | 4 | 5 | 9 | 7 | 9 | 7 | 10 | 6 | 8 | 5 | 6 |
| High body mass index | 5 | 7 | 3 | 9 | 8 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 10 | 5 |
| Physical inactivity and low physical activity | 6 | 4 | 5 | 6 | 6 | 4 | 5 | 7 | 6 | 5 | 5 | 4 | 6 | 7 |
| Household air pollution from solid fuels | 7 | 10 | 9 | 5 | 4 | N/A | 8 | 11 | N/A | 8 | 10 | N/A | 3 | 3 |
| High total cholesterol | 8 | 6 | 8 | 8 | 7 | 5 | 6 | 6 | 4 | 6 | 7 | 5 | 8 | 10 |
| High fasting plasma glucose | 9 | 8 | 7 | 7 | 9 | 7 | 9 | 8 | 8 | 7 | 8 | 7 | 7 | 8 |
| Alcohol use | 10 | 11 | 11 | 10 | 11 | 10 | 11 | 5 | 10 | 11 | 11 | 10 | 11 | 11 |
| Lead exposure | 11 | 9 | 10 | 11 | 10 | 8 | 10 | 10 | 9 | 9 | 9 | 9 | 9 | 9 |

Source: Moran A E et al, 2014[72]

**Figure 1.8:** Showing diet and cardiovascular pathways and mechanisms.




Source: Mozaffarian D et al, 2016[73]

**Figure 1.9:** PRISMA flow diagram of literature review

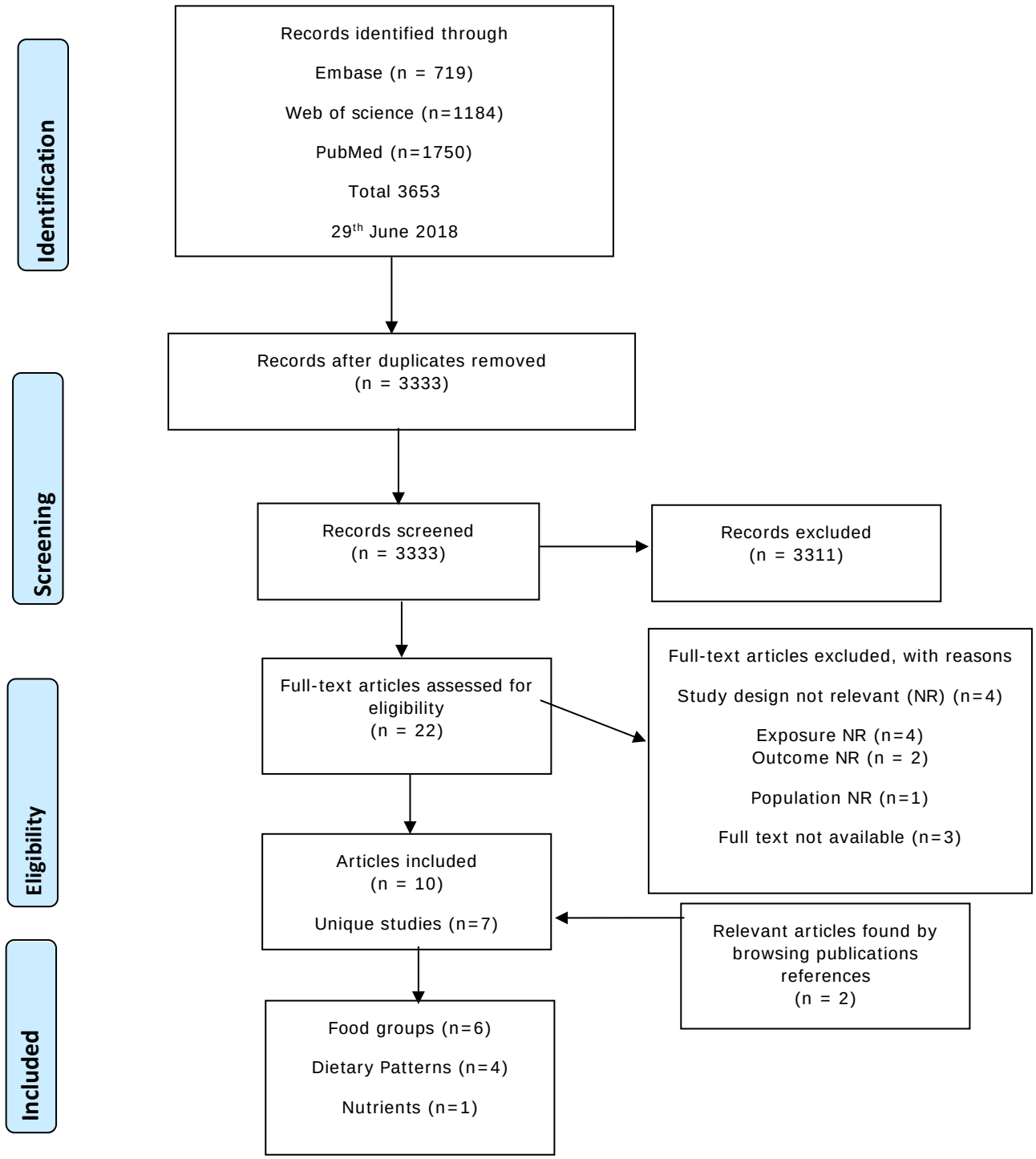


**Table 1.1:** Dietary guidelines by Food and Agriculture Organization (FAO) in Bangladesh.

| Food items | Dietary recommendation set by FAO |
|---|---|
| **Rice and whole grains** | Eat rice or wheat or a combination of cereals around 270-450 g which is equivalent to 9-15 servings daily |
| **Protein intake** | Eat 1 to 4 medium size pieces of fish, meat, poultry and 1/3 to 1/2 cup of pulses daily. |
| **Fruits** | Eat 2 seasonal fruits every day, one from citrus, another from vitamin A sources. |
| **Vegetables** | Eat at least 100g leafy and 200g non-leafy vegetables daily. |
| **Dairy products** | Take at least one cup (150 ml) milk or one cup (100 ml) milk product such as plain curd or yoghurt as a good source of calcium for healthy bones and teeth |
| **Oil** | Vegetable oil, like mustard and soybean oil should be used in moderation daily instead of ghee, butter, and palm oil |
| **Fat intake** | Total fat should provide between 15-30% of the daily energy intake. Saturated fat should provide less than 10% of the daily energy intake. |

**Table 1.2:** Strengths and limitations of the main dietary assessment methods used in epidemiological studies.

| Dietary instrument | Rationale for use | Strengths | Weaknesses |
|---|---|---|---|
| Food Frequency Questionnaires | To assess past diet for a relatively long period of time. | • Low participant burden<br>• Long term diet captured<br>• Lower cost than other dietary assessment methods | • Recall bias<br>• Foods often grouped<br>• May over or underestimate energy intake |
| 24-hour recalls | To assess current diet | • Low participant burden<br>• Immediate recording<br>• Standardised interview<br>• Low cost | • Recall bias<br>• Multiple recalls needed<br>• Difficulty to assess portion size<br>• May over or under estimate energy |
| Food diaries | To assess current diet | • Real time capture<br>• Provides detailed information | • High participant burden<br>• Generalizability<br>• Infrequent foods may not be captured<br>• May underestimate energy<br>• High coding costs |

Source: Naska et al, 2017[93]

**Table 1.3**: Inclusion and exclusion criteria and search terms of literature review.

**Inclusion criteria:**

- Cohort studies and case-control studies investigating the relationship between different food groups with fatal and non-fatal CHD
- Cohort studies and case-control studies investigating the relationship between different dietary patterns and fatal and non-fatal CHD.
- Cohort studies and case-control studies investigating the relationship between different nutrients and fatal and non-fatal CHD.
- Studies from the South Asian population (immigrant or indigenous)

**Exclusion criteria:**

- Cross-sectional studies
- Letters, reviews, animal studies.
- Studies looking at risk factors of CHD as outcomes
- Studies that had grouped results on CVD (CHD and stroke).

Systematic searches were conducted in PubMed using the following **search strategy**:

1. "heart diseases"[MeSH Terms] OR “Cardiovascular disease" OR "coronary artery disease" OR “coronary heart disease” OR "heart disease" OR "myocardial infarction"
2. AND (diet OR food OR "food groups" OR food habits OR “dietary patterns” OR “food patterns” OR nutrient* OR macronutrient OR “fatty acid” OR “saturated fat” OR “polyunsaturated fat” or “monounsaturated fat” OR “unsaturated fat” OR pufa OR mufa OR sfa OR carbohydrate OR protein OR meat OR rice OR dairy OR poultry OR pulse OR grain OR vegetable OR fruit OR egg OR fish OR potato OR spice)
3. AND (pakistan OR india OR bangladesh OR sri lanka OR afghanistan OR nepal OR bhutan OR maldives)))) OR (pakistan OR india OR bangladesh OR sri lanka OR afghanistan OR nepal OR bhutan OR maldives [MeSH Terms]) OR (south asian OR indian OR pakistani OR bangladeshi OR sri lankan OR nepali OR afghani )
4. AND cohort studies [MeSH Terms] OR "case-control studies"[MeSH Terms] OR "cross-sectional studies"[MeSH Terms] OR "follow-up studies"[Mesh] OR "prospective" OR longitudinal OR “nested case-control” OR "case cohort"
5. Filters Human

There was no restriction for language or time of publication. Similar search was conducted in Web of Science using the following search terms:

1. TS= ("heart diseases" OR “Cardiovascular disease" OR "coronary artery disease" OR “coronary heart disease” OR "myocardial infarction")
2. AND TS=( diet OR food OR "food groups" OR food habits OR “dietary patterns” OR “food patterns” OR nutrient* OR macronutrient OR “fatty acid” OR “saturated fat” OR “polyunsaturated fat” or “monounsaturated fat” OR “unsaturated fat” OR pufa OR mufa OR sfa OR carbohydrate OR protein OR meat OR rice OR dairy OR poultry OR pulse OR grain OR vegetable OR fruit OR egg OR fish OR potato OR spice)
3. AND TS= (pakistan OR india OR bangladesh OR sri lanka OR afghanistan OR bhutan OR Maldives OR nepal OR “south asian” OR indian OR pakistani OR bangladeshi OR sri Lankan OR nepali OR afghani)
4. AND TS= ("cohort studies" OR "case-control studies" OR "cross-sectional studies" OR "follow-up studies" OR prospective OR longitudinal OR "nested case-control" OR "case cohort")

Embase

1. ((exp heart disease/ OR exp cardiovascular disease/ OR exp coronary artery disease/ OR exp coronary heart disease/ OR exp heart infarction/))
2. AND ((exp diet/ OR exp food/ OR exp food intake/ OR dietary pattern.mp. OR food pattern.mp. OR exp nutrient/ OR exp macronutrient/ OR exp fatty acid/ OR exp saturated fat/ OR exp polyunsaturated fat/ or exp monounsaturated fat/ OR exp unsaturated fat/ OR exp carbohydrate/ OR exp protein/ OR exp meat/ OR exp rice/ OR exp dairy product/ OR exp poultry/ OR exp legume/ OR Expo whole grain/ OR exp refined grain/ exp vegetable/ OR exp fruit/ OR exp egg/ OR exp fish/ OR exp potato/ OR exp spice/))
3. AND ((exp Pakistan/ OR exp india/ OR exp Bangladesh/ OR exp sri lanka/ OR exp afghanistan/ OR exp Bhutan/ OR exp Maldives/ OR exp nepal/ OR exp “south asian”/ OR exp indian/ OR exp Pakistani/ OR exp Bangladeshi/ OR exp sri Lankan/))
4. AND (cohort studies.mp. OR exp cohort analysis/ OR exp case-control study/ OR exp cross-sectional study/ OR exp follow-up / OR exp prospective study/ OR exp longitudinal study/ OR exp nested case-control.mp./ OR exp case cohort/))
5. NOT (animals)

**Table 1.4:** Summary of studies examining the association between dietary food groups with the risk of CHD in South Asians.

| Author | Study design | Location | Population number | Age | Exposure assessment | Outcome assessment | Confounders adjusted for | Summary of results |
|---|---|---|---|---|---|---|---|---|
| **Rastogi et al, 2004** | Matched case-control study | Urban hospitals in New Delhi and Bangalore, India | 350 cases and 700 controls | 21-74 | FFQ | Cases with first MI | Age, sex, smoking, BMI, waist-to-hip ratio, leisure time physical exercise, history of hypertension, history of diabetes, history of high cholesterol, family history of CHD, alcohol, education, household income and Hindu religion | A median of 3.5 servings/week of green leafy vegetables had a 67% lower relative risk<br><br>Cereal intake and mustard oil were also associated with lower risk of first MI |
| **Rafique et al, 2014** | Matched case-control study | Five major hospitals in Lahore, Pakistan | 190 cases and 380 controls | 35-55 | FFQ | Cases with first MI | Age, sex, smoking and BMI | Eggs, sweets, butter, desi ghee, desserts and beef were significant risk factors<br><br>Fish and fruit were significant protective dietary predictors<br><br>Vegetables showed no association |
| **Karim et al, 2015** | Prospective observational study | Dhaka, Bangladesh | 50 patients less than 40 years old and 50 more than 40 years old | young group : | Questionnaire | Patients with first acute ST Segment Elevation Myocardial | Education and socioeconomic status | Taking rice ≥2 times daily had 3.5-fold higher risk of AMI in the younger group compared to the older group |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 36.5 ± 4.6<br><br>old group : 57.0 ± 9.1 | | Infarction (STEMI) or AMI | | Taking beef ≥2 times daily had 4.5 fold risk of AMI in the younger group compared to the older group<br><br>Taking vegetables ≥2 times daily had an inverse association with AMI in the younger group compared to the older group |
| **Miller et al, 2017 (PURE)** | Prospective | Multi-centre including south Asian population | 135 335 individuals India: 25324 Pakistan: 1477 Bangladesh: 2759 | 35-70 years | FFQ | CVD, fatal and non-fatal MI and total mortality | Age, sex, centre, energy intake, current smoker, diabetes, urban or rural location, physical activity, education level, and tertiles of white meat, red meat, breads, and cereals intake. | An inverse association of fruits, vegetables and legumes with the risk of MI in the age and sex adjusted models. However, after multivariate adjustment for confounders this association became non-significant. |
| **Iqbal et al, 2008 (INTERHEART)** | Case-control | Multi-centre including | 5761 cases and 10 646 controls South Asian population India: 470 cases, 940 controls Pakistan: 637 cases, 655 controls; Bangladesh: 228 cases, | | FFQ | AMI | Age, sex, region, education, smoking, physical activity, BMI, region, household income, psychosocial factors, and ApoB/ApoA1 tertiles. | Highest quintiles vs lowest quintile of green leafy vegetables, other raw vegetables, cooked vegetables, fruits had a significant inverse association with the risk of MI.<br><br>Highest intake of meat and grains were not associate with the risk of MI.<br><br>Highest quintile of fried foods and salty foods had a higher risk of MI |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 238 controls;<br>Sri Lanka: 153 cases, 132 controls;<br>Nepal: 244 cases, 239 controls) | | | | |
| **Joshi et al, 2007 (INTERHEART)** | Case-control | Multi- centre South Asian region | 1732 AMI cases and 2204 controls<br><br>India: 470 cases, 940 controls<br>Pakistan: 637 cases, 655 controls;<br>Bangladesh: 228 cases, 238 controls;<br>Sri Lanka: 153 cases, 132 controls;<br>Nepal: 244 cases, 239 controls) | FFQ | AMI | Age, sex, and smoking status | Intakes of fruits and vegetables >1 portion a day had an inverse association with the risk of MI |

**Table 1.5:** Summary of studies examining the association between dietary patterns with the risk of CHD in South Asians.

| Author | Study design | Location | Population number | Age | Exposure assessment | Outcome assessment | Confounders adjusted for | Results |
|---|---|---|---|---|---|---|---|---|
| **Pais et al, 1996** | Matched case-control study | Bangalore, India | 200 cases and 200 controls | 30-60 | Questionnaire<br><br>Vegetarian and non-vegetarian diet | Cases with first MI | Age, sex, smoking, HDL, LDL, triglycerides, blood glucose | Vegetarianism had a protective effect. |
| **Iqbal et al, 2015** | Case-control study | Karachi, Pakistan | 203 cases 205 controls | <45 | FFQ<br><br>Dietary patterns:(i)" prudent dietary pattern", characterised by high intakes of legumes, cooked and uncooked vegetables, wheat, chicken and fruits; (ii) "combination dietary pattern", which was characterised by high consumption of eggs, fish, fruits, juices and coffee; and "western dietary pattern", which was characterised by high intake of meat, fish and tea with milk. | AMI | BMI, ferritin, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol | An inverse association of prudent diet and combination dietary pattern with MI.<br><br>No association was observed between western diet and risk of MI. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Chen et al, 2013 (HEALS)** | Prospective cohort study | Araihazaar, Bangladesh | 11,116 | 18-75 | 39 item FFQ<br><br>Dietary patterns: (i) a "balanced" pattern, comprised of steamed rice, red meat, fish, fruit and vegetables; (ii) an "animal protein" diet, which was more heavily weighted towards eggs, milk, red meat, poultry, bread, and vegetables; and (iii) a "gourd and root vegetable" diet that heavily relied on a variety of gourds, radishes, pumpkin, sweet potato, and spinach | CVD deaths<br><br>Defined by ICD 10 | Age, sex, energy intake, BMI and smoking status | Increased association of CHD with animal protein diet. |
| **Iqbal et al, 2008 (INTERHEART)** | Case-control | Multi-centre including south Asian population | 5761 cases and 10 646 controls<br><br>India: 470 cases, 940 controls<br>Pakistan: 637 cases, 655 controls; Bangladesh | | FFQ<br><br>Dietary patterns: (i) Oriental (high intake of tofu and soy and other sauces), (ii) Western (high in fried foods, salty snacks, eggs, and meat), and (iii) prudent (high in fruit and vegetables). | MI | Age, sex, region, education, smoking, physical activity, BMI, region, household income, psychosocial factors, and | Prudent diet had an inverse association with AMI.<br><br>The Western pattern showed a U-shaped association with AMI. However, there was no association when stratified by South Asian region.<br><br>The Oriental pattern had no association with AMI. |

| | | |
|---|---|---|
| | : 228 cases, 238 controls; Sri Lanka: 153 cases, 132 controls; Nepal: 244 cases, 239 controls) | ApoB/ApoA1 tertiles. |

**Table 1.6:** Summary of evidence examining the association between dietary nutrients with the risk of CHD in South Asians.

| Author | Study design | Location | Population number | Age | Exposure assessment | Outcome assessment | Confounders adjusted for | Results |
|---|---|---|---|---|---|---|---|---|
| **Dehgan et al, 2017 (PURE)** | Prospective | Multi-centre including south Asian population | 135 335 individuals<br><br>India: 25324<br>Pakistan: 1477<br>Bangladesh : 2759 | 35-70 years | FFQ | CVD, fatal and non-fatal MI and total mortality | Age, sex, education, waist-to-hip ratio, smoking, physical activity, diabetes, urban or rural location, and energy intake | Highest vs lowest quintile of carbohydrates, total fats and protein was not associated with the risk<br><br>Subtypes of fats were also not associated with the risk of MI.<br><br>In substitution analyses, replacement of carbohydrates with SFA, PUFA, MUFA and proteins had no association with the risk of MI |

# Chapter 2: Description of the Bangladesh Risk of Acute Vascular Events (BRAVE) case-control study

## 2.1 Chapter summary

BRAVE is a large hospital-based case-control of about 8000 cases and 8000 controls matched by age and sex in Dhaka, Bangladesh. This study has overlapping data on lifestyle (including dietary determinants), biochemical, genetic and environmental risk factors for coronary heart disease (CHD) in the Bangladeshi population. BRAVE was initiated in 2011 by the University of Cambridge, in collaboration with the Chronic Non-communicable Disease Unit at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and the National Institute of Cardiovascular Disease (NICVD) in Bangladesh. The aims of this chapter are to (1) summarise the study design of BRAVE case-control study which has been previously published; (2) explain the harmonization of dataset used in subsequent analyses in this thesis; (3) describe baseline characteristics of cases and controls and (4) investigate the association of conventional and local risk factors of CHD specific to Bangladesh.

The data reports that: (1) the mean age of cases is 52.4 and controls is 51.2; (2) as compared to controls, cases have significantly higher waist circumference, history of diabetes, history of hypertension, family history of myocardial infarction (MI), level of low density lipoproteins (LDL-C) and were more likely to be currently smoking and less physically active; (3) as for the dietary intake, the controls have higher consumption of potatoes, vitamin C rich fruits, deep fried snacks, savoury snacks, sweets, eggs, milk and yoghurt as compared to the cases; (4) for conventional cardiovascular risk factors such as history of disease, blood lipids, cigarette smoking, and waist-to-hip ratio are significant risk factors of CHD; (5) as for the local socio-economic and behavioural factors, parental consanguinity, living below the poverty line, lifelong urban residence, high levels of stress and day-time nap are each significantly associated with increased risk of acute MI (AMI) in this population.

## 2.2 BACKGROUND

Cardiovascular disease (CVD) is the leading cause of death and disability in the world, with 80% burden occurring in low-and middle-income countries (LMIC). Quarter of the world's population lives in South Asia and it is projected that over the next years the burden of CVD is projected to increase more in this area than other geographic areas worldwide.[1,2] South Asian populations remain largely understudied with regards to the potential determinants of CVD. In particular in Bangladesh, a country with about 165 million population and in which coronary outcomes (predominately myocardial infraction (MI)) are the leading causes of death and disability, remains understudied.[3,4] Despite the need, previous epidemiological studies are scarce, limited with insufficient power and have methodological issues such as based solely on rural populations or using solely self-report methods to ascertain vascular and nonvascular outcomes. This highlights the pressing need for reliable evidence on the determinants of CHD derived from large epidemiological studies. The investigation of these risk factors will extend the relevance of established and suspected determinates relevant to this population such as those related to local lifestyles, dietary habits and environmental contaminants.

In this context, the objective of BRAVE study is to investigate lifestyle (including dietary determinants), biochemical, genetic and environmental risk factors for first MI, main clinical manifestation of CHD in the Bangladeshi population. BRAVE is a hospital based study that was initiated in 2011 at the icddr,b and the NICVD in Dhaka, Bangladesh.[5] The icddr,b is the project's national collaborating centre and houses the local laboratory facilities for the study **(Figure 2.1)**. The NICVD, Bangladesh's largest cardiology care centre, treats acute MI (AMI) patients from Dhaka (the capital city; population ~20 million) as well as from surrounding semi-urban and rural areas. The study recruitment was completed in 2016 and it has recruited about 8000 cases and 8000 controls. BRAVE is serving as a huge bio-resource with extensive baseline questionnaire and physical measures, as well as stored blood samples to investigate different determinants of AMI. For lifestyle factors such as dietary consumption, this study is a largest and most detailed investigation on how food groups, dietary patterns and dietary nutrients affect the risk of AMI in Bangladesh.

## 2.3 METHODS

### 2.3.1 Study location

The study is based in Dhaka as shown in **Figure 2.1**. Dhaka is the capital of Bangladesh with about 20 million population as of 2018.[6] NICVD, is the largest referral hospital in Dhaka that receives patients from the entire country. Icddr,b is a research organisation based in Bangladesh that has the study clinic to collect data from the participants of the study. This hospital is the key tertiary and referral centre in Dhaka for all divisions (urban and rural) and therefore appropriate for recruiting cases and controls

### 2.3.2 Ascertainment of the Study Outcomes and Controls

**Figure 2.2** shows the BRAVE study flow diagram. Following screening by medically-qualified research officers, patients (male or female; aged at least 20 years) admitted to the emergency rooms of the NICVD hospital were eligible for inclusion as AMI cases if they fulfilled all of the following criteria: (1) presentation at the hospital within 48 hours of the onset of sustained clinical symptoms suggestive of AMI lasting longer than 20 min; (2) presence of ECG changes indicative of AMI (i.e., new pathologic Q waves, at least 1 mm ST elevation in any 2 or more contiguous limb leads or a new left bundle branch block, or new persistent ST-T wave changes diagnostic of a non-Q wave MI); (3) increased cardiac troponin-I (cTnI) levels; (4) no previous CVD, defined as self-reported history of angina, MI, coronary revascularisation, transient ischaemic attack, stroke, other CVD or evidence of CHD on prior ECG, or in other medical records; and (5) not concurrently hospitalised for any other CVD events.[5].

Controls were recruited within 48 hours of recruiting index cases and they were “frequency-matched” to cases by sex and age (in 5-year age bands).[5] Controls were without a previous self-reported history of MI (as defined above) drawn from individuals concurrently identified in the same hospital as index cases, and recruited in the following order of priority: (1) visitors of patients attending the out-patient department (excluding the visitors of the included cases); (2) visitors of in-patients who were not part of the BRAVE study; and (3) visitors of index AMI cases who were not their blood relatives. All controls went through same study questions as the cases. The approach described above was chosen to potentially achieve a balance between feasibility and scientific rigour. Although it could be argued that population-based controls should have been used in principle, however they are expensive to recruit, require more time and still may not guarantee representation of the catchment area from which cases are derived, particularly since reference patterns of hospitals are complex. Furthermore, since the controls were

recruited from the visitors of outpatients and non-blood relatives of the AMI cases this would minimize the potential bias with recruitment of conventional hospital-based controls. In addition, participants were not recruited if they (1) had a history of acute viral or bacterial infection in the previous 2 weeks; (2) documented chronic conditions, such as malignancy, any chronic infection, leprosy, malaria, tuberculosis or other bacterial/parasitic infections, chronic inflammatory disorders, chronic hepatitis or chronic kidney disease on past medical history; (3) had a recent history of any surgery; (4) were pregnant; or (5) were unable to provide consent.[5]

### 2.3.3 Assessment of covariates

A pre-piloted questionnaire of over 350 items of information on demographic factors, tobacco consumption, dietary intake, physical activity, personal and family medical history was administered to the participants of the study.[5] In addition to the main outcome and dietary exposures, several other covariates were considered as possible confounders or effect modifiers to be included in the statistical analyses, based on prior knowledge.

Past medical history was assessed by the question “Have you ever diagnosed with diabetes?” It also included list of conditions: CHD, stroke, arterial fibrillation, hypertension, high blood cholesterol, peripheral vascular disease, valvular disease, chronic liver disease, acute febrile illness (>4 days during past 6 months), malignant tumour and surgical history. Family history (defined as maternal and paternal) of MI, angina, stroke, hypertension, diabetes and sudden death were also asked in the questionnaire as yes or no.

Physical activity was evaluated by asking participants questions about the activity at work; travel to and from places, recreational activities and sedentary behaviour. Participants were further asked about how much time they spend doing vigorous and moderate intensity activities at work in a typical day, how much time they spend walking or bicycling on a typical day, how much time they spend doing vigorous and moderate intensity sports, fitness or recreational activities on a typical day and how much time do they spend sitting or reclining on a typical day. Smoking status was assessed by asking the participants about their smoking status (current, ex, never) and participants were also asked about type of tobacco related products used. The questionnaire also collected information on social-demographic factors such as level of education, annual income, location of residence and occupation of the participants. In addition, information was also collected on psychosocial factors, assessing the stress at work and home. Information on day time nap was collected by asking participants “In a typical week, how much time did you sleep at noon in average in last 3 months?”

Trained research nurses used standardised and calibrated equipment to collect measurements of height, weight, waist-to-hip ratio, systolic and diastolic blood pressures and heart rate. Waist circumference was assessed over the abdomen at the widest diameter between the costal margin and the iliac crest, and hip circumference has been assessed at the level of the greater trochanters (i.e., the widest diameter around the buttocks). For both cases and controls, anthropometric measurements were performed in a standing position.

### 2.3.4 Collection of biological samples

Non-fasting blood samples (with the time since last meal recorded) were drawn by trained study staff nurses from each participant and centrifuged (@10,000 rpm for 10 min) within 45 min of venipuncture. For AMI cases, blood sampling was conducted within 48 hour of the onset of clinical symptoms (time since onset of pain is recorded) and prior to the administration of any thrombolytic medication.[5] As the blood sample is typically obtained from AMI cases while they are in a recumbent position (e.g., at about 45°), the sampling in the controls was carried out in the same manner to limit the possibility of systematic differences. Biological samples were stored in long-term repositories in both Cambridge, UK, and in Dhaka, Bangladesh.[5] Blood lipid concentrations (total cholesterol, LDL-C and high density lipoprotein (HDL-C)) were obtained from these samples.

### 2.3.5 Data harmonisation and preparation

Distributions of continuous variables were assessed by visual inspection of boxplots and histograms in STATA. Waist-to-hip ratio showed a skewed distribution, so it was log transformed.

For the analyses, physical activity was calculated using frequency and intensity of each activity (walking, moderate-intensity activities and vigorous intensity activities) collected by questionnaire based on International Physical Activity Questionnaires (IPAQ)[7] in terms of metabolic equivalent tasks (METs/min/week). One metabolic equivalent is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml $O_2$ per kg body weight x min.[8] These values were obtained by multiplying the average energy expenditure (3.3 MET for walking, 4.0 MET for moderate intensity, and 8.0 MET for vigorous intensity) by min/week for each physical activity and then summed to obtain total physical activity.[7] The METs were converted into two categories $<600$ and $\geq 600$ according to the cut offs defined by World Health Organisation (WHO).[7]

Categorical variables were analysed using the standard categories provided in the questionnaire or alternatively combined into fewer categories where there were low numbers of participants. Highest level of education achieved by the participant was categorised as no schooling, primary, secondary and university/vocational. Occupation was categorised as business/professional (e.g., self-employed, office executive, government or NGO staff), manual labour (agriculture/construction based, labour), non-manual labour (indoor industry based) and unemployed/student/retired). These categories were used to separate more physically active professions with, professions with pre-dominantly sedentary nature. These categories have been broadly based on the Bangladesh Demographic Health Survey but clumped together due to fewer number of people in each categories.[9] Life-long rural residence was defined as those people who were living in rural areas and had never migrated. The annual income was converted into four categories. The first category was defined as income below the poverty line by using the international poverty line cut-off of $1.90 per day as of 2015, which is equal to approximately 56000 Bangladeshi Taka yearly.[10] The low income was defined as >56000 – 99999 Bangladeshi Taka yearly, medium income as >99999 -199999 Bangladeshi Taka yearly and high income as >199999-999999. High stress was defined as those who reported having stress often and always. Day time nap was categorised as those who slept at noon and those who did not. **Table 2.1** shows the list of main covariates that have been used in the analyses in subsequent chapters. For baseline variables, mean (standard deviation (SD)) values and frequencies were calculated separately for AMI cases and controls and compared using t-test for mean intakes or Chi-squared test for categorical variables presented in **Table 2.2**.

### 2.3.6 Assessment of Dietary exposure and data preparation

Dietary information was obtained using a semi-quantitative Food Frequency Questionnaire (FFQ) which has been developed and validated in Bangladesh and was further adapted for the BRAVE study.[11] In the validation study the correlations of macronutrients and common micronutrients comparing FFQ and 7 day food diary ranged from 0·30 to 0·76.[12] The FFQ consisted of 145 items and asked participants to recall their diet over the past year **(Appendix 2)**. Participants were asked to choose from a list of nine consumption frequencies ranging from no intake to six or more portions per day. To estimate the daily intake of food items the servings were first converted into portion/day by converting the frequencies in FFQ into a multiplier as shown in **Table 2.3.** Standard portion sizes were determined by taking measurements of FFQ items in the study clinic of the BRAVE study (e.g. one apple, bowl of yoghurt). Grams/day intakes were then calculated by multiplying the portion size of the FFQ items by the chosen multiplier. The FFQ was administered by an interviewer and collected usual intake over the past year. It is important to note as multiple FFQ assessments were not done this may not have accounted for seasonal variation in consumption of some foods such as fruits and vegetables. However, as FFQ measures average intake over past year this may not have a substantial effect. The household members of the participants also confirmed the consumption of food items by cases. FFQ items were collated into groups based on nutrient content and culinary usage as shown in **Table 2.4**. Consumption of butter, cheese, alcohol and western foods were minimal in the population and therefore excluded from the analysis. These food groupings are broadly similar to those which have been reported in national representative Household, Income and Expenditure (HIES) survey conducted in Bangladesh.[13] The 12 major food groupings in the BRAVE study are similar to the HIES survey including fruits, vegetables, eggs, milk, red meat, white meat, rice, bread, pulses etc. Additionally, BRAVE has included separate categories for the relatively less consumed foods such as savoury snacks, deep fried snacks, sweets, biryani and sugar sweetened beverages that HIES were not able to include. Therefore, BRAVE study has a more comprehensive capture of the Bangladeshi diet.

Distributions of dietary intake variables were assessed by visual inspection of histograms in STATA **(Appendix 2, Figure 1)**. Median intakes were used as food intakes were ordinal and were not normally distributed. Food groups were converted into quintiles which has been done by previous studies of diet and risk of CHD in South Asian populations and rest of the world.[14–17] When it was not possible to convert a food item into quintiles non-consumers were treated as a separate group and then quartiles were created between consumers to form 5 categories. However, in case of white meat, red meat, sugar sweetened beverages (SSB), egg, yoghurt, boiled white rice and biryani, I was unable to

categorize the values in 5 groups due to low intakes. Median intakes of food groups with inter quartile range are presented within consumers for cases and controls in **Table 2.5.** Differences in percentage of consumers between cases and controls was done by two sample proportions test. Differences in median intakes between consumers in cases and controls was done by Mann–Whitney U test. To account for multiple testing Bonferroni correction was done in **Table 2.5**.

### 2.3.7 Calculation of total energy intake

To estimate the total energy intake of a participant as assessed by dietary questionnaire, a food composition table was constructed relevant to the data in the BRAVE study. The underlying food composition data are primarily based on the publications from Bangladesh.[18] Where not available, the Indian food composition table was consulted.[19] For mixed dishes, recipe calculations were made using local Bangladeshi websites as references[20–22] and McCance and Widdowson's Composition of Foods,[23] where nutrient values were unavailable from the food composition tables. The details of recipe calculation of mixed dishes are in **Appendix 3**. The data taken from food composition tables corresponding to the food items was manually entered in an excel spread sheet. Energy intake per day (kcal/d) was calculated by multiplying the frequency of consumption of each FFQ item by the energy content (taken from the food composition table) of the standard portion size, divided by 100. These were then summed across all foods for each individual to get an estimated total daily energy intake from food and beverages.

For example:

Energy intake from boiled white (kcal/day) = (frequency of boiled white rice (g/day) x energy of boiled white rice (kcal)) /100

### 2.3.8 Ethical approval

BRAVE has received approval from the relevant research ethics committee of each of the institutions involved in participant recruitment. Written informed consent was obtained from each participant prior to recruitment, including for use of stored samples for biochemical, genetic and other analyses.[5] Data collected in this research are subject to the core data protection principles and requirements of the UK Data Protection Act 1998. The investigators and institutional review boards are committed to ensure that research is conducted according to the latest version of the Declaration of Helsinki, the Universal Declaration on the Human Genome and Human Rights adopted by UNESCO, and other relevant legislation.[5]

### 2.3.9 Participants used in the present analyses

The present analyses were confined to 7066 cases and 8079 controls that had relevant information on age, sex, smoking status, history of diabetes and history of hypertension. **Table 2.6** shows the missing data for variables in these cases and controls. 94% of the participants had complete data on the above-mentioned variables.

### 2.3.10 Association with cardiovascular risk factors

*Association of conventional risk factors with acute myocardial infraction*

Multivariate unconditional logistic regression models were used to assess the association between conventional risk factors (history of diabetes, history of hypertension, family history of MI, waist-to-hip ratio, total cholesterol, LDL-C and HDL-C) and risk of AMI. Progressive adjustments of the exposure-outcome associations were conducted to gain insight into the confounding effects of different factors in the BRAVE study.

Four models were applied:

1) Model 1- The minimally adjusted model was adjusted for age and sex.

2) Model 2- additionally adjusted for smoking status (never, ex and current)

3) Model 3- additionally adjusted for history of disease related variables: history of diabetes (yes or no), history of hypertension (yes or no) and family history of MI (yes or no) as appropriate (e.g. History of diabetes was only adjusted for history of hypertension and family history of MI and history of hypertension for diabetes and family history of MI).

4) Model 4 -additionally adjusted for total cholesterol, LDL-C and HDL-C as appropriate (e.g. Total cholesterol and LDL-C was only adjusted for HDL-C, and HDL-C was only adjusted for LDL-C).

The results of sequential adjustments are presented in **Table 2.7**.

Subgroup analyses

To investigate if the associations were modified by age (<50 years or ≥50 years- as mean age was 50 years), sex (males, females) and geographical location (urban/rural), interaction terms were included between exposure variables and each of the indicated stratifying variables **(Figures 2.8-2.10)**.

Shapes of association of conventional risk factors

To characterize shapes of associations of continuous variables , ORs were calculated using deciles of risk factors; the corresponding 95% confidence intervals (CIs) were estimated from floated absolute variances (FAR) that reflect the amount of information underlying each group (including the reference group).[24] Patterns of smoking was adjusted for age, sex, LDL-C and HDL_C, history of diabetes, history of hypertension, family history of MI, physical activity and total energy intake. Physical activity was adjusted for age, sex, LDL-C, HDL-C, smoking status, history of diabetes, history of hypertension, family history of MI, smoking status and total energy intake. The results are presented in **Figures 2.4-2.6**

*Sensitivity analysis*

A sensitivity analysis was done to impute the missing data using multiple imputation with chained equations only for conventional risk factors (such as history of disease, waist-to-hip ratio and blood lipids) with MI. This method uses the distribution of the observed data to estimate a set values for the missing data. Predictive mean matching algorithm (pmm) for continuous variables, logistic regression (logit) for binary variables, and logistic regression (mlogit) for categorical variables. The results are in **Appendix 4**. They are not marginally different from the main results; therefore, multiple imputation was not carried out for other analyses in the thesis.

*Association of local risk factors with acute myocardial infraction*

Multivariate unconditional logistic regression models were used to assess the association between local risk factors (occupation, income, residence, stress levels, intermarriage and day time nap) and risk of AMI. Progressive adjustments of the exposure-outcome associations were conducted to gain insight into the confounding effects of different factors in the BRAVE study.

Three models were applied:

1) Model 1- The minimally adjusted model was adjusted for age and sex.

2) Model 2- additionally adjusted for smoking status (never, ex and current), history of diabetes (yes or no) and history of hypertension (yes or no)

3) Model 3 -additionally adjusted for income and occupation as appropriate (e.g. income was adjusted for occupation only and occupation for income only).

The results of sequential adjustment are presented in **Table 2.8**.

All analyses were performed using Stata (version 14, StataCorp, College Station, TX)

## 2.4 RESULTS

### 2.4.1 General characteristics of the participants in BRAVE

**Table 2.2** summarises the baseline characteristics of cases (N=7066) and controls (N=8079) in BRAVE. The cases and controls have been matched for age and sex. Mean age of cases was 52.4 years old (SD:10.4) and about 89% of the cases were males. The proportion with a history of diabetes was 18.4%, history of hypertension 26.3%, and family history of MI was 15.6%. Mean LDL-C and HDL-C levels were 3.22 (SD:1.03) and 0.82 (SD:0.22) mmol/L. Waist-to-hip ratio had a mean of 0.97 (SD:0.07) and body mass index (BMI) was 22.95 (SD:3.80) kg/m$^2$. Current tobacco users were about 77% and about 66% had physical activity greater than 600 METs/min/week. 37.6% of the cases had no formal schooling, whereas 11.2% had university education. About half of the cases were business/professionals by occupation and about 33% were unemployed. 26.5% of the cases had annual income below the poverty line. 47% of the cases were from urban areas and 53% from rural areas.

Mean age of controls was 51.2 years old (SD:10.3) and about 88% of the controls were males. The proportion with a history of diabetes was 10.3%, history of hypertension 13.6%, and family history of MI was 7.2%. Mean LDL-C and HDL-C levels were 2.81 (SD:0.89) and 0.85 (SD:0.23) mmol/L. Waist-to-hip ratio had a mean of 0.96 (SD:0.07) and BMI was 23.30 (SD:5.0) kg/m$^2$. Current tobacco users were about 60.5% and about 77.3% had physical activity greater than 600 METS/min/week. 34.7% of the controls had no formal schooling, whereas 11.8% had university education. About half of the controls were business/professionals by occupation and about 30% were unemployed. 24% of the controls had annual income below the poverty line. 45% of the controls were from urban areas and 55% from rural areas.

Compared with the controls, cases had significantly higher ($p < 0.05$) waist circumference, history of diabetes, history of hypertension, family history of MI, levels of LDL-C, were more likely to be currently smoking and less physically active and had higher percentage of people living with income below the poverty line.

### 2.4.2 Food group consumption in BRAVE participants

**Table 2.5** summarises the food group consumption among consumers in cases and controls. In the BRAVE controls, the highest intake was reported for: boiled white rice (median intake 1161 g/day), followed by other vegetables (404.8 g/day), other fruits (339.8 g/day), sweet water fish (208.3 g/day), vitamin C rich fruits (119.7 g/day), green

leafy vegetables (98.6 g/day) and pulses (77.7 g/day). Food groups such as biryani, deep fried snacks, savoury snacks, sweets, red meat, sea water fish, eggs and yoghurt had median intakes of less than 35 g/day. Cases followed similar trends of highest consumption of boiled white rice, followed by other vegetables, other fruits and sweet water fish.

In crude comparisons between proportion of consumers, controls were more likely to consume potatoes, vitamin C rich fruits, deep fried snacks, savoury snacks, sweets, eggs, milk and yoghurt (p value <0.001 for all). Whereas the percentage of consumers of other vegetables, other fruits, pulses, boiled white rice, bread, SSB and sweet water fish were similar in cases and controls (p>0.05 for all).

### 2.4.3 Conventional and local risk factors and risk of acute myocardial infraction

*Conventional risk factors and risk of AMI*:

Adjusted ORs and corresponding were 1.84 (95% CI 1.66-2.04) for history of diabetes, 2.20 (95% CI 2.01-2.41) for history of hypertension, and 2.14 (95% CI 1.91-2.40) for family history of AMI **(Figure 2.3)**. The adjusted ORs were 1.07 (95% CI 1.04-1.11), 1.40 (95% CI 1.35-1.46), 1.71 (95% CI 1.64-1.78) and 0.82 (95% CI 0.79-0.85) per 1-SD higher baseline levels of waist-to-hip ratio, total cholesterol, LDL-C and HDL-C respectively **(Figure 2.3)**. There were approximately log-linear associations for LDL-C and HDL-C with AMI **(Figure 2.4)**. There was a curvilinear association between numbers of cigarettes smoked per day and risk of AMI **(Figure 2.4)**. Sequential adjustment did not substantially change the estimates except for waist-to-hip ratio that became non-significant after adjustment of other confounders **(Table 2.7)**.

Additionally, total cholesterol, HDL-cholesterol, waist-hip ratio, history of diabetes and history of hypertension were more strongly associated with risk of AMI among individuals less than 50 years of age (P<0.05; **Figure 2.8**). There was no apparent difference in risk estimates when stratified by sex, expect for history of diabetes, which had a stronger association in females and current smoking which has higher association only for males (**Figure 2.9)**. Major lipids were the only risk factors that differed by place of residence, with stronger associations among participants from an urban setting **(Figure 2.10)**.

*Local behavioural and socio-demographic factors and risk of AMI*

(1) Indigenous tobacco products: Compared with never-consumers of tobacco, ORs were 4.11 (95% CI 3.18-5.31) with *biri* only, and 2.07 (95% CI 1.50-2.83) with chewable tobacco (*gul, jarda, tamak or khoyer)* **(Figure 2.5)**. The corresponding ORs were 1.07 (95% CI 0.86-1.34) in individuals who used betel leaf and betel nut (*paan/supari*) without chewing tobacco, 1.30 (95% CI 1.15-1.47) for using *paan/supari* with chewing tobacco, 5.43 (95% CI 3.50-8.43) for

using both cigarettes and *biri,* and 3.58 (95% CI 3.16-4.10) in people who both chewed and smoked tobacco, respectively **(Figure 2.5)**.

(2) Local physical activity patterns: Physical activity yielded an inverse association with risk of AMI in this population that reduced with increasing levels of overall activity **(Figure 2. 6)**.

(3) Local socio-demographic factors: Lifelong urban residence compared to lifelong rural residence had increased association with risk of AMI (OR 1.52 (95% CI 1.34-1.72); **(Figure 2.7)**. Individuals who lived below poverty line, were at higher risk of AMI, OR 1.19 (95% CI 1.08-1.31) **(Figure 2.7)**. In the analyses comparing various professions, compared with office/business-based professionals, ORs were 1.30 (95% CI 1.14-1.49) for indoor-based industry-based workers, 0.65 95% CI (0.60-0.70) for agriculture and construction workers, and 1.02 (95% CI 0.95-1.10) for unemployed, retired or students **(Figure 2.7)**. Among other socio-economic and behavioural factors, parental consanguinity (OR 1.22 (95% CI 1.02-1.45)), high level of stress at home and work (ORs: 1.08 (95% CI 1.03-1.14) and 1.25 (95% CI 1.18-1.33), respectively) and day-time nap (OR 1.18 (95% CI 1.10-1.28) were each significantly associated with higher risk of AMI. Sequential adjustment did not substantially change the estimates **(Table 2.8)**.

## 2.5 DISCUSSION

In Bangladesh the burden of CHD, with MI as its main clinical manifestation is increasing at an alarming rate,[25] however the evidence on its potential determinants is sparse in this population. In addition to the high CHD, it is postulated that South Asians experience heart disease at a younger age and of higher severity as compared to Western populations.[26,27] Resultantly, high CHD mortality in young age in Bangladesh leads to loss of productive years of life. The present analysis had 7066 cases and 8079 controls with about half residing in rural areas and other half in urban areas. Overall, cases had significantly higher waist circumference, history of diabetes, history of hypertension, family history of MI, levels of LDL-C, were more likely to be currently smoking and less physically active and higher percentage of people with income below the poverty line than the controls. The study reported that participants had highest consumption of boiled white rice followed by other vegetables, other fruits and sweet water fish. However, the controls reported higher consumption of potatoes, vitamin C rich fruits, deep fried snacks, savoury snacks, sweets, eggs, milk and yoghurt than the cases.

Prior to BRAVE, the Bangladesh component of the INTERHEART case-control study (228 cases, 238 controls from Bangladesh) reported that as compared to other South Asian countries Bangladesh had highest prevalence of most risk factors (smoking, hypertension, obesity, depression and elevated ApoB100 /Apo-I ratio) among controls.[28] On the other hand, Bangladesh had lowest prevalence for regular physical activity and daily intake of fruits and vegetables. There are two other large studies that have data from Bangladesh (1) HEALS[11] cohort study of 11,746 participants was established to evaluate the effects of arsenic exposure on various health outcomes in Araihazar, Bangladesh and (2) the recent PURE[29] prospective study of 18 countries which has about 2700 participants from Bangladesh. However, the BRAVE study is the most powerful and detailed data on AMI involving a South Asian population which has quantified associations of various conventional and locally-relevant cardiovascular risk factors of MI. As summarised below, BRAVE has yielded findings that highlight, for CHD prevention, a need to consider both conventional cardiovascular risk factors and certain other modifiable exposures that might be distinctive to South Asians.

The results reinforce the relevance of several conventional cardiovascular risk factors to AMI in Bangladesh, showing strong risk of AMI with each of the following: amount of tobacco smoked, concentration of pro-atherogenic lipids, history of disease and waist-to-hip ratio. Second, the current study showed that use of smokeless tobacco products (such as tobacco chewing and dipping) is associated with higher risk of AMI in Bangladesh. Furthermore, the analyses found evidence that the effects of some conventional risk factors on AMI are modified by age, such as the proportional impact of HDL-cholesterol and diabetes mellitus on AMI being

greater in the cases who were less than 50 years of age. These findings suggest that younger Bangladeshis may be particularly vulnerable to the cardiovascular consequences of lipids and metabolic dysfunction. In addition, there may be possibility of residual confounding as younger individuals have healthier lifestyle behaviours than the older group. A stronger association was also seen for lipids in urban setting as compared to rural setting. This may be because of the additional risk at urban setting of air pollution or water pollution which may not be as prevalent in the rural areas. Additionally, when history of diabetes was stratified by sex, a stronger association was observed in females as compared to males. Future research is required to elucidate the exact reasons, but this may be due to chance due to low number of females in the study.

The magnitude of associations of tobacco with AMI was broadly comparable to those previously reported with tobacco chewing and dipping in non-Western populations. Furthermore, when stratified by sex, males who were currently smoking showed increased association with AMI as compared to no association for females. This may be due to the higher percentage of male smokers than the females and therefore an earlier presentation of AMI in males. However, as the females in the study are low these findings can also be due to chance. These findings reinforce earlier suggestions[30] that cardio-toxic elements in tobacco are intrinsic to tobacco itself, and not just confined to the smoked form. The findings may encourage tobacco control strategies that address consumption of smoked tobacco as well as smokeless tobacco products.[31,32] The study also observed that physical activity was inversely associated with the risk of AMI, the association however reduced with increasing levels of overall activity.

Furthermore, the data reported that those who had income below the poverty line were at a higher risk of AMI. It is postulated that people with low income are more exposed to risk factors of CHD.[33] In addition, participants who worked in indoor industries had higher risk of AMI. It may be postulated that exposure to unhealthy indoor environment may be possible for the observed risk. A significant higher association of parental consanguinity with AMI was observed in Bangladesh - a finding among South Asians linking this preventable social practice with risk of CHD. Such increased risk of recessive disease in people with high rates of consanguineous marriages arises from the higher likelihood of carrying deleterious alleles in the homozygous state, due to inheritance of identical segments of chromosomal DNA from both parents.[34,35] An increased association was observed between daytime napping and risk of AMI – an observation previously reported in European[36] and Asian populations.[37] Although causality cannot be assumed, potential biological explanations include sympathetic nervous activation owing to an abrupt increase in blood pressure and heart rate upon awakening from a nap[38] and the prothrombotic effects following a daytime nap which may trigger a coronary

event.[39] The generally increased associations with the level of stress observed in this population are in line with the prior reports based in Western populations.[40]

### 2.5.1 Strengths and limitations

The strengths and potential limitations of this study merit consideration. BRAVE studied 5 times as many events as the previous largest study of AMI involving South Asians.[28] AMI cases were clinically-validated using a contemporary criteria. A wide range of conventional and other risk factors were recorded, extending from biochemical to behavioural factors. Where appropriate locally developed and pre-piloted instruments for data collection were used. As this study involved largest referral hospital of Bangladesh with approximately similar number of participants from both rural and urban settings, the results should be broadly generalizable in Bangladesh.

*Selection and information bias:*

Nevertheless, retrospective case-control studies may be liable to potential biases, such as selection and information bias (e.g. recall bias). Selection bias can occur if there is systematic error in ascertainment of cases and controls. The BRAVE study hospital is the largest tertiary hospital in Bangladesh where the majority of the AMI cases from all the cities are referred to. As not all cities have CVD care units, the hospital has a good representation of the majority of AMI cases in the country. In addition, in case-control studies selection bias can also occur if the selection of controls enrolled in the study is different from the general population. However, following the example of previous case-control studies of AMI,[41,42] various measures in this study help to reduce such potential biases. For example, to reduce the scope for selection bias, controls were sampled from approximately the same source population as the cases. Information bias may occur if there are recording errors in self-administered questionnaires, misreporting due to memory and misinterpretation of information. There is a possibility of recall bias as cases may recall differentially than controls. For example, the cases may be more likely to overestimate risk factors of CHD like smoking and physical inactivity inflating the ORs and controls may be likely to underestimate. To reduce the scope for recall bias, an incident case-control study of AMI was conducted and sought information from cases within hours of the index event. In addition, information from the questionnaires from cases was confirmed by household members by the interviewers to minimize errors.

*Limitations of dietary assessment methods:*

The dietary instrument used in this study had inherent limitation of bias that can lead to under or over-reporting. Non-differential misclassification may occur when the degree of

misclassification of exposure status among those with and those without the disease is the same; e.g. random error which will attenuate the associations observed. Differential misclassification may cause bias in either direction and is particularly likely, when exposure reporting is associated with a characteristic such as higher BMI may be associated with underreporting of food items. In addition, the calculation of energy intake depends on the number of items in the FFQ and accuracy of the food composition tables. Food composition tables often do not cover all food items and do not capture the variability in food composition.

*Residual confounding:*
As with all observational studies the bias due to residual confounding cannot be ruled out. Residual confounding can be caused by unmeasured confounding factors, additional confounding factors that were not adjusted for (e.g. use of nutritional supplements) and not precisely measured confounding factors (e.g. socioeconomic factors). Although the analyses were well adjusted, self-reported measures, such as medical history, and a lack of data on relevant variables such as disparities to access CVD health care, food contamination, other environmental factors such as water pollution (water used in cooking of foods) and nutritional deficiencies (such as iron and vitamin D), may have had an impact.

### 2.5.2 Implications

CHD is a major public health concern. Previously thought to be the disease of developed countries, now there is growing evidence that the majority of the CVD burden occurs in developing countries. As South Asia holds quarter of the developing world's population, it is important to study the determinants of CVD in this population. BRAVE is a pioneering large study that investigates association of conventional and local risk factors for the risk of CHD in an understudied population of Bangladesh.

Indigenous tobacco products showed an increase risk with CHD. Findings, therefore encourage tobacco control strategies focusing on all types of tobacco products and not just cigarettes alone. Furthermore, intermarriage and day time nap was associated with increased risk, which emphasises the importance of evaluating and integrating novel risk factors, into guidelines of CHD risk assessment in order to improve identification of people at risk of CHD. However, as the case-control studies are unable to establish causality this study has implications for future research. To prove causality a randomized controlled trial (RCT) has to be done, however, that will be ethically challenging for some risk factors (e.g. smoking status). A prospective cohort study with a long follow up period will provide invaluable information between the association of conventional and local risk factors and CHD in Bangladesh. In addition, genetic

information from genome wide association study (GWAS) could be done to investigate the genetic determinants of risk factors of CHD in the BRAVE study.

### 2.5.3 Conclusion

In conclusion, BRAVE is the largest and most powerful study of CHD from South Asia. The findings of the study indicate that while conventional CHD risk factors remain relevant among Bangladeshis with respect to risk of AMI, several local socio-demographic factors (such as parental consanguinity, use of non-smoking tobacco products, and daytime nap) are also associated significantly with AMI. Future efforts to reduce the burden of AMI in Bangladesh should, therefore, consider both conventional and local determinants distinctive to this region.

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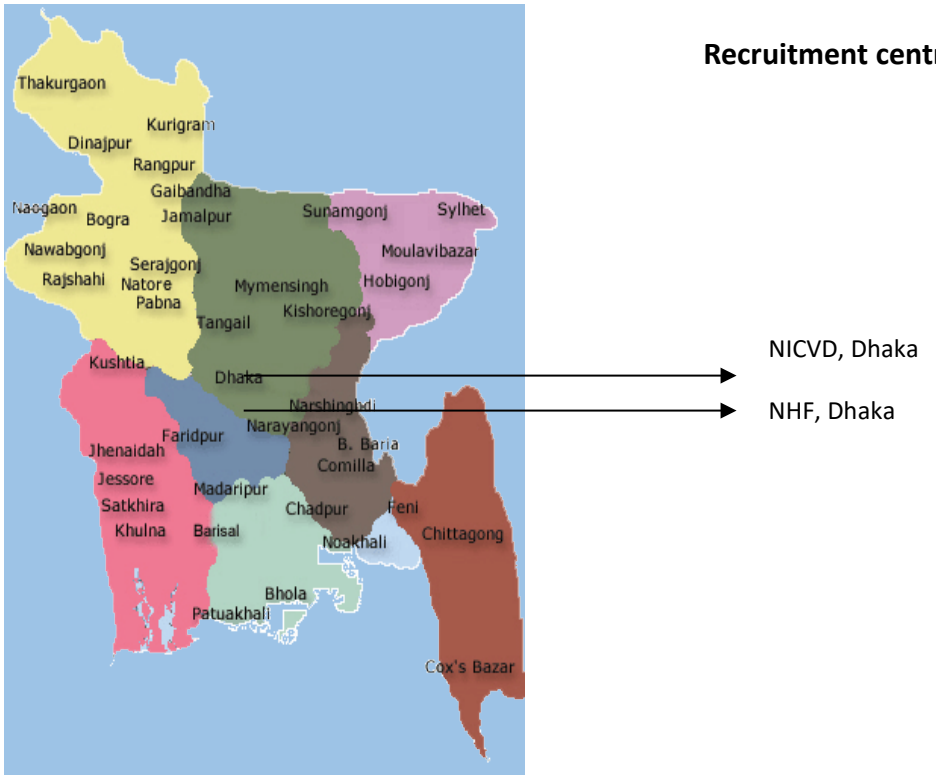
**Figure 2.1:** Recruitment **centres** for the BRAVE case-control study




NICVD- National Institute of Cardiovascular Diseases

NHF- National Heart Foundation

**Figure 2.2:** BRAVE study flow diagram

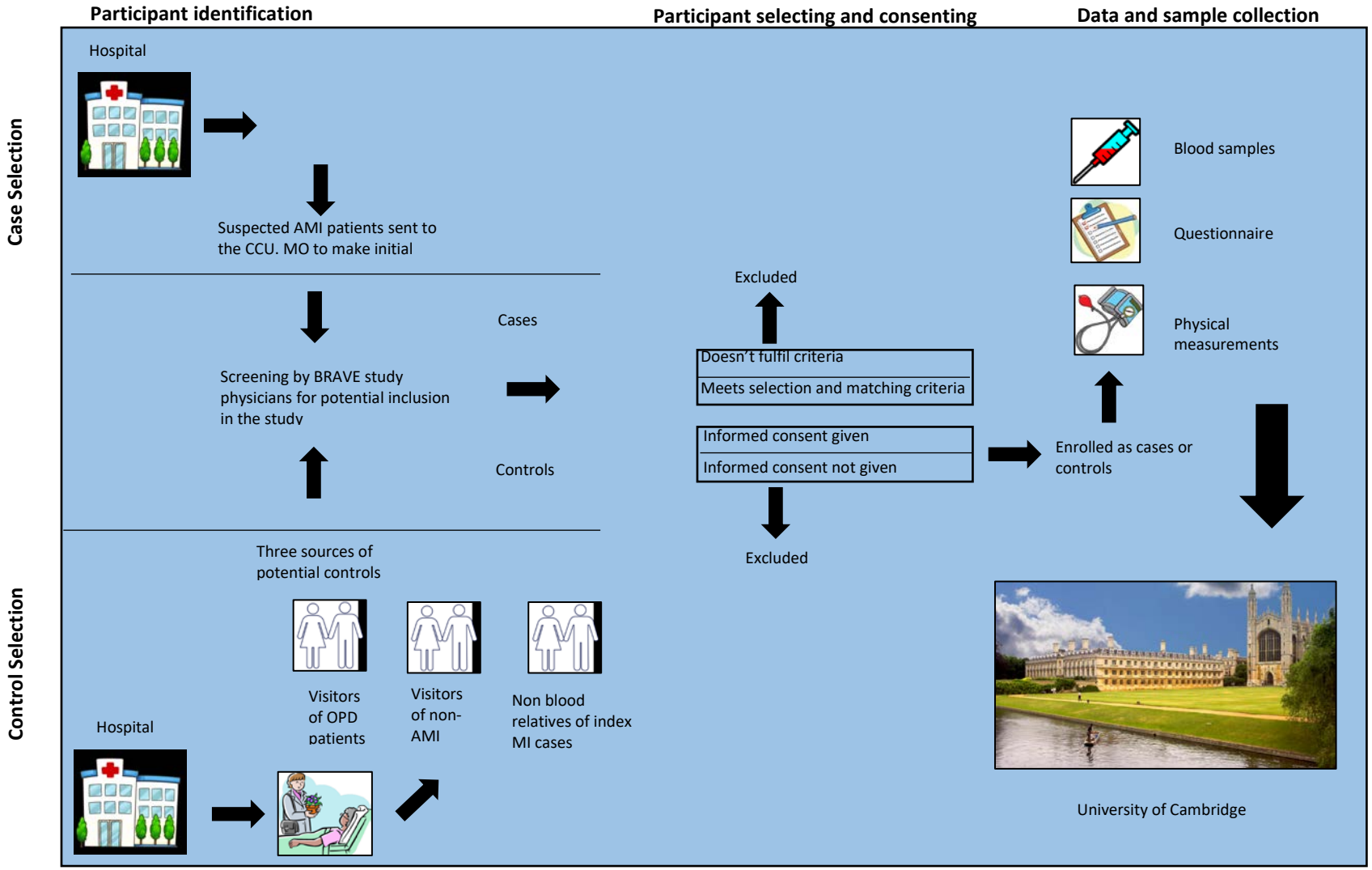
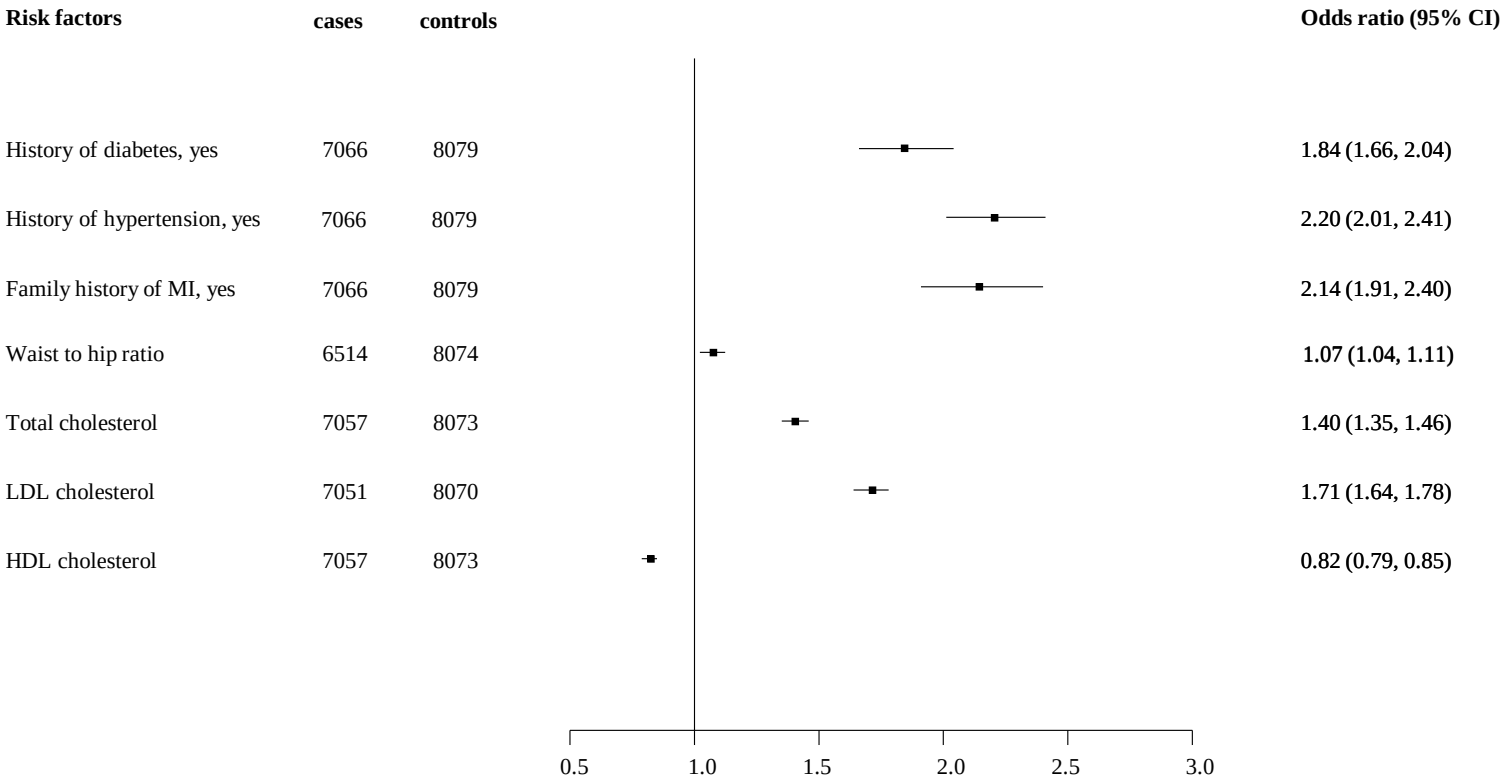
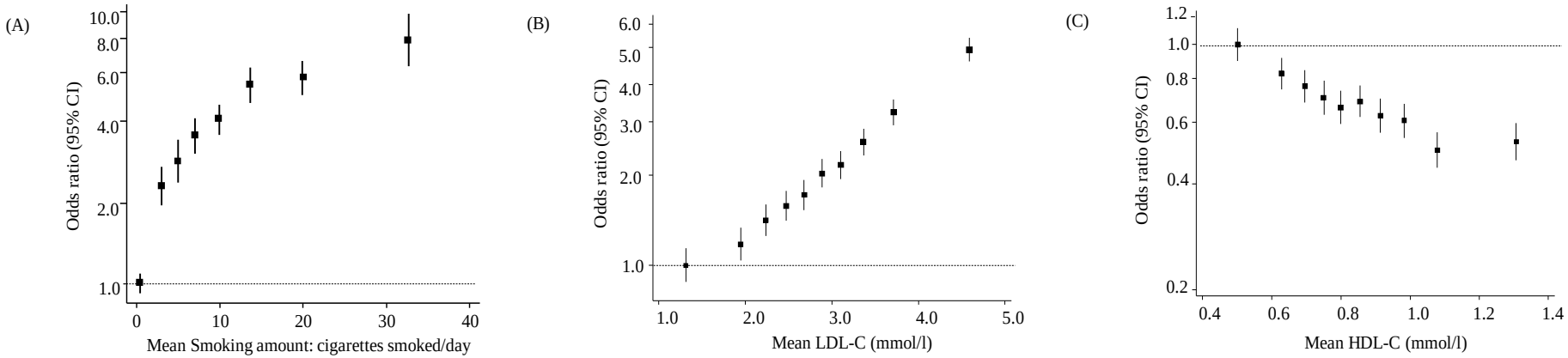


**Figure 2.3**: Associations of traditional risk factors with risk of AMI in Bangladesh




Adjusted for: age, sex, tobacco use and (where appropriate) for LDL and HDL cholesterol, history of diabetes, history of hypertension, and family history of MI.
Waist to hip ratio was adjusted for age, sex and tobacco use only.

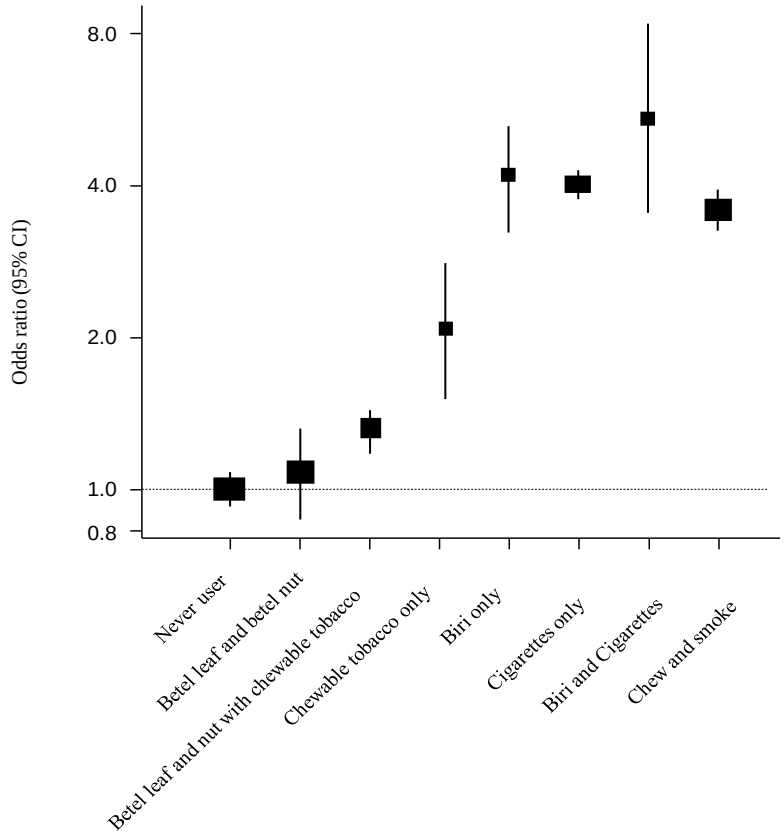
**Figure 2.4**: Shape of associations for various continuous factors and AMI risk




(Panel A): Adjusted for age, sex, LDL and HDL cholesterol, history of diabetes, history of hypertension, family history of MI, physical activity and total energy intake.
Cases =4569 and controls =5141

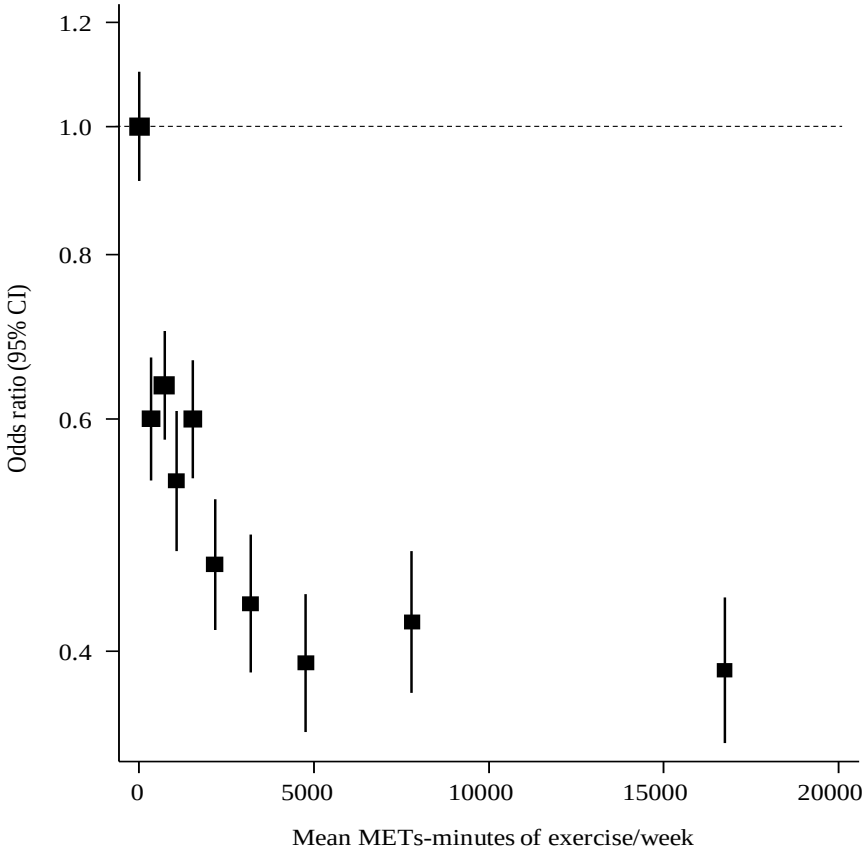
(Panel B and C): Adjusted for: age, sex, tobacco use and (where appropriate) for LDL and HDL cholesterol, history of diabetes, history of hypertension, and family history of MI.
Cases 7051, controls 8070

**Figure 2.5:** Patterns of tobacco use and related products and risk of AMI in Bangladesh




Adjusted for age, sex, LDL and HDL cholesterol, history of diabetes, history of hypertension, family history of MI, physical activity and total energy intake
Former smoker OR: 1.58 [1.35-1.84]
Betel and betel nut = paan and supari
Chewable tobacco = noshhi, gul, jarda, tamak and khoyer
Cases =6980 and controls =8011

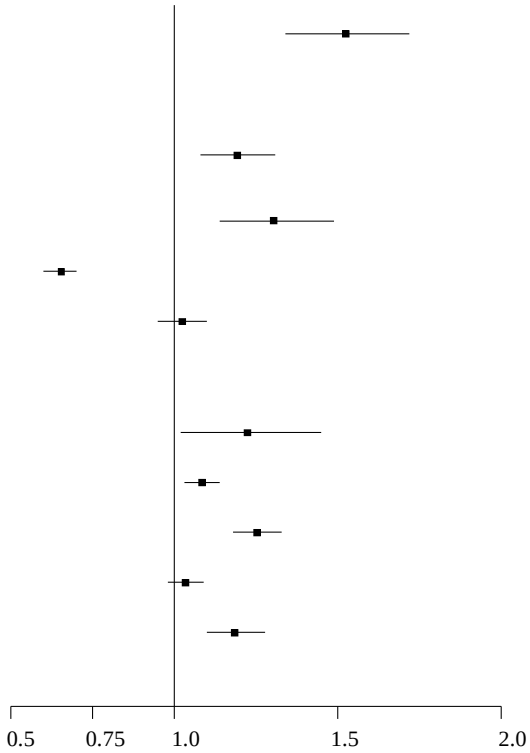
**Figure 2.6:** Physical activity and risk of AMI in Bangladesh.




Adjusted for age, sex, LDL, HDL, smoking status, history of diabetes, history of hypertension, family history of MI, smoking status and total energy intake
MET >600: OR 0.62 (0.57-0.67)
Cases =6980, controls = 8011

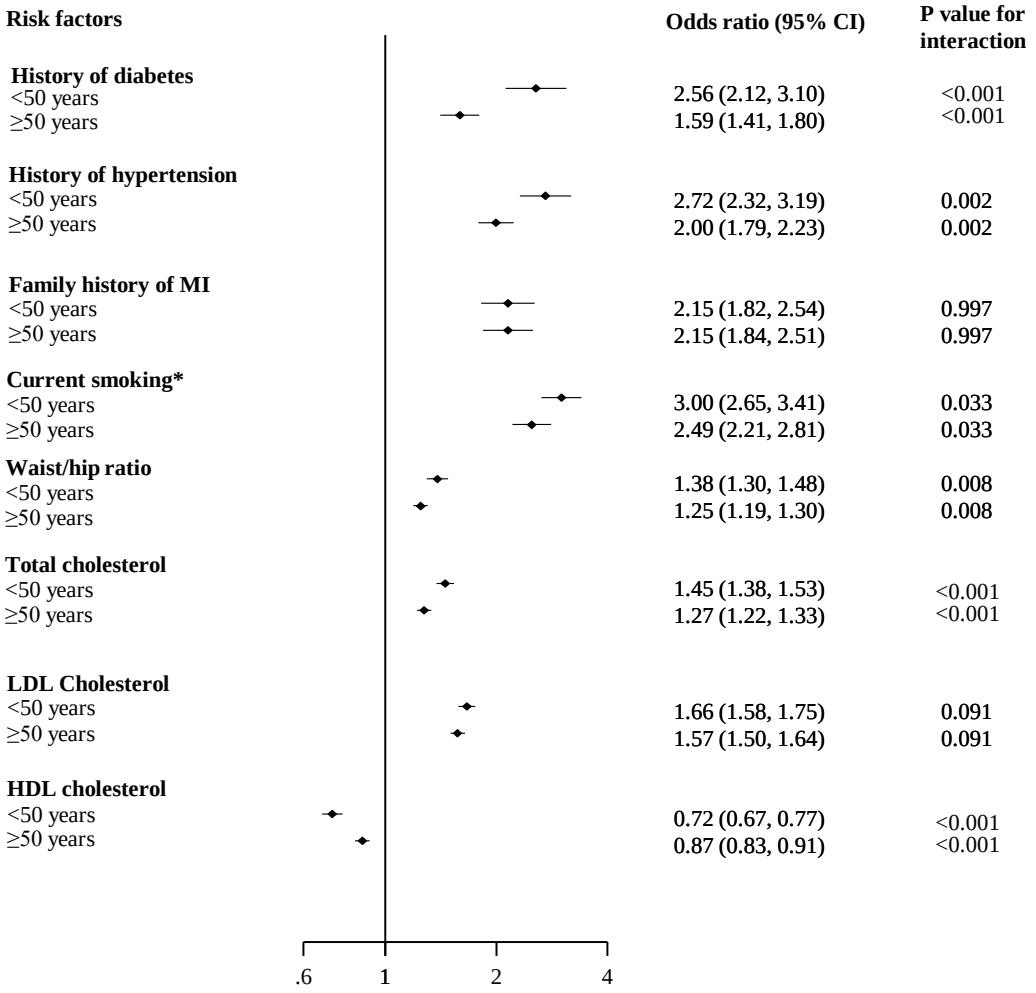
**Figure 2.7:** Socio-demographic factors and risk of AMI in Bangladesh.

| | Risk factors* | cases | controls | Odds ratio (95% CI) |
|---|---|---|---|---|
| **Residence** | | | | |
| | Life long urban residence[1] | 3950 | 4467 | 1.52 (1.34, 1.72) |
| **Economic** | | | | |
| *Income* | Annual income below poverty line[2] | 6898 | 7970 | 1.19 (1.08, 1.31) |
| *Occupation* | Labour, indoor industry based[3] | 7025 | 8070 | 1.30 (1.14, 1.49) |
| | Labour, agri/construction based | 7025 | 8070 | 0.65 (0.60, 0.70) |
| | Unemployed/retired/student | 7025 | 8070 | 1.02 (0.95, 1.10) |
| **Psycho-social** | | | | |
| | Intermarriage[4] | 6977 | 8056 | 1.22 (1.02, 1.45) |
| | Stress at home (high/severe)[5] | 6997 | 8002 | 1.08 (1.03, 1.14) |
| | Stress at work (high/severe) | 6997 | 8001 | 1.25 (1.18, 1.33) |
| | Financial stress (high/severe) | 6996 | 8000 | 1.03 (0.98, 1.09) |
| | Day time nap[6] | 6817 | 7759 | 1.18 (1.10, 1.28) |



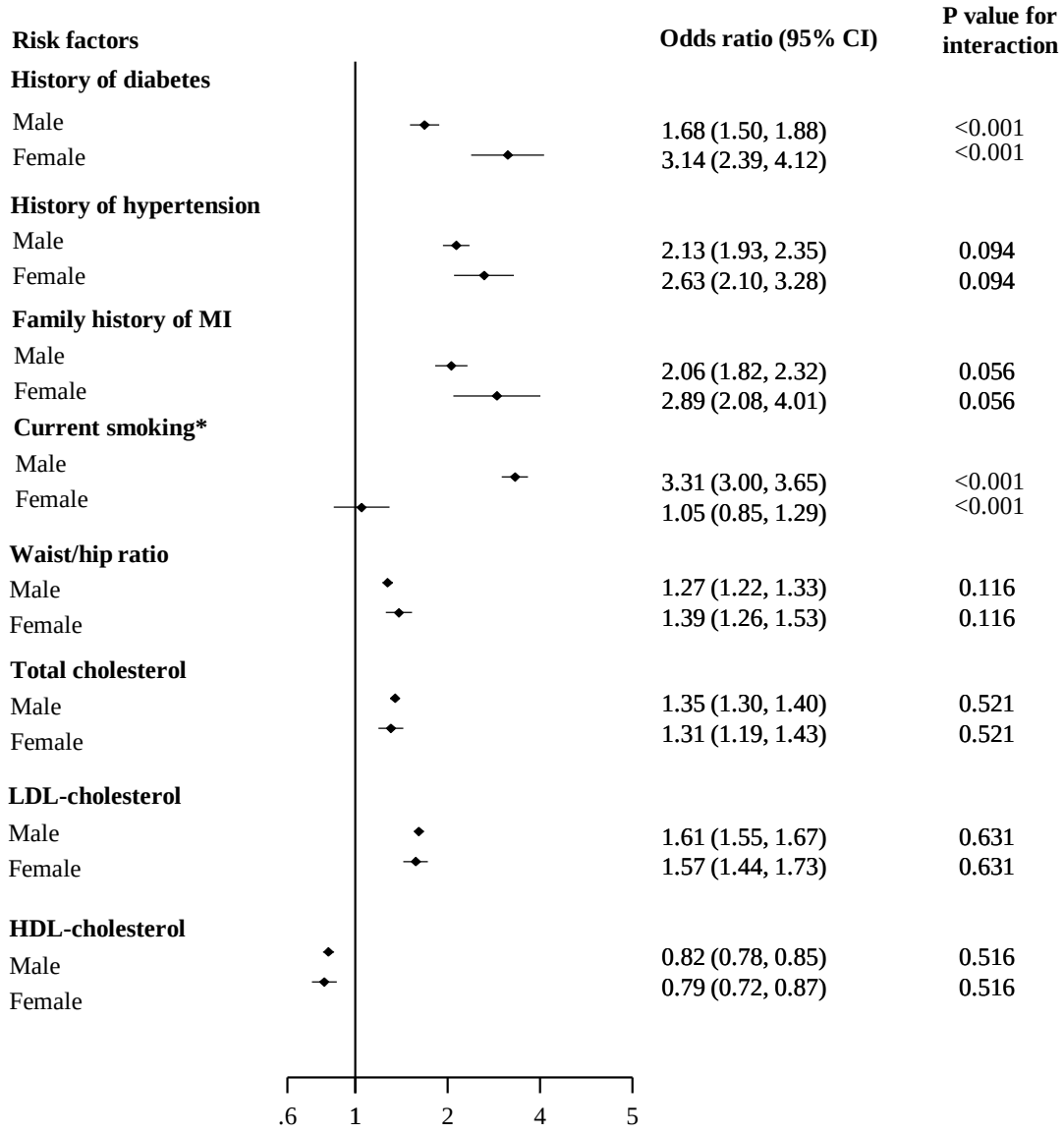

*All adjusted for age, sex, history of diabetes, history of hypertension and tobacco use
**1.** Reference lifelong rural residence. **2**. Reference annual income above poverty line. **3.** Reference business/professional. **4.** Reference no intermarriage. **5.** Reference no stress. **6.** Reference not taking day time nap.

**Figure 2.8:** Associations of traditional risk factors with risk of AMI in participants grouped below and beyond the age of 50



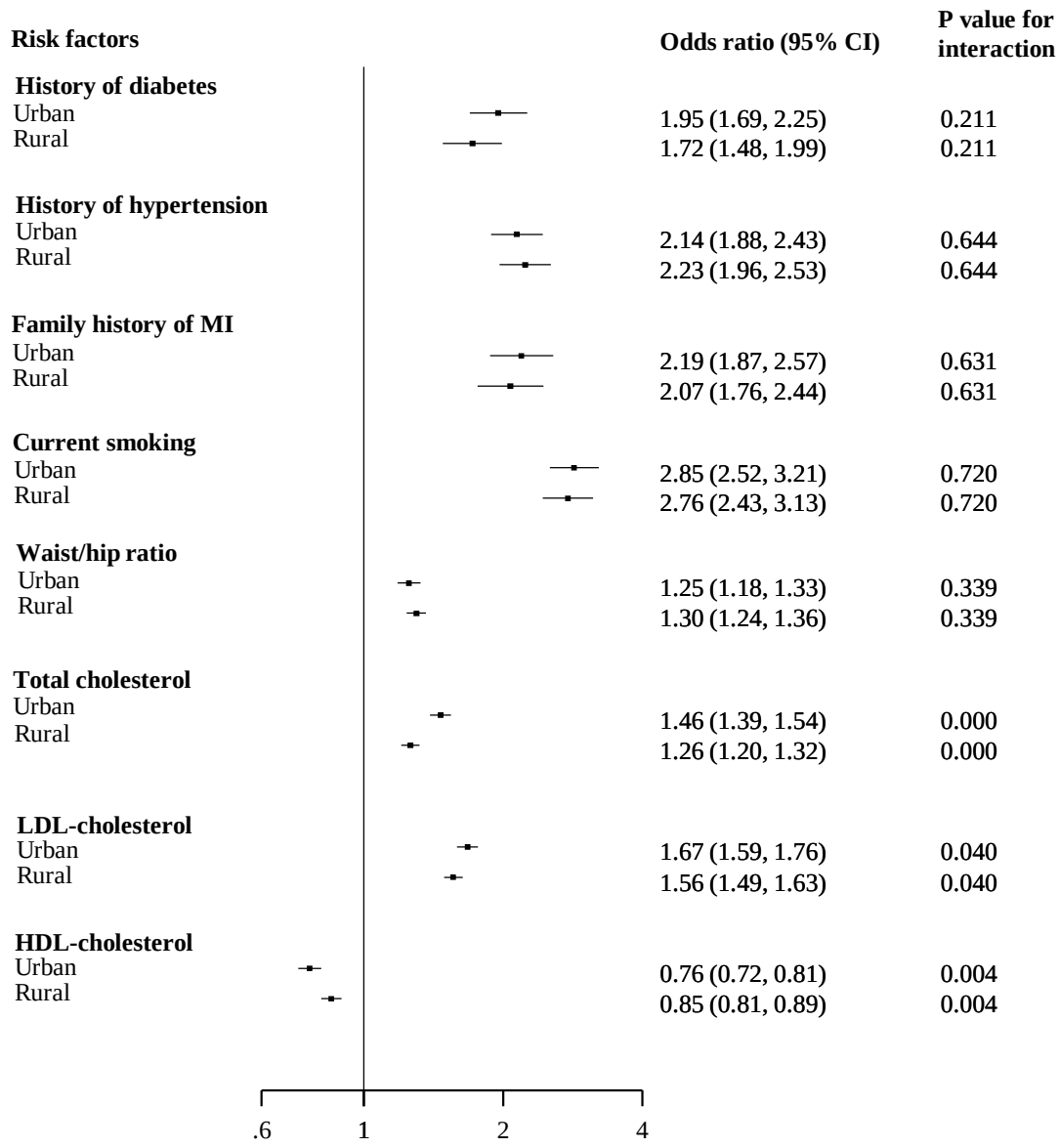

Adjusted for: age, sex, tobacco use and (where appropriate) for LDL and HDL cholesterol, history of diabetes, history of hypertension, and family history of MI.
Waist/hip ratio are adjusted for age, sex and smoking only.
*Tobacco use includes both smoked/inhaled and chewed tobacco; reference category is never users

**Figure 2.9:** Associations of traditional risk factors with risk of acute MI, stratified by sex




Adjusted for: age, sex, tobacco use and (where appropriate) for LDL and HDL cholesterol, history of diabetes, history of hypertension, and family history of MI.
Waist/hip ratio are adjusted for age, sex and smoking only.
*Tobacco use includes both smoked/inhaled and chewed tobacco; reference category is never users

**Figure 2.10:** Associations of traditional risk factors with risk of acute MI, stratified by place of residence (urban or rural).




Adjusted for: age, sex, tobacco use and (where appropriate) for LDL and HDL cholesterol, history of diabetes, history of hypertension, and family history of MI.
Waist/hip ratio are adjusted for age, sex and smoking only.
*Tobacco use includes both smoked/inhaled and chewed tobacco; reference category is never users

**Table 2.1:** List of covariates used in the analyses in subsequent chapters

| **Covariate** | **Type** | **Categories** |
|---|---|---|
| Age | Continuous | |
| Sex | Binary | Male<br>Female |
| BMI | Continuous | |
| Waist-to-hip ratio | Continuous | |
| History of diabetes | Binary | Yes<br>No |
| History of hypertension | Binary | Yes<br>No |
| Family history of MI | Binary | Yes<br>No |
| LDL-C | Continuous | |
| HDL-C | Continuous | |
| Education level | Ordinal | No schooling<br>Primary<br>Secondary<br>Vocational/university |
| Annual income (Taka) | Ordinal | Income below the poverty line ≤56000<br>Low income (>56000 – 99999)<br>Medium income (>99999 - 199999)<br>High income (>199999-999999) |
| Occupation | Nominal | Business professional<br>Non-manual labour<br>Manual labour<br>Unemployed/retired/students |
| Smoking status | Nominal | Never user<br>Ex-user<br>Current user |
| Physical activity | Nominal | < 600<br>≥ 600 |
| Location | Nominal | Urban<br>Rural |

**Table 2.2:** Characteristics of BRAVE cases and controls

| Characteristics | Cases | | Controls | | P value |
|---|---|---|---|---|---|
| | *N* | *Mean (SD) or %* | *N* | *Mean (SD) or %* | |
| **Demographic factors** | | | | | |
| *Age (years)* | 7066 | 52.39 (10.4) | 8079 | 51.23 (10.3) | Matched |
| *Sex (%)* | | | | | |
| Males | 6275 | 88.81 | 7082 | 87.66 | |
| Females | 791 | 11.19 | 997 | 12.34 | Matched |
| *Location (%)* | | | | | |
| Urban | 3301 | 47.23 | 3619 | 45.05 | |
| Rural | 3688 | 52.77 | 4414 | 54.95 | 0.008 |
| **Conventional risk factors** | | | | | |
| *Waist-to-hip ratio* | 6514 | 0.97 (0.07) | 8074 | 0.96 (0.07) | <0.001 |
| *Body mass index* | 6506 | 22.95 (3.80) | 8075 | 23.30 (5.00) | <0.001 |
| *History of diabetes (%)* | | | | | |
| Yes | 1301 | 18.41 | 835 | 10.34 | |
| No | 5765 | 81.59 | 7244 | 89.66 | <0.001 |
| History of hypertension (%) | | | | | |
| Yes | 1857 | 26.28 | 1101 | 13.63 | |
| No | 5209 | 73.72 | 6978 | 86.37 | <0.001 |
| Family history of MI (%) | | | | | |
| Yes | 1105 | 15.64 | 580 | 7.18 | |
| No | 5961 | 84.36 | 7499 | 92.82 | <0.001 |
| **Blood lipid measurements** | | | | | |
| *HDL-C, mmol/L* | 7057 | 0.82 (0.22) | 8073 | 0.85 (0.23) | <0.001 |
| *LDL-C, mmol/L* | 7051 | 3.22 (1.03) | 8070 | 2.81 (0.89) | <0.001 |
| **Lifestyle factors** | | | | | |
| *Tobacco consumption (%)* | | | | | |
| Never | 1170 | 16.56 | 2597 | 32.15 | |
| Ex | 463 | 6.55 | 591 | 7.32 | |
| Current | 5433 | 76.89 | 4891 | 60.54 | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| *Total MET-minutes of exercise/week (%)* | | | | | |
| < 600 | 2387 | 33.78 | 1831 | 22.66 | |
| ≥ 600 | 4679 | 66.22 | 6248 | 77.34 | |
| *Education level (%)* | | | | | |
| No formal education | 2640 | 37.63 | 2802 | 34.72 | |
| Primary | 2139 | 30.49 | 2399 | 29.72 | |
| Secondary | 1447 | 20.63 | 1919 | 23.78 | |
| University/vocational | 789 | 11.25 | 951 | 11.78 | <0.001 |
| *Occupation (%)* | | | | | |
| Business professional | 3221 | 45.85 | 3553 | 44.03 | |
| Manual labour | 1013 | 14.42 | 1669 | 20.68 | |
| Non-manual labour | 495 | 7.05 | 434 | 5.38 | |
| Unemployed/retired/students | 2296 | 32.68 | 2414 | 29.91 | <0.001 |
| *Annual income (%)* | | | | | |
| Income below the poverty line ≤56000 | 1831 | 26.54 | 1911 | 23.98 | |
| Low income | 1227 | 17.79 | 1680 | 21.08 | |
| Medium income | 2117 | 30.69 | 2477 | 31.08 | |
| High income | 1723 | 24.98 | 1902 | 23.86 | <0.001 |

Differences in categorical variables was done by Chi squared test

Differences in means between cases and controls was done using t-test

**Table 2.3:** Conversion of FFQ frequencies to portion per day

| **Frequencies indicated in FFQ** | **Portion per day consumption (multiplier)** |
|---|---|
| Never | 0.0 |
| 1-3 per month | 0.066 |
| Once a week | 0.142 |
| 2-4 per week | 0.428 |
| 5-6 per week | 0.786 |
| Once a day | 1.0 |
| 2-3 per day | 2.5 |
| 4-5 per day | 4.5 |
| 6+ per day | 6.0 |

**Table 2.4:** Food groups used in the BRAVE study.

| | Food group | No. of FFQ items | Contents |
|---|---|---|---|
| Fruits and vegetables | Leafy and stem vegetables | 5 | Amaranth stem, drumstick leaves, colocasia leaves, spinach and cabbage |
| | Potatoes | 2 | Potatoes and sweet potatoes |
| | Other vegetables | 17 | Ash gourd, brinjal, okara, bitter gourd, bottle gourd, ridge gourd, snake gourd, unripe papaya, pointed gourd, water lilly, tomato, cucumber, green long beans, banana blossom, sponse gourd, carrots and runner beans |
| | Vitamin C rich fruits | 3 | Guava, orange and grapefruit |
| | Other fruits | 17 | Banana, custard apple, hog plum, burmese grapes, papaya, emblic (amla), monkey jack, wood apple, jambolan, pineapple, coconut, mango, jackfruit, sapota, apple, grapes and asian pears |
| Animal based foods | White meat | 2 | Chicken and duck meat |
| | Red meat | 2 | Beef and goat meat |
| | Sweet (fresh) water fish | 21 | Rohu, catla, clown knife, hilsa, pangas, catfish, walking catfish, climbing perch, tilapua, tengra, barb, mola carplet, striped snake-head, spotted snake-head, byne, nola, silve carp, mirgel carp, koral, air and rissha |
| | Sea water fish | 11 | Lobster, shrimps, pomfret, bombay duck, croaker, rita, churi, hangori, parsey, giant sea perch and goby |
| | Egg | 1 | Chicken, eggs |
| | Milk | 1 | Whole milk |
| | Yoghurt | 1 | Yoghurt |
| Individual foods | Pulses | 3 | Green gram (muger daal), lentil (musrur daal) and bengal gram (buter daal) from dishes cooked by using oil and water. |
| | Boiled white rice | 1 | Boiled white rice |
| | Biryani | 1 | Biryani/Polau/Tehari -Mixed rice dish with meat, cooked by using salt and oil |
| | Bread | 4 | Ruti, paratha, chapatti and bread loaf |
| | Deep fried local snacks | 6 | Puri, singarao aloo, singara koliza, samosa, chop roll and pakora |
| | Savoury snacks | 10 | Jhal pattie, muglai paratha, chanachur, haleem, chotpoti, jhal muri, desi pitha, chana boot, crisps and noodles |
| | Sweets | 3 | Cakes/pastries, laddu and sweet biscuits |
| | SSB | 2 | Carbonated soft drinks (coke, pepsi) and bottled juice drinks with sugar |
| Spices | | 6 | Coriander powder, cumin powder, garlic, ginger, turmeric powder and mixed spices (garam masala) |

**Footnotes table 2.4:** 99% of the study population did not consume alcohol which is expected in a Bangladeshi population due to religious reasons. In addition, 99% of the study population did not consume dairy products such as cheese, butter, mayonnaise and margarine. Other foods (mostly western foods) were also excluded from the analysis due to their extremely low consumption in the study population. The fish were classified into sweet water and fresh water fish based on interviews with the locals and using literature. Carp fishes and catfish are the major fishes in pond culture and are therefore classified as sweet/fresh water fish. Lobster, shrimps, pomfret, bombay duck, croaker, rita, churi, hangori, parsey, giant sea perch and goby are the major sea water fish. However, it is important to note that 76 species of fish in Bangladesh move between sea water and fresh water.

**Table 2.5:** Descriptive characteristics of food and drink intake by case-control status

| | **Cases** | | | **ControlsControls** | | | P-values[1] |
|---|---|---|---|---|---|---|---|
| **Food group (g/day)** | Number | Among consumers Median (IQR), g/day | | Number | Among consumers Median (IQR), g/day | | |
| | | | | | | | |
| Green leafy and stem vegetables | 6979 | 85.2 | (52.7, 158.4) | 7998 | 98.6 | (57.9, 174.6) | S |
| Potatoes | 6988 | 50.0 | (39.3, 77.9) | 7998 | 50.0 | (39.3, 125) | S |
| Other vegetables | 6838 | 349.2 | (236.2, 559.2) | 7885 | 404.8 | (263.3, 627.2) | S |
| Vitamin C rich fruits | 6217 | 55.6 | (26.3, 136.9) | 7338 | 119.7 | (35.6, 144.8) | S |
| Other fruits | 6967 | 268 | (157.6, 488.7) | 7980 | 339.8 | (198.7, 672.5) | S |
| Pulses | 6861 | 77 | (53.4, 179) | 7848 | 77.7 | (53.4, 179) | NS |
| Boiled white rice | 6989 | 1161 | (645, 1161) | 8016 | 1161 | (645, 1161) | S |
| Biryani | 4974 | 27.7 | (27.7, 27.7) | 4470 | 27.7 | (27.7, 27.7) | NS |
| Bread | 6339 | 97.0 | (26.31, 132.6) | 7261 | 65.5 | (22.4, 129.5) | NS |
| Deep fried snacks | 4754 | 21.6 | (7.4, 59.0) | 5799 | 21.6 | (9.1, 59.0) | NS |
| Savoury snacks | 5191 | 17.7 | (6.4, 46.4) | 6407 | 21.0 | (9.0, 50.0) | S |
| Sweets | 6605 | 12.7 | (6.4, 28.2) | 7652 | 14.7 | (8.0, 31.6) | S |
| Sugar sweetened beverages | 3166 | 33 | (16.5, 107.3) | 3726 | 33 | (16.5, 107.3) | NS |
| White meat | 6323 | 40.3 | (13.4, 48.0) | 7316 | 40.3 | (13.4, 48.0) | S |
| Red meat | 4494 | 6.0 | (6.0, 13.0) | 5312 | 6.0 | (6.0, 13.0) | NS |
| Sweet water fish | 6958 | 210.4 | (129.4, 327.8) | 7957 | 208.3 | (125.8, 328.2) | NS |
| Sea water fish | 5650 | 8.6 | (1.3, 25.4) | 6345 | 7.5 | (1.3, 21.4) | S |
| Egg | 6186 | 25.3 | (8.4, 25.3) | 7247 | 25.3 | (8.4, 25.3) | NS |
| Milk | 4554 | 107.3 | (16.5, 107.3) | 5380 | 107.3 | (11916.5, 107.3) | NS |
| Yoghurt | 1579 | 16.5 | (16.5, 16.5) | 2320 | 16.5 | (16.5, 16.5) | NS |

1-Differences in median intake between consumers in cases and controls using Mann–Whitney U test
P value <0.001 are considered to be significant after applying Bonferroni correction.
S- Significant NS- non-significant

**Table 2.6 (1):** Missing data by cases and controls

| Variables | Cases | Controls |
|---|---|---|
| | 7066* | 8079* |
| Education level | 51 | 8 |
| Occupation | 41 | 9 |
| Wait to hip ratio | 552 | 5 |
| LDL | 15 | 9 |
| HDL | 9 | 6 |
| Income | 168 | 106 |
| Total energy | 71 | 59 |
| Rice | 71 | 59 |
| Bread | 72 | 64 |
| Red meat | 72 | 70 |
| White meat | 73 | 42 |
| Dairy | 76 | 95 |
| Pulses | 76 | 81 |
| Vegetables | 74 | 75 |
| Fruits | 75 | 84 |
| Fish | 74 | 89 |
| Fried snacks | 76 | 94 |
| Savoury snacks | 76 | 95 |
| Sweets | 76 | 95 |
| SSB | 74 | 74 |
| Spices | 74 | 84 |

* The present analysis was confined to 7066 cases and 8079 controls that had relevant information on age, sex, smoking, history of diabetes and history of hypertension.

**Table 2.6 (2):** Patterns of missing data

| Percent | Pattern 1 2 3 4 | 5 6 7 8 | 9 10 11 12 | 13 14 15 16 |
|---|---|---|---|---|
| 94% | 1 1 1 1 | 1 1 1 1 | 1 1 1 1 | 1 1 1 1 |
| | 1 1 1 1 | | | |
| 3 | 1 1 1 1 | 1 1 1 1 | 1 1 1 1 | 1 1 1 1 |
| | 1 1 1 0 | | | |
| 1 | 1 1 1 1 | 1 1 1 1 | 1 1 1 1 | 1 1 1 1 |
| | 1 1 0 1 | | | |

Variables are

Row 1: (1) HDL-C (2) LDL-C (3) Occupation (4) Education level (5) Rice (6) Total energy (7) Bread (8) Red meat (9) White meat (10) SSB (11) Vegetables (12) Pulses (13) Fruits (14) Fish (15) Fired snacks (16) Dairy products

Row 2: (1) Savoury snacks (2) Sweets (3) Annual Income (4) Waist to hip ratio

*94% of the variables used in the multivariate analyses had complete data

*Please note this is an excerpt of the table

**Table 2.7:** Traditional risk factors and risk of acute myocardial infraction

| Risk factors | Model 1 | Model 2 | Model 3 | Model 4 |
|---|---|---|---|---|
| History of diabetes | 1.93 (1.75-2.12) | 2.17 (1.97-2.39) | 1.79 (1.62-1.98) | 1.84 (1.66-2.04) |
| History of hypertension | 2.26 (2.08-2.46) | 2.52 (2.31-2.74) | 2.26 (2.07-2.47) | 2.20 (2.01-2.41) |
| Family history of MI | 2.46 (2.21-2.74) | 2.50 (2.24-2.78) | 2.40 (2.15-2.68) | 2.14 (1.91-2.40) |
| Waist-to-hip ratio | 1.00 (0.97-1.04) | 1.07 (1.04-1.11) | 0.97 (0.94-1.00) | 0.88 (0.85-0.92) |
| Total Cholesterol | 1.35 (1.31-1.39) | 1.35 (1.31-1.39) | 1.31 (1.28-1.36) | 1.40 (1.35-1.46) |
| LDL-C | 1.52 (1.47-1.57) | 1.52 (1.47-1.57) | 1.51 (1.46-1.56) | 1.71 (1.54-1.65) |
| HDL-C | 0.89 (0.86-0.92) | 0.91 (0.88-0.94) | 0.93 (0.90-0.97) | 0.82 (0.79-0.85) |

Model 1: Age and sex

Mode 2: additionally, adjusted for smoking status (never, ex and current)

Model 3: additionally adjusted for history of disease related variables: history of diabetes (yes or no), history of hypertension (yes or no) and family history of MI (yes or no) as appropriate (e.g. History of diabetes was only adjusted for history of hypertension and family history of MI).

Model 4: additionally adjusted for total cholesterol, LDL-C and HDL-C as appropriate (e.g. Total cholesterol and LDL-C was only adjusted for HDL-C, and HDL-C was only adjusted for LDL-C).

**Table 2.8:** Local risk factors and risk of acute myocardial infarction

| Risk factors | Model 1 | Model 2 | Model 3 |
|---|---|---|---|
| Lifelong urban residence | 1.55 (1.38-1.75) | 1.52 (1.34-1.72) | 1.41 (1.24-1.61) |
| Income below the poverty line | 1.19 (1.09-1.31) | 1.19 (1.08-1.31) | 1.23 (1.13-1.39) |
| Non-manual | 1.30 (1.13-1.49) | 1.30 (1.14-1.49) | 1.31 (1.14-1.50) |
| Manual | 0.64 (0.58-0.70) | 0.65 (0.60-0.70) | 0.63 (0.58-0.68) |
| Unemployed | 1.04 (0.95-1.13) | 1.02 (0.95-1.10) | 0.95 (0.89-1.03) |
| Intermarriage | 1.23 (1.04-1.45) | 1.22 (1.02-1.45) | 1.20 (1.00-1.45) |
| Stress at home | 1.14 (1.08-1.21) | 1.08 (1.03,1.14) | 1.12 (1.06,1.18) |
| Stress at work | 1.31 (1.24-1.38) | 1.25 (1.18-1.33) | 1.20 (1.13-1.28) |
| Financial stress | 1.01 (1.02-1.13) | 1.03 (0.98-1.09) | - |
| Day time nap | 1.22 (1.13-1.31) | 1.18 (1.10-1.28) | 1.17 (1.08-1.26) |

Model 1: Age and sex

Model 2: additionally adjusted for smoking status (never, ex and current), history of diabetes (yes or no) and history of hypertension (yes or no)

Model 3: additionally adjusted for income and occupation as appropriate

# Chapter 3: Distribution and cross-sectional correlates of diet in BRAVE

## 3.1 Chapter summary

It is important to study the potential determinants of diet to 1) improve understanding of the context in which diet is consumed in this population, 2) enhance the interpretation of epidemiological evidence on association between diet and risk of acute myocardial infraction (AMI), discussed in subsequent chapters and 3) highlight the relevance of potential confounding factors.

This chapter describes the cross-sectional associations of food groups with a range of lifestyle (such as smoking, physical activity), biochemical (such as lipids), dietary and other socioeconomic characteristics (such as income, education, occupation) recorded in the BRAVE case-control study. In summary, findings from these analyses indicate that 1) the highest consumed foods in BRAVE controls are boiled white rice followed by vegetables, fruits and sweet water fish. On the other hand, consumption of meat from other sources than fish, dairy products, snacks, sweets and sugar sweetened beverages (SSB) are relatively low in this population; 2) dietary food groups show modest association with various characteristics (indicating the role of potential confounding); 3) higher consumption of fruits and vegetables is associated with healthy lifestyle behaviours such as non-smoking and being physically active; 4) by contrast, higher consumption of red meat and biryani is associated with physical inactivity; 5) strong correlations across various food groups, characterising how foods are eaten together in this population and 6) as compared to the western studies the diet in Bangladesh is very different.

## 3.2 BACKGROUND

As discussed in **Chapter 1**, the evidence on the association between diet and risk of coronary heart disease (CHD) is scarce in South Asia. However, prior to the assessment of diet and its association with disease, reliable characterisation of associations that may exist across diet and various lifestyle, socio-demographic, anthropometric and other dietary factors is essential to (1) better understand the determinants of dietary factors and (2) identify potential sources of confounding in epidemiological studies investigating the association of diet with CHD or other outcomes. The role of a wide array of potential risk factors such as smoking habits, socio-demographic factors, and physical activity have been extensively researched with the risk of CHD. In addition, previous studies have shown such risk factors may also be associated with dietary intake and are important targets for health promotion.[1] However, whether such correlations exist between diet and potential risk factors of CHD in a South Asian population remains uncertain.

In this context, this chapter describes the cross-sectional associations of consumption of main food groups with a wide range of potential CHD risk factors including lifestyle, socio-demographic, anthropometric and other dietary factors from the Bangladesh based BRAVE study. The specific aims of this chapter are to: (1) describe the diet of controls in the BRAVE study in terms of food groups and compare it with a national diet survey, and (2) examine the cross-sectional correlates of dietary food groups with various characteristics.

## 3.3 METHODS

### 3.3.1 Participants

Details of population, data collection, and harmonization for the analyses in BRAVE study have been provided in **Chapter 2**. Briefly, BRAVE is a hospital-based case-control study set in Dhaka, Bangladesh. For the analyses of food groups 8079 controls of the BRAVE study were used as they may represent the general population as compared to the cases.

### 3.3.2 Food group consumption in BRAVE controls

The details of the dietary assessment for BRAVE participants are described in **Chapter 2**. Briefly, a semi-quantitative food frequency questionnaire (FFQ) was used to assess the diet of participants in the past year. FFQ items were collated into groups based on nutrient content and culinary usage **(Table 2.4, Chapter 2)**. Median intakes of food groups in controls by sex (male vs female), age group (<50 years vs ≥ 50 years- median age) and geographical location (urban vs rural) were presented. Distributions of dietary intake variables were assessed by visual inspection of histograms in STATA **(Appendix 2, Figure 1)**. Median intakes were used as food intakes were ordinal and are not normally distributed. In order to assess the comparability of the BRAVE population with the general Bangladesh population, food group intakes in BRAVE controls were compared to a Bangladesh nationwide cross-sectional household survey, Household Income and Expenditure Survey (HIES), 2010.[2] This survey includes 12,240 households, of which 7,840 were from rural areas and 4,400 from urban areas. The age range of participants in this survey was 0 to 60 plus and it had about 50% males and females. In this survey food consumption of BRAVE controls was compared to dietary consumption in households of the HIES survey according to urban and rural areas. The 12 major food groupings in the BRAVE study are similar to the HIES survey including fruits, vegetables, eggs, milk, red meat, white meat, rice, bread, pulses etc. Additionally, BRAVE has included separate categories for the relatively less consumed foods such as savoury snacks, deep fried snacks, sweets, biryani and sugar sweetened beverages that HIES were not able to include. Therefore, BRAVE study has a more comprehensive capture of the Bangladeshi diet. The HIES survey reports intakes in grams per capita which can be taken as average consumptions per individuals.

### 3.3.3 Percentage of controls meeting the dietary guidelines

The Food and Agriculture Organisation (FAO) have published dietary guidelines specific to Bangladesh.[3] These guidelines were used to assess the percentage of controls that met the daily food intake requirements as these are the only dietary guidelines available that are specific to Bangladesh. To make reliable comparison, the FAO guidelines were first converted into standard portion sizes (g/d) and then compared to the intakes of BRAVE controls. This was done to observe which foods were not meeting the guidelines in the BRAVE population and to emphasise which foods are eaten more in Bangladesh as compared to others.

### 3.3.4 Correlates of food groups

Mean daily food group intakes (g/d) were ranked and grouped into quintiles. Quintile comparison is a standard nutrition epidemiology presentation. This has been done in previous studies from South Asia.[4–6] Quintile approach has also been used by the Nurses' Health Study, Health Professional Study and PURE study.[4,7] In order to remain consistent and compare the estimates of BRAVE study with other benchmark studies the quintile approach was used. Baseline characteristics of controls were presented comparing Quintile 1 vs Quintile 5 of the distribution of major food groups with continuous variables reported as means and standard deviations, and categorical variables as percentages. Correlations between food groups were calculated using spearman's correlation coefficient as food groups are not normally distributed. This was further adjusted for Bonferroni correction to account for Type 1 error. The analysis was done to justify if foods are eaten together and the use of dietary pattern analysis in **Chapter 5**. **Appendix 6** shows the use of linear mixed models adjusted for age to assess the shape of the association of food groups with lipids and waist-to-hip ratio.

## 3.4 RESULTS

*3.4.1 Food group consumption in BRAVE controls*

Overall, BRAVE controls reported highest intake of boiled white rice followed by vegetables, fruits and sweet water fish. **Tables 3.1-3.2** show the sex, age and location (place of residence) specific intakes of food group intake among controls of the BRAVE study. In general, men reported higher median intakes of food groups consumption than women. As for those in the older age group (≥ 50 years) reported marginally lower intakes of the majority of the food groups than the younger age group (<50 years). However, only for milk and sweet water fish there was a modestly higher consumption reported by older age group. Those who resided in rural areas reported higher intakes of leafy vegetables, other vegetables, vitamin C rich fruits, other fruits, boiled white rice, sweet water fish, milk and yoghurt. On the contrary, those residing in urban areas reported higher intakes of pulses, biryani, bread, deep fried snacks, savoury snacks, sweets, sugar sweetened beverages (SSB), white meat, red meat, sea water fish and egg consumption, as compared to participants living in rural areas. As compared to HIES 2010 survey, the reported intakes of food items were higher in BRAVE controls **(Table 3.2)**. Specifically, daily consumption (g/day) of green leafy vegetables was 131.0 vs. 36.1 and for rice 990.3 vs 416.0 for BRAVE controls as compared to the HIES survey. Overall the trends of highest to lowest consumption of food groups in the BRAVE controls were similar to the HIES data.[2]

*3.4.2 Percentage of controls meeting the dietary guidelines*

**Table 3.3** shows the percentage of controls meeting the FAO Bangladeshi dietary guidelines for specific foods.[3] Overall, 99% of the controls met the guidelines for rice intake, 87.6% for fish intake and 60% for intake of pulses. As for fruits and vegetables, less than half of the controls met the guidelines. Recommendations to consume 150 ml of milk and 100 ml of yoghurt daily were met by 15.9% and 3.4% of the controls only. Guidelines about whole grains were met by 4.5% of the population. As for chicken only about 6% met the guidelines and for red meat less than 1% controls met the guidelines.

*3.4.3 Association of food groups with baseline characteristics among controls*

BRAVE controls in the highest quintile (as compared to the lowest quintile) of total vegetable consumption tended to be younger (mean age 50.5 (9.9) vs 52.4 (10.7)), more educated (lower percentage of participants with no schooling), marginally less likely to be currently smoking (59.1% vs 62.5 %), more physically active (78.9% vs 74.9%), had

higher percentage of history of diabetes (11.4% vs 9.5%), history of hypertension (15.3% vs 12.4%) and family history of MI (7.8% vs 5.0%) and were mostly residing in rural areas **(Table 3.4)**. For total fruit consumption, controls in the highest quintile were also younger (mean age 50.2 (10.3) vs 53.3 (10.5)), more educated, marginally less likely to be currently smoking (59.1% vs 65.4 %), more physically active (83.4% vs 71.0%), had higher percentage of family history of MI (8.0% vs 4.9%) and were mostly residing in rural areas. However, those in the highest consumption of total fruits had lower percentage of history of diabetes (8.5% vs 11.5%).

For pulses intake, those who had highest intake were more educated, had higher percentage of history of hypertension (14.6% vs 10.3%) and were mostly residing in urban areas. People consuming highest quintile of boiled white rice were younger (49.8 (9.9) vs 52.9 (10.4)), had lower history of diabetes (3.4% vs 17.0%), hypertension (7.8% vs 17.9%) and family history of MI (5.4% vs 8.5%), and were mostly residing in rural areas as compared to those in the lowest quintile. For biryani, those who had the highest intake were younger (47.5 (10.0) vs 53.3 (10.0)), had higher waist-to-hip ratio, had slightly higher percentage of hypertension, and were more educated, less likely to be current smokers, less physically active and mostly residing in urban areas. Those who consumed high amounts of bread were marginally younger, more educated, had higher history of hypertension and diabetes and mostly residing in urban areas. Controls in the highest quintiles of snacks intake were younger, more educated and higher family history of MI and were more physically active as compared to those in the lowest quintile. People who consumed highest intake of SSB were younger, had less percentage of history of diabetes and hypertension, were more educated and mostly resided in urban areas.

Controls who consumed more white meat and eggs were younger, more educated and less likely to be current smokers. Highest consumption of red meat was correlated with being younger (48.7 (10.4) vs 53.1 (10.1)), more educated, being less physically active (73.2 vs 80.8%), having lower history of hypertension (11.9% vs 15.9%) and higher family history of MI (9.8% vs 5.7%). Those consuming high amounts of total fish were more educated, had higher family history of MI (8.2% vs 5.6%), more physically active (80.1% vs 75.0%) and mostly resided in rural areas. For milk those in the highest quintile were older (53.1 (10.6) vs 50.6 (10.2)), more educated, physically active (80.3% vs 73.7%) and were mostly residing in rural areas. Those with highest intake of yoghurt consumption were more educated, more physically active (80.8% vs 76.5%), had higher history of hypertension (18.6% vs 12.2%), diabetes (13.9% vs 9.4%) and family history of MI (9.6% vs 6.3%).

Overall, higher consumption of most of the food groups was associated with being younger, having a higher energy intake, higher education level, higher income level and

they were less likely to be currently smoking. As for occupation, the majority of the people consuming high amounts of food groups were business professionals followed by being retired, unemployed or students**.** In addition, highest quintiles of most of the food groups was associated with higher BMI and waist-to-hip ratio.

*3.4.4 Correlations between food groups*

Correlations between food groups were significantly strong **(Table 3.5)**. The strongest positive correlations were found for intake of green leafy vegetables with other vegetables (rho=0.76), vitamin C rich fruits with other fruits (0.55), biryani with red meat (0.42), deep fried snacks with savoury snacks (0.40) and other vegetables with other fruits (0.38). There was also a strong negative association observed between boiled white rice and bread (rho=-0.38). Overall there were fewer negative than positive correlations**.**

## 3.5 DISCUSSION

### *3.5.1 Summary of main findings*

This chapter reports the consumption of food groups in BRAVE controls. It also describes in detail the cross-sectional associations of food groups with various characteristics. The study reported that the BRAVE controls had the highest consumption (in g/day) of rice followed by vegetables, fruits and sweet water fish. Whereas consumption of meat from other sources than fish, dairy products, snacks, sweets and SSB were low. These trends were similar to the national representative HIES study in Bangladesh. However, the reported intakes were higher for BRAVE controls. There were slight differences in food group intakes by sex, age and location. Overall, dietary food groups showed modest association with various characteristics showing the role of potential confounding. In particular, higher consumption of fruits, vegetables were associated with healthy lifestyle behaviours such as less likely to be currently smoking and being more physically active. On the contrary, higher consumption of red meat and biryani was associated with being less physically active. There were few strong correlations between food groups suggesting how foods are eaten together.

### *3.5.2 Food groups consumption in BRAVE controls*

The data of 8079 controls from the BRAVE study reported that people in Bangladesh mostly consumed boiled white rice, followed by vegetables, fruits and fish. Whereas there was low daily intake of red meat, dairy products, sweets and local snacks. According to the dietary recommendations set by FAO in Bangladesh, more than half of the population met the guidelines for the consumption of rice, fish and pulses.[3] However, less than half of the population met guidelines for consumption of fruits, vegetable, dairy products, whole grains and white meat. This again emphasises that in the population there is low consumption of white and red meat, dairy products and whole grains. Consistent to this study, the national nutritional surveys and previous studies conducted in Bangladesh also reported that the majority of the calories consumed by Bangladeshis come from rice, whereas there is low consumption of animal and dairy based food products.[2,8,9] In particular the HIES data (2010) highlighted that major contributor (about 70%) to diet was rice.[2] Whereas protein and micronutrient-rich foods like fish, meat, eggs, milk products, fats and oils accounted for lower consumption (less than 10%).[2] However, this is a cross-sectional survey conducted in various cities in Bangladesh with younger mean age and different proportion of males and females so it may not be directly comparable to

participants of BRAVE. Nevertheless, the BRAVE controls reported similar trends of consumption as in the HIES data, highlighting the homogenous food consumption in different areas of Bangladesh. The BRAVE controls reported higher intakes of foods than the HIES survey. This could be because the HIES survey includes children and adolescents (60% of the population between 0-19 years of age), therefore the low reported intakes of food as compared to the BRAVE study which recruited people above 18 years of age (mean age was 50 years). Nevertheless, the possibility of over-reporting by the BRAVE controls cannot be ruled out. In addition, BRAVE study is based in Dhaka and has a greater proportion of people living in urban areas as compared to the HIES, whereas the HIES covered 6 divisions in Bangladesh with more households from rural areas as compared to urban areas. This may explain the differences in consumption patterns as well. Additionally, the higher intakes of BRAVE controls than the HIES survey may also affect the percentage of controls meeting the FAO dietary guidelines. The national survey may have lesser portion of people meeting the guidelines than the BRAVE controls.

As compared to western studies the food intakes in BRAVE controls is very different. Many western studies have reported higher intakes of processed and unprocessed red meat, whereas in Bangladesh the consumption of red meat (mostly unprocessed) is extremely low.[10–13] In Bangladesh dairy consumption is relatively low and is mostly from milk followed by yoghurt, whereas western studies have reported higher general consumption of dairy products such as milk, cheese and yoghurt.[14] Furthermore, fish consumption in BRAVE controls is relatively higher in those reported in some of the western studies.[13,15] In addition, in western populations rice is not the major contributor to total calorie intake.

The present study also reported differences in dietary intake by different subgroups in the BRAVE controls. The older age group reported lower intakes of all food groups (except milk and sweet water fish) as compared to the younger group. As for different sexes, men reported higher intakes of the majority of the food groups as compared to women. This may be because men have higher energy requirements than women and as older people have less fat free mass and have lower physical activity levels therefore, they may have less energy needs due to less energy expenditure. However, there may also be a case of under-reporting. There is evidence that that older people and women may tend to under-report their diets more than younger people and men respectively.[16] However, whether that is true for a South Asian population is not established. There was also considerable variation of food consumption in urban and rural areas in this study. Those who resided in rural areas reported higher intakes of vegetables, fruits, boiled white rice, sweet water fish and dairy products. On the contrary, those residing in urban areas reported higher intakes of pulses, biryani, bread, snacks, sweets, SSB, meat, sea water fish and eggs. It may be postulated that in rural areas people tend to consume less of expensive foods such

as meat and sweets as compared to urban areas. The urban-rural differences in types of food consumed in this study is broadly consistent with the evidence from HIES 2010 report in Bangladesh.[17] This report also highlighted that as compared to rural areas in urban areas there was higher consumption of pulses, meat, fish, eggs, sweets and sugar, and there was lower consumption of rice, potatoes, vegetables and cereals.

In terms of sociodemographic correlates, those who were more educated and had higher income levels reported higher consumption of all food groups. Education and income may influence the lifestyle of the people. Higher education may facilitate the understanding of dietary information and create awareness about other health promoting behaviours.[18] A systematic review reported that higher socio-economic status (measured by education and occupation) was associated with higher consumption of fruits and vegetables.[18] This may mean that people may have financial means to buy expensive and more energy dense foods.

Furthermore, this study found that higher consumption of fruits, vegetables was associated with healthy lifestyle behaviours such as being less likely to be currently smoking and being more physically active. On the contrary, higher consumption of red meat and biryani was associated with being less physically active. These associations are similar to a meta-analysis of 98 studies that reported that fruit and vegetable consumption were associated with healthy lifestyle behaviours, on the contrary red meat was associated with unhealthy lifestyle behaviours.[19] As for fish intake, higher consumption was associated with being more physically active. Whereas for boiled white rice there were not much differences in proportion of smokers and physically active people in highest versus lowest intake. As for history of disease overall there were slight differences in those with the highest and lowest intake of food group consumption. Those with higher vegetable, fruits, pulses, biryani, bread white meat, fish and yoghurt consumption reported higher percentage of history of hypertension. Those with higher vegetable, pulses, biryani, bread, white meat, fish, egg, milk and yoghurt reported having higher percentage of diabetes. Higher consumption of most food groups was associated with having higher family history of MI.

The study reported strong correlations between some food groups highlighting the importance of studying dietary patterns as these foods may be eaten together. The strongest positive correlation was observed between green leafy vegetables and other vegetables followed by vitamin C rich fruits and other fruits. There was also a correlation between consumption of biryani and red meat. These also mean potential healthy or unhealthy foods may be eaten together.

### *3.5.3 Strengths and limitations*

The strengths and limitations of this study merit consideration. The present analysis is the largest and most detailed study of cross-sectional correlates of food groups in a South Asian population. The study had sufficient information on food intake to quantify the associations with wide array of non-dietary and dietary variables, including potential risk factors of CHD. The associations highlight the role of potential clustering of risk factors that may confound the associations between diet and CHD if associated both with the exposure and outcome. In addition, the study provides a better understanding of diet consumed in different subgroups (sex, age and location) in the population that would better elucidate the associations in the subsequent chapters.

However, as the current analyses were based on observational cross-sectional assessment, the current study cannot make causal inference about diet and their correlates. The associations may be affected by reverse causation (e.g., subjects who may have changed their dietary habits because of being diagnosed with diabetes or hypertension). This might partly explain the general lack of associations for food groups with some of the risk factors such as lipids. In addition, as the study is based on self-reported measures of risk factors such as smoking, history of disease, physical activity etc., there may be possibility of reporting bias. There may be also a possibility of residual confounding due to unmeasured factors.

FFQs rely on respondent memory to recall the diet retrospectively so there may be possibility of recall bias if cases and controls recall their diets differentially. The dietary assessment was based on an FFQ in BRAVE, which is prone to random and systematic measurement errors, inherent to any standard self-reported dietary instrument. When the error originates from variations in individual food choices, which may simply differ from one day to another, the error is characterised as random and is common to all individuals in a population. As this error occurs at random or is unpredictable, this would lead to attenuation of the observed associations. On the other hand, if the error is due to systematic underreporting or overreporting this leads to a bias and no longer considered as random error. The direction of bias can be in either direction. For instance, if persons are likely to give socially desirable answers to specific questions (e.g., under consumption of unhealthy snacks or over-consumption of healthy fruits and vegetables), this could consequently affect the estimated amount of energy intake and the food groups consumed.

### *3.5.4 Implications*

This chapter reports the dietary intake of the BRAVE study population and shows that Bangladeshis mostly consume boiled white rice, followed by vegetables, fruits, and fish. They tended to consume red meat, dairy products, sweets and local snacks the least. More than half of the study population do not meet the current FAO dietary guidelines for consumption of fruits, vegetables, dairy products, whole grains and white meat. This emphasizes that the population mostly consumes carbohydrate-rich diet and that the diet is not very diversified. Higher consumption of fruits and vegetables were each associated with healthy lifestyle behaviors such as less likely to be currently smoking and being more physically active. On the contrary, higher consumption of red meat and biryani was associated with being less physically active. These findings indicate potential sources of confounding in observational studies of associations between dietary factors and CHD risk and warrants appropriate accounting of these factors.

### *3.5.5 Conclusion*

In conclusion, the results of this chapter show that a typical diet in the BRAVE population comprises principally of rice, which is then followed by vegetables, fish and fruits. These trends were similar to the national representative HIES study in Bangladesh, suggesting a homogenous diet consumption in Bangladesh. Food groups were associated, albeit modestly, with various individual characteristics, emphasizing the possibility of confounding by these attributes. There were a few strong correlations across various food groups, which characterises various ways how foods are eaten together in Bangladesh and highlights the importance of studying dietary patterns in relation to AMI risk.

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**Table 3.1:** Median intakes (g/day) of food groups by sex and age in BRAVE controls

| **Food group (g/day)** | **Sex** | | **P-value** | **Age** | | **P-value** |
|---|---|---|---|---|---|---|
| | Males | Females | | <50 years | ≥ 50 years | |
| Green leafy and stem vegetables | 132.5 | 120.0 | S | 133.3 | 129.3 | NS |
| Potatoes | 68.4 | 56.5 | S | 69.2 | 65.2 | S |
| Other vegetables | 494.9 | 442.3 | S | 501.6 | 478.7 | S |
| Vitamin C rich fruits | 117.3 | 100.7 | S | 124.8 | 108.2 | S |
| Other fruits | 509.0 | 400.5 | S | 526.6 | 472.8 | S |
| Pulses | 141.7 | 106.9 | S | 144.9 | 131.9 | S |
| Boiled white rice | 1012.3 | 834.5 | S | 1040.3 | 953.7 | S |
| Biryani | 30.3 | 17.6 | S | 35.5 | 23.7 | S |
| Bread | 86.3 | 62.0 | S | 85.8 | 81.5 | S |
| Deep fried snacks | 36.1 | 11.0 | S | 43.0 | 25.7 | S |
| Savoury snacks | 34.1 | 17.2 | S | 39.2 | 26.7 | S |
| Sweets | 22.6 | 15.3 | S | 23.5 | 20.3 | S |
| Sugar sweetened beverages | 31.5 | 10.9 | S | 42.3 | 19.1 | S |
| White meat | 38.9 | 26.3 | S | 42.4 | 33.6 | S |
| Red meat | 10.8 | 5.0 | S | 12.0 | 8.6 | S |
| Sweet water fish | 262.1 | 238.5 | S | 257.3 | 260.6 | NS |
| Sea water fish | 16.9 | 17.0 | S | 17.6 | 16.5 | NS |
| Egg | 22.5 | 17.0 | S | 24.4 | 19.9 | S |
| Milk | 71.4 | 65.8 | S | 65.8 | 74.3 | NS |
| Yoghurt | 9.6 | 5.6 | S | 9.1 | 9.1 | NS |

**Footnotes Table 3.1**

The p values were obtained using Mann U Whitney test.

P value <0.001 considered to be significant according to the Bonferroni correction.

S- Significant

NS- Non-significant

**Table 3.2:** Median intakes (g/day) of food groups by location in BRAVE controls compared to HIES, 2010 survey

| **Food group (g/day)** | **BRAVE controls** | | | **HIES 2010** | | |
|---|---|---|---|---|---|---|
| | Rural | Urban | Total | Rural | Urban | Total |
| Green leafy and stem vegetables | 140.7 | 119.7 | 131.0 | 36.1 | 36.3 | 36.1 |
| Potatoes | 67.0 | 66.7 | 66.9 | 71.5 | 66.7 | 70.3 |
| Other vegetables | 512.0 | 461.4 | 488.4 | 133.9 | 118.6 | 129.9 |
| Vitamin C rich fruits | 123.2 | 105.7 | 115.2 | | | |
| Other fruits | 532.5 | 451.2 | 495.6 | 42.6 | 50.4 | 44.7 |
| Pulses | 121.8 | 157.1 | 137.4 | 13.2 | 17.2 | 14.3 |
| Boiled white rice | 1017.0 | 958.5 | 990.3 | 441.6 | 344.2 | 416.0 |
| Biryani | 24.8 | 33.2 | 28.7 | | | |
| Bread | 68.3 | 101.7 | 83.3 | 23.3 | 33.6 | 26.0 |
| Deep fried snacks | 28.7 | 38.2 | 33.0 | | | |
| Savoury snacks | 29.5 | 34.6 | 32.0 | | | |
| Sweets | 20.3 | 23.3 | 21.7 | | | |
| Sugar sweetened beverages | 25.5 | 33.0 | 28.9 | | | |
| White meat | 34.1 | 41.5 | 37.3 | 9.0 | 17.4 | 11.2 |
| Red meat | 8.7 | 11.7 | 10.1 | 5.2 | 13.4 | 7.4 |
| Sweet water fish | 283.5 | 229.4 | 259.2 | 45.8 | 59.9 | 49.5 |
| Sea water fish | 15.5 | 18.8 | 16.9 | | | |
| Egg | 19.9 | 24.1 | 21.8 | 5.8 | 10.9 | 7.2 |
| Milk | 82.0 | 57.0 | 70.7 | 31.8 | 39.2 | 33.7 |
| Yoghurt | 9.2 | 8.8 | 9.1 | | | |

**Footnotes Table 3.2**

Household Income and Expenditure Survey (HIES) is the core survey conducted in Bangladesh in 2010 to provide data on income, expenditure, consumption and poverty situation. A two-stage sample design was adopted for the survey. The sample size was 12,240 households where 7,840 were from rural area and 4,400 from urban area. The age range of participants was 0 to 60 years plus and there were about 50% males and females. The HIES survey was conducted in Chittagong, Dhaka, Khulna, Barisal, Sylhet and Rajshahi. The HIES survey shows the average per capita per day consumption.

**Table 3.3**: Percentage of controls meeting the FAO Bangladeshi dietary guidelines

| Food items | Dietary recommendation set by FAO | Percentage of BRAVE controls meeting guidelines |
|---|---|---|
| **Rice and whole grains** | Eat rice or wheat or a combination of cereals around 270-450 g which is equivalent to 9-15 servings daily | Rice 99.0 %<br>Whole grains 4.5% |
| **Meat** | Eat 1 to 4 medium size pieces of fish, meat, poultry a day<br>90 grams | Fish 87.6%<br>Red meat 0.75%<br>Poultry 5.6% |
| **Pulses** | 68 grams a day | 60% |
| **Fruits** | Eat 2 seasonal fruits every day, one from citrus, another from vitamin A sources a day<br>80 grams per fruit | 48.6% |
| **Vegetables** | Eat at least 100g leafy and 200g non-leafy vegetables daily. | 49.9% |
| **Milk** | 150 ml | 15.9% |
| **Yoghurt** | 100 ml | 3.4% |
| **Fat intake** | Total fat should provide between 15-30% of the daily energy intake. Saturated fat should provide less than 10% of the daily energy intake. | 33.6% |

**Footnotes Table 3.3**

The Food and Agriculture guidelines of Bangladesh were converted into grams or ml by day using standard portion sizes. These guidelines are for men and women of all age groups.

**Table 3.4:** Characteristics of controls in quintile 1 versus quintile 5 of different food groups

| | Vegetables | | Fruits | | Pulses | |
|---|---|---|---|---|---|---|
| Characteristics | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 |
| | | | | | | |
| Age (years) | 52.4 (10.7) | 50.5 (9.9) | 53.3 (10.5) | 50.2 (10.3) | 52.0 (10.2) | 50.4 (10.1) |
| Sex (%) | | | | | | |
| Males | 1125 (82.8) | 1590 (90.4) | 1050 (79.7) | 1796 (91.3) | 1260 (81.5) | 1443 (92.7) |
| Females | 234 (17.2) | 169 (9.6) | 268 (20.3) | 171 (8.7) | 286 (18.5) | 114 (7.3) |
| BMI ($kg/m^2$) | 22.8 (5.8) | 23.8 (6.7) | 22.6 (3.9) | 23.5 (5.4) | 22.8 (4.0) | 23.7 (3.8) |
| Waist-to-hip ratio | 0.95 (0.08) | 0.96 (0.08) | 0.95 (0.07) | 0.96 (0.08) | 0.95 (0.08) | 0.96 (0.07) |
| History of diabetes (%) | | | | | | |
| Yes | 129 (9.5) | 200 (11.4) | 152 (11.5) | 167 (8.5) | 148 (9.6) | 160 (10.3) |
| No | 1230 (90.5) | 1559 (88.6) | 1166 (88.5) | 1800 (91.5) | 1398 (90.4) | 1397 (89.7) |
| History of hypertension (%) | | | | | | |
| Yes | 169 (12.4) | 270 (15.3) | 153 (11.6) | 252 (12.8) | 160 (10.3) | 228 (14.6) |
| No | 1190 (87.6) | 1489 (84.7) | 1165 (88.4) | 1715 (87.2) | 1386 (89.7) | 1329 (85.4) |
| Family history of MI (%) | | | | | | |
| Yes | 68 (5.0) | 138 (7.8) | 64 (4.9) | 158 (8.0) | 94 (6.1) | 121 (7.8) |
| No | 1291 (95.0) | 1621 (92.2) | 1254 (95.1) | 1809 (92.0) | 1452 (93.9) | 1436 (92.2) |
| LDL-C, (mmol/L) | 2.78 (0.88) | 2.83 (0.87) | 2.78 (0.88) | 2.80 (0.97) | 2.76 (0.86) | 2.82 (0.89) |
| HDL- C, (mmol/L) | 0.87 (0.24) | 0.83 (0.22) | 0.87 (0.23) | 0.84 (0.23) | 0.87 (0.24) | 0.82 (0.22) |
| Education level (%) | | | | | | |
| No schooling | 596 (43.9) | 523 (29.7) | 642 (48.7) | 537 (27.3) | 665 (43.0) | 438 (28.1) |
| Primary | 386 (28.4) | 527 (30.0) | 395 (30.0) | 574 (29.2) | 441 (28.5) | 490 (31.5) |
| Secondary | 270 (19.9) | 460 (26.1) | 202 (15.3) | 603 (30.7) | 316 (20.4) | 396 (25.5) |
| Vocational/university | 106 (7.8) | 249 (14.2) | 79 (6.00) | 252 (12.8) | 124 (8.01) | 232 (14.9) |
| Annual income (Taka) (%) | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Income below the poverty line ≤56000 | 423 (31.6) | 317 (18.2) | 479 (36.6) | 342 (17.7) | 492 (32.2) | 242 (15.8) |
| Low income | 315 (23.5) | 321 (18.5) | 341 (26.1) | 371 (19.1) | 351 (22.9) | 280 (18.3) |
| Medium income | 382 (28.5) | 573 (33.0) | 334 (25.5) | 656 (33.9) | 434 (28.4) | 499 (32.6) |
| High income | 219 (16.4) | 527 (30.3) | 154 (11.7) | 569 (29.4) | 253 (16.5) | 511 (33.4) |
| Occupation (%) | | | | | | |
| Business professional | 525 (38.6) | 822 (46.7) | 415 (31.5) | 959 (48.8) | 513 (33.2) | 810 (52.1) |
| Manual labour | 303 (22.3) | 371 (21.1) | 341 (25.9) | 408 (20.8) | 438 (28.4) | 239 (15.4) |
| Non-manual labour | 88 (6.5) | 85 (4.8) | 73 (5.5) | 85 (4.3) | 77 (5.0) | 109 (7.0) |
| Unemployed/retired/students | 443 (32.6) | 481 (27.4) | 488 (37.1) | 514 (26.1) | 517 (33.5) | 398 (25.6) |
| Smoking status (%) | | | | | | |
| Never | 404 (29.7) | 588 (33.4) | 352 (26.7) | 670 (34.1) | 518 (33.5) | 497 (31.9) |
| Ex | 106 (7.8) | 132 (7.5) | 104 (7.9) | 134 (6.8) | 102 (6.6) | 115 (7.4) |
| Current | 849 (62.5) | 1039 (59.1) | 862 (65.4) | 1163 (59.1) | 926 (59.9) | 945 (60.7) |
| Total MET-minutes of exercise/week (%) | | | | | | |
| < 600 | 341 (25.1) | 371 (21.1) | 382 (29.0) | 327 (16.6) | 349 (22.6) | 371 (23.8) |
| ≥ 600 | 1018 (74.9) | 1388 (78.9) | 936 (71.0) | 1640 (83.4) | 1197 (77.4) | 1186 (76.2) |
| Total Energy intake (kcal/day) | 2480.8 (776.9) | 3703.3 (1067.4) | 2369.5 (649.9) | 3919.0 (978.9) | 2633.3 (851.1) | 3706.5 (1006.4) |
| Location (%) | | | | | | |
| Urban | 676 (50.3) | 671 (38.2) | 647 (49.7) | 736 (37.6) | 528 (34.5) | 863 (55.5) |
| Rural | 667 (49.7) | 1084 (61.8) | 655 (50.3) | 1224 (62.4) | 1002 (65.5) | 692 (44.5) |

| | Boiled white rice | | Biryani | | Bread | | Snacks | | Sugar sweetened beverages | |
|---|---|---|---|---|---|---|---|---|---|---|
| Characteristics | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 |
| | | | | | | | | | | |
| Age (years) | 52.9 (10.4) | 49.8 (9.9) | 53.3 (10.0) | 47.5 (10.0) | 52.4 (10.4) | 50.8 (9.9) | 54.5 (10.0) | 47.8 (10.2) | 53.34(9.9) | 46.5 (10.2) |
| Sex (%) | | | | | | | | | | |
| Males | 2930 (82.0) | 1445 (95.1) | 2522 (82.8) | 963 (94.6) | 1372 (81.0) | 1425 (92.7) | 1006 (70.8) | 1640 (96.4) | 3494 (81.7) | 1467 (95.8) |
| Females | 643 (18.0) | 75 (4.9) | 524 (17.2) | 55 (5.4) | 321 (19.0) | 112 (7.3) | 415 (29.2) | 61 (3.6) | 785 (18.3) | 65 (4.2) |
| BMI ($kg/m^2$) | 23.5 (5.6) | 23.1 (3.6) | 22.3 (3.7) | 24.7 (4.1) | 22.2 (3.6) | 24.5 (5.7) | 22.7 (3.9) | 23.7 (4.0) | 22.4 (3.9) | 24.1 (5.7) |
| Waist-to-hip ratio | 0.96 (0.08) | 0.96 (0.07) | 0.94 (0.08) | 0.97 (0.07) | 0.94 (0.08) | 0.97 (0.07) | 0.95 (0.08) | 0.96 (0.08) | 0.95 (0.08) | 0.96 (0.07) |
| History of diabetes (%) | | | | | | | | | | |
| Yes | 606 (17.0) | 51 (3.4) | 293 (9.6) | 108 (10.6) | 45 (2.7) | 416 (27.1) | 137 (9.6) | 162 (9.5) | 486 (11.4) | 125 (8.2) |
| No | 2966 (83.0) | 1469 (96.6) | 2752 (90.4) | 909 (89.4) | 1648 (97.3) | 1121 (72.9) | 1284 (90.4) | 1539 (90.5) | 3793 (88.6) | 1407 (91.8) |
| History of hypertension (%) | | | | | | | | | | |
| Yes | 639 (17.9) | 119(7.8) | 385 (12.6) | 148 (14.5) | 155 (9.2) | 311 (20.2) | 214 (15.1) | 227 (13.4) | 610 (14.3) | 171 (11.2) |
| No | 2933 (82.1) | 1401 (92.2) | 2660 (87.4) | 869 (85.5) | 1538 (90.8) | 1226 (79.8) | 1207 (84.9) | 1474 (86.7) | 3669 (85.7) | 1361 (88.8) |
| Family history of MI (%) | | | | | | | | | | |
| Yes | 302 (8.5) | 82 (5.4) | 155 (5.1) | 94 (9.24) | 90 (5.3) | 151 (9.8) | 70 (4.9) | 161 (9.5) | 250 (5.8) | 146 (9.5) |
| No | 3269 (92.5) | 1437 (94.6) | 2888 (94.9) | 923 (90.8) | 1603 (94.7) | 1386 (90.2) | 1351 (95.1) | 1540 (90.5) | 4029 (94.2) | 1386 (90.5) |
| LDL-C, (mmol/L) | 2.87 (0.90) | 2.70 (0.83) | 2.79 (0.86) | 2.86 (0.90) | 2.78 (0.85) | 2.79 (0.89) | 2.84 (0.89) | 2.81 (0.87) | 2.80 (0.88) | 2.83 (0.91) |
| HDL- C, (mmol/L) | 0.85 (0.24) | 0.84 (0.22) | 0.87 (0.24) | 0.81 (0.21) | 0.87 (0.23) | 0.82 (0.23) | 0.86 (0.25) | 0.84 (0.22) | 0.86 (0.24) | 0.82 (0.22) |
| Education level (%) | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No schooling | 1186 (33.2) | 581 (38.3) | 1438 (47.2) | 189 (18.6) | 783 (46.3) | 386 (25.1) | 728 (51.3) | 406 (23.9) | 1803 (42.1) | 354 (23.1) |
| Primary | 1018 (28.5) | 457 (30.1) | 810 (26.6) | 346 (34.0) | 455 (26.9) | 465 (30.3) | 363 (25.6) | 542 (31.9) | 1197 (28.0) | 500 (32.7) |
| Secondary | 903 (25.3) | 342 (22.5) | 563 (18.5) | 307 (30.1) | 336 (19.9) | 456 (29.7) | 232 (16.3) | 484 (28.5) | 871 (20.4) | 453 (29.6) |
| Vocational/university | 464 (13.0) | 139 (9.2) | 234 (7.7) | 176 (17.3) | 118 (7.0) | 229 (14.9) | 98 (6.9) | 266 (15.7) | 407 (9.5) | 223 (14.6) |
| Annual income (Taka) (%) | | | | | | | | | | |
| Income below the poverty line ≤56000 | 983 (28.0) | 299 (19.8) | 991 (32.8) | 101 (10.2) | 597 (35.4) | 234 (15.6) | 601 (42.8) | 187 (11.2) | 1399 (33.0) | 153 (10.18) |
| Low income | 602 (17.1) | 405 (26.8) | 807 (26.7) | 128 (12.9) | 419 (24.8) | 244 (16.2) | 275 (19.6) | 342 (20.5) | 963 (22.7) | 222 (14.8) |
| Medium income | 972 (27.7) | 536 (35.5) | 814 (27.0) | 321 (32.5) | 444 (26.3) | 494 (32.9) | 312 (22.2) | 592 (35.4) | 1142 (27.0) | 566 (37.7) |
| High income | 953 (27.2) | 269 (17.8) | 408 (13.5) | 439 (44.4) | 227 (13.5) | 532 (35.4) | 216 (15.4) | 550 (32.9) | 733 (17.3) | 562 (37.4) |
| Occupation (%) | | | | | | | | | | |
| Business professional | 1596 (44.7) | 611 (40.2) | 978 (32.1) | 622 (61.1) | 508 (30.0) | 863 (56.2) | 402 (28.3) | 965 (56.8) | 1427 (33.4) | 950 (62.1) |
| Manual labour | 484 (13.6) | 508 (33.4) | 872 (28.6) | 94 (9.2) | 515 (30.4) | 182 (11.8) | 299 (21.0) | 283 (16.7) | 1066 (24.9) | 191 (12.5) |
| Non-manual labour | 157 (4.4) | 95 (6.3) | 154 (5.1) | 58 (5.7) | 69 (4.1) | 80 (5.2) | 54 (3.8) | 112 (6.6) | 213 (5.0) | 91 (5.9) |
| Unemployed/retired/students | 1335 (37.4) | 305 (20.1) | 1041 (34.2) | 244 (24.0) | 600 (35.5) | 412 (26.8) | 666 (46.9) | 339 (20.0) | 1571 (36.7) | 299 (19.5) |
| Smoking status (%) | | | | | | | | | | |
| Never | 1106 (21.0) | 532 (35.0) | 809 (26.6) | 367 (36.1) | 539 (31.8) | 495 (32.2) | 409 (28.8) | 543 (31.9) | 1301 (30.4) | 514 (33.6) |
| Ex | 301 (8.4) | 89 (5.9) | 212 (7.0) | 78 (7.7) | 88 (5.2) | 139 (9.0) | 103 (7.3) | 108 (6.4) | 320 (7.5) | 100 (6.5) |
| Current | 2166 (60.6) | 899 (60.5) | 2025 (66.5) | 573 (56.3) | 1066 (63.0) | 903 (58.8) | 909 (64.0) | 1050 (61.7) | 2658 (62.1) | 918 (59.9) |
| Total MET-minutes of exercise/week (%) | | | | | | | | | | |
| < 600 | 717 (20.1) | 323 (21.3) | 555 (18.2) | 282 (27.7) | 412 (24.3) | 304 (19.8) | 372 (26.2) | 345 (20.3) | 948 (22.2) | 358 (23.4) |
| ≥ 600 | 2856 (79.9) | 1197 (78.7) | 2491 (81.8) | 736 (72.3) | 1281 (75.7) | 1233 (80.2) | 1049 (73.8) | 1356 (79.7) | 3331 (77.8) | 1174 (76.6) |
| Total Energy intake (kcal/day) | 2732.0 (901.1) | 3551.1 (930.6) | 2715.1 (850.2) | 3788.7 (1091.5) | 2801.7 (873.4) | 3395.7 (1053.4) | 2519.0 (799.7) | 3789.7 (996.3) | 2841.9 (892.3) | 3558.3 (1028.0) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location (%) | | | | | | | | | | |
| Urban | 1705 (48.0) | 562 (37.1) | 1121 (36.9) | 585 (57.8) | 496 (29.5) | 908 (59.2) | 519 (36.6) | 892 (52.9) | 1671 (39.2) | 813 (53.5) |
| Rural | 1846 (52.0) | 951 (62.9) | 1914 (63.1) | 427 (42.2) | 1188 (70.5) | 626 (40.8) | 898 (63.4) | 795 (47.1) | 2593 (60.8) | 707 (46.5) |

| | White meat | | Red meat | | Fish | | Egg | | Milk | | Yoghurt | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristics | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 | Q1 | Q5 |
| | | | | | | | | | | | | |
| Age (years) | 53.2 (10.2) | 49.7 (10.3) | 53.1 (10.1) | 48.7 (10.4) | 51.2 (10.5) | 51.3 (10.2) | 53.7 (10.2) | 48.9 (10.7) | 50.6 (10.2) | 53.1 (10.6) | 51.4 (10.2) | 50.7 (10.3) |
| Sex (%) | | | | | | | | | | | | |
| Males | 1936 (82.7) | 1498 (92.6) | 2156 (79.9) | 1111 (95.4) | 1383 (84.4) | 1422 (90.1) | 595 (78.3) | 1127 (92.0) | 2249 (86.4) | 1124 (88.5) | 4866 (85.9) | 472 (92.5) |
| Females | 404 (17.3) | 119 (7.4) | 541 (20.1) | 54 (4.6) | 256 (15.6) | 157 (9.9) | 165 (21.7) | 98 (8.0) | 355 (13.3) | 146 (11.5) | 798 (14.1) | 38 (7.5) |
| BMI ($kg/m^2$) | 22.3 (3.8) | 24.2 (7.0) | 22.7 (5.9) | 24.2 (4.1) | 22.7 (3.9) | 23.4 (4.0) | 23.0 (4.0) | 23.5 (3.6) | 23.4 (3.9) | 23.0 (3.7) | 23.0 (3.9) | 24.2 (8.2) |
| Waist-to-hip ratio | 0.94 (0.08) | 0.97 (0.07) | 0.94 (0.08) | 0.97 (0.07) | 0.95 (0.07) | 0.96 (0.08) | 0.95 (0.07) | 0.96 (0.07) | 0.96 (0.08) | 0.96 (0.07) | 0.95 (0.08) | 0.97 (0.07) |
| History of diabetes (%) | | | | | | | | | | | | |
| Yes | 199 (8.5) | 197 (12.2) | 283 (10.5) | 111 (9.5) | 144 (8.8) | 162 (10.3) | 65 (8.5) | 155 (12.7) | 234 (9.0) | 153 (12.1) | 531 (9.4) | 71 (13.9) |
| No | 2141 (91.5) | 1420 (87.8) | 2414 (89.5) | 1054 (90.5) | 1495 (91.2) | 1417 (89.7) | 695 (91.5) | 1070 (87.4) | 2370 (91.0) | 1117 (87.9) | 5133 (90.6) | 439 (86.1) |
| History of hypertension (%) | | | | | | | | | | | | |
| Yes | 239 (10.2) | 265 (16.4) | 428 (15.9) | 138 (11.9) | 202 (12.3) | 220 (13.9) | 154 (20.3) | 140 (11.4) | 370 (14.2) | 157 (12.4) | 690 (12.2) | 95 (18.6) |
| No | 2101 (89.8) | 1352 (83.6) | 2269 (84.1) | 1027 (88.1) | 1437 (87.67 | 1359 (86.1) | 606 (79.7) | 1085 (88.6) | 2234 (85.8) | 1113 (87.6) | 4974 (87.8) | 415 (81.4) |
| Family history of MI (%) | | | | | | | | | | | | |
| Yes | 122 (5.2) | 145 (9.0) | 154 (5.7) | 114 (9.8) | 91 (5.5) | 129 (8.2) | 54 (7.1) | 110 (9.0) | 191 (7.3) | 106 (8.4) | 358 (6.3) | 49 (9.6) |
| No | 2218 (94.8) | 1472 (91.0) | 2543 (94.3) | 1051 (90.2) | 1548 (94.5) | 1450 (91.8) | 706 (92.9) | 1115 (91.0) | 2413 (92.7) | 1164 (91.7) | 5306 (93.7) | 461 (90.4) |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LDL-C, (mmol/L) | 2.78 (0.88) | 2.83 (0.89) | 2.78 (0.88) | 2.87 (0.91) | 2.79 (0.87) | 2.81 (0.88) | 2.78 (0.91) | 2.82 (0.89) | 2.80 (0.90) | 2.83 (0.87) | 2.79 (0.87) | 2.84 (0.89) |
| HDL- C, (mmol/L) | 0.87 (0.24) | 0.83 (0.22) | 0.87 (0.24) | 0.82 (0.21) | 0.86 (0.24) | 0.84 (0.23) | 0.86 (0.24) | 0.83 (0.23) | 0.84 (0.23) | 0.86 (0.24) | 0.85 (0.23) | 0.81 (0.23) |
| Education level (%) | | | | | | | | | | | | |
| No schooling | 1133 (48.5) | 366 (22.6) | 1197 (44.4) | 279 (24.0) | 683 (41.7) | 490 (31.2) | 343 (45.1) | 310 (25.3) | 960 (36.9) | 426 (33.5) | 2194 (38.7) | 120 (23.7) |
| Primary | 700 (29.9) | 476 (29.4) | 740 (27.5) | 359 (30.8) | 463 (28.3) | 519 (32.9) | 204 (26.8) | 390 (31.8) | 747 (28.7) | 397 (31.3) | 1677 (29.6) | 144 (28.4) |
| Secondary | 373 (16.0) | 483 (29.9) | 516 (19.1) | 360 (30.9) | 349 (21.3) | 384 (24.4) | 157 (20.7) | 330 (26.9) | 596 (22.9) | 301 (23.7) | 1242 (21.9) | 150 (29.6) |
| Vocational/university | 132 (5.6) | 292 (18.1) | 243 (9.0) | 167 (14.3) | 144 (8.8) | 184 (11.7) | 56 (7.4) | 195 (15.9) | 301 (11.5) | 146 (11.5) | 551 (9.7) | 93 (18.3) |
| Annual income (Taka) (%) | | | | | | | | | | | | |
| Income below the poverty line ≤56000 | 793 (34.3) | 240 (15.2) | 889 (33.3) | 148 (13.2) | 468 (28.8) | 301 (19.4) | 279 (37.0) | 207 (17.3) | 665 (25.9) | 302 (24.2) | 1520 (27.1) | 82 (16.5) |
| Low income | 648 (28.0) | 236 (14.9) | 691 (25.9) | 165 (14.5) | 418 (25.7) | 273 (17.6) | 153 (20.3) | 193 (16.1) | 57 (22.5) | 238 (19.0) | 1283 (22.9) | 59 (11.9) |
| Medium income | 617 (26.6) | 504 (31.9) | 698 (26.1) | 374 (32.9) | 468 (28.8) | 521 (33.6) | 173 (22.9) | 378 (31.6) | 768 (29.8) | 381 (30.5) | 1728 (30.8) | 147 (29.5) |
| High income | 259 (11.2) | 602 (38.0) | 393 (14.7) | 450 (39.6) | 274 (16.8) | 455 (29.4) | 149 (19.8) | 420 (35.1) | 559 (21.8) | 329 (26.3) | 1082 (19.3) | 210 (42.2) |
| Occupation (%) | | | | | | | | | | | | |
| Business professional | 716 (30.6) | 882 (54.6) | 882 (32.7) | 702 (60.3) | 611 (37.3) | 746 (47.3) | 265 (34.9) | 658 (53.7) | 1141 (43.8) | 528 (41.6) | 2275 (40.2) | 267 (52.6) |
| Manual labour | 691 (29.6) | 245 (15.2) | 714 (26.5) | 141 (12.1) | 358 (21.8) | 335 (21.2) | 165 (21.7) | 160 (13.1) | 497 (19.1) | 304 (23.9) | 1293 (22.8) | 74 (14.6) |
| Non-manual labour | 144 (6.2) | 69 (4.3) | 143 (5.3) | 52 (4.5) | 97 (5.9) | 67 (4.3) | 30 (4.0) | 69 (5.6) | 164 (6.3) | 40 (3.2) | 339 (6.0) | 14 (2.8) |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unemployed/retired/students | 787 (33.7) | 421 (26.0) | 957 (35.5) | 270 (23.2) | 573 (35.0) | 429 (27.2) | 300 (39.5) | 338 (27.6) | 801 (30.8) | 398 (31.3) | 1756 (31.0) | 153 (30.1) |
| Smoking status (%) | | | | | | | | | | | | |
| Never | 614 (26.2) | 578 (35.8) | 856 (31.7) | 375 (32.2) | 521 (31.8) | 482 (30.5) | 195 (25.7) | 414 (33.8) | 766 (29.4) | 396 (31.2) | 1754 (31.0) | 158 (31.0) |
| Ex | 159 (6.8) | 133 (8.2) | 210 (7.8) | 80 (6.9) | 130 (7.9) | 108 (6.8) | 54 (7.1) | 87 (7.1) | 192 (7.4) | 103 (8.1) | 386 (6.81 | 42 (8.2) |
| Current | 1567 (67.0) | 906 (56.0) | 1631 (60.5) | 710 (60.9) | 988 (60.3) | 989 (62.6) | 511 (67.4) | 724 (59.1) | 1646 (63.2) | 771 (60.7) | 3524 (62.2) | 310 (60.8) |
| Total MET-minutes of exercise/week (%) | | | | | | | | | | | | |
| < 600 | 492 (21.0) | 341 (21.1) | 518 (19.2) | 312 (26.8) | 410 (25.0) | 315 (20.0) | 187 (24.6) | 312 (25.5) | 684 (26.3) | 250 (19.7) | 1333 (23.5) | 98 (19.2) |
| ≥ 600 | 1848 (79.0) | 1276 (78.9) | 2179 (80.8) | 853 (73.2) | 1229 (75.0) | 1264 (80.0) | 573 (75.4) | 913 (74.5) | 1920 (73.7) | 1020 (80.3) | 4331 (76.5) | 412 (80.8) |
| Total Energy intake (kcal/day) | 2704.2 (838.9) | 3544.4 (1068.9) | 2764.9 (878.5) | 3593.4 (1073.3) | 2455.1 (714.1) | 3954.1 (1050.5) | 2751.0 (920.9) | 3524.7 (1050.7) | 2784.8 (854.4) | 3516.0 (1029.8) | 2908.2 (866.1) | 3725.5 (1140.6) |
| Location (%) | | | | | | | | | | | | |
| Urban | 894 (38.4) | 783 (48.5) | 1080 (40.2) | 599 (51.8) | 868 (53.2) | 586 (37.4) | 309 (41.0) | 650 (53.4) | 1388 (57.3) | 342 (31.7) | 2523 (44.7) | 218 (43.1) |
| Rural | 1432 (61.6) | 833 (51.5) | 1608 (59.8) | 557 (48.2) | 764 (46.8) | 982 (62.6) | 445 (59.0) | 568 (46.6) | 1035 (42.7) | 738 (68.3) | 3122 (55.3) | 287 (54.9) |

**Footnotes Table 3.4:**

Values are percentages or mean (standard deviation). Quintiles were constructed on the distribution of consumption of different food groups in BRAVE controls. When it was not possible to convert a food item into quintiles I treated non-consumers as a separate group and created quartiles between consumers. However, in case of white meat, red meat, SSB, egg, yoghurt, boiled white rice and biryani, I was unable to categorize the values in 5 groups due to low intakes. Vegetables include green leafy and stem vegetables, potatoes and other vegetables. Fruits include vitamin C rich fruits and other fruits. Snacks include deep fried and savoury snacks. Fish includes sweet water and sea water fish.

**Table 3.5:** Spearman's rank correlations between intakes of food and drink components in BRAVE controls.

| | Green leafy stem | Potatoes | Other vegetables | Vitamin C rich fruits | Other fruits | Pulses | Boiled white rice | Biryani | Bread | Deep fried snacks | Savoury snacks | Sweets | SSB | White meat | Red meat | Sweet water fish | Sea water fish | Egg | Milk |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Potatoes** | 0.03 | | | | | | | | | | | | | | | | | | |
| **Other vegetables** | 0.76* | 0.02 | | | | | | | | | | | | | | | | | |
| **Vitamin C rich fruits** | 0.15* | -0.07 | 0.27* | | | | | | | | | | | | | | | | |
| **Other fruits** | 0.26* | 0.04 | 0.38* | 0.55* | | | | | | | | | | | | | | | |
| **Pulses** | 0.18* | 0.03 | 0.23* | 0.14* | 0.19* | | | | | | | | | | | | | | |
| **Boiled white rice** | 0.04 | 0.11* | -0.07 | -0.19* | -0.09* | -0.04 | | | | | | | | | | | | | |
| **Biryani** | 0.02 | 0.02 | 0.08* | 0.19* | 0.18* | 0.14* | 0.00 | | | | | | | | | | | | |
| **Bread** | 0.01 | -0.04 | 0.09* | 0.21* | 0.15* | 0.18* | -0.38* | 0.21* | | | | | | | | | | | |
| **Deep fried snacks** | 0.01 | 0.10* | 0.01 | 0.08* | 0.07* | 0.10* | 0.08* | 0.22* | 0.18* | | | | | | | | | | |
| **Savoury snacks** | 0.04 | 0.11* | 0.08* | 0.30* | 0.27* | 0.15* | -0.04 | 0.28* | 0.21* | 0.40* | | | | | | | | | |
| **Sweets** | 0.04 | 0.09* | 0.10* | 0.22* | 0.25* | 0.15* | -0.07* | 0.20* | 0.19* | 0.26* | 0.31* | | | | | | | | |
| **SSB** | -0.02 | 0.09* | 0.05 | 0.27* | 0.22* | 0.14* | -0.07* | 0.33* | 0.23* | 0.30* | 0.35* | 0.29* | | | | | | | |
| **White meat** | 0.13* | 0.03 | 0.22* | 0.26* | 0.27* | 0.20* | -0.10* | 0.31* | 0.20* | 0.15* | 0.26* | 0.20* | 0.27* | | | | | | |
| **Red meat** | -0.04 | 0.08* | 0.00 | 0.16* | 0.16* | 0.14* | 0.04 | 0.42* | 0.14* | 0.20* | 0.30* | 0.19* | 0.33* | 0.29* | | | | | |
| **Sweet water fish** | 0.28* | 0.05 | 0.30* | 0.23* | 0.27* | 0.07* | 0.08* | 0.15* | 0.03 | 0.09* | 0.22* | 0.13* | 0.10* | 0.19* | 0.11* | | | | |
| **Sea water fish** | 0.17* | -0.02 | 0.22* | 0.22* | 0.23* | 0.16* | -0.10* | 0.18* | 0.15* | 0.06* | 0.21* | 0.13* | 0.15* | 0.23* | 0.12* | 0.27* | | | |
| **Egg** | 0.02 | 0.06* | 0.09* | 0.20* | 0.19* | 0.13* | -0.05 | 0.20* | 0.16* | 0.16* | 0.20* | 0.18* | 0.23* | 0.23* | 0.21* | 0.09* | 0.12* | | |
| **Milk** | 0.10* | 0.01 | 0.13* | 0.19* | 0.23* | 0.06* | -0.03 | 0.11* | 0.04 | -0.00 | 0.12* | 0.12* | 0.08* | 0.09* | 0.10* | 0.16* | 0.08* | 0.16* | |
| **Yoghurt** | -0.00 | 0.07* | 0.06* | 0.27* | 0.23* | 0.09* | -0.13* | 0.20* | 0.16* | 0.09* | 0.32* | 0.20* | 0.25* | 0.22* | 0.24* | 0.16* | 0.19* | 0.13* | 0.18* |

* indicates level of significance with Bonferroni correction

# Chapter 4: Food groups and risk of acute myocardial infarction in Bangladesh

## 4.1 Chapter summary

The main objective of this chapter is to investigate, reliably and in more detail than previously possible, the associations between different food groups and risk of acute myocardial infarction (AMI) in the BRAVE study in Bangladesh. Diet was assessed by food frequency questionnaire (FFQ). Foods were grouped based on nutrient content and culinary use and were converted into quintiles. Logistic regression with multivariate adjustments (age, sex, smoking status, history of diabetes, history of hypertension, family history of MI, physical activity, education, income, occupation and total energy intake) were used to assess the association of different food groups and risk of AMI.

The results from this analysis indicate that: (1) higher consumption of fruits, vegetables, yoghurt and certain spices (cumin, coriander and garlic) are associated significantly and independently with lower risk of AMI; (2) higher consumption of sweet water fish, sea water fish and biryani are associated with significantly higher risk of AMI; (3) consuming red meat and white meat have no significant association with the risk of AMI; (4) food group-AMI associations were generally unaltered by stratified analyses according to various individual characteristics such as age, sex, place of residence, smoking status, body mass index (BMI) and physical activity; (5) when stratified by age, the associations tend to be generally more extreme among younger compared to the older age groups for other fruits and stronger in older age groups for highest quintile of milk; (6) when stratified by smoking status stronger associations for biryani in current smokers and bread in ex-smokers are observed and (7) sensitivity analysis by excluding people with high and low energy reporters, did not change these results materially.

These findings, based on a first-ever detailed investigation of food groups with the risk of AMI in Bangladesh, highlight the importance of diet-coronary heart disease (CHD) research in relatively under-studied non-Western settings, where the diet and their clinical consequences might differ importantly from those observed in the Western populations.

## 4.2 BACKGROUND

As discussed in **Chapter 1**, South Asia has high burden of CHD, however the evidence on its dietary determinants is scarce. According to the Global Burden of Disease 2010 study, suboptimal diet was the leading cause of cardio-metabolic deaths in South Asia, with population-attributable fraction of 40.7% in Bangladesh, emphasizing the pivotal role of diet and nutrition in maintaining cardio-metabolic health in this region.[1] In addition, dietary habits of South Asia are different from western countries therefore the evidence on diet-CHD associations from the west may not be generalised to South Asian countries.[2]

Traditional approaches to investigate diet-disease associations from Western countries were mainly focused on the effect of nutrients on cardiovascular disease (CVD) with often conflicting results.[3] It is important to consider, however, that nutrients studied are derived from a variety of food groups and the food sources of these nutrients might be very different between countries leading to different conclusions. In addition, the various nutrients that constitute food groups might act synergistically or counterbalance each other's effect. Therefore, it is essential to study the association of the whole food matrix rather than looking at isolated nutrients.[3,4]

As summarised in **Chapter 1**, to date, there is limited evidence on how diet influences the risk of CHD in Bangladesh. The previous largest studies investigating food groups and risk of MI were the INTERHEART study that had about 3900 participants from South Asia and the recent Prospective Urban and Rural Epidemiology (PURE) study that had about 30,000 participants.[3,5] However, these studies did not report separate estimates from Bangladesh, as they were underpowered. There was one hospital based study that was the only study that reported estimates for the association of food groups with CHD from Bangladesh.[6] However, this study investigated only selected food groups and had only 100 participants. Therefore the aim of this chapter is to report the first detailed large-scale study analysing a wide range of food groups with the risk of AMI solely focusing on a Bangladeshi population, specifically addressing: (1) the association of different food groups with the risk of MI, adjusting for potential confounders; (2) the potential effect of mediators and (3) the potential effect modification by age, sex, geographical location, smoking status, body mass index (BMI) and physical activity.

The present analyses differ from previous studies on food groups and CHD in Bangladesh in several important ways. First, it is the largest and most detailed investigating of food groups and CHD filling research gaps about locally consumed foods such as spices and biryani. Second, this study adjusted for several potential confounders and adjusted for total energy intake based on food composition tables from Bangladesh. Finally, it

characterises the shape of associations with CHD for various food groups in an understudied population.

## 4.3 METHODS

### 4.3.1 Participants

Details of baseline population, data collection, and harmonization for the analyses in BRAVE study have been provided in **Chapter 2.** Briefly, this is a hospital-based case-control study set up in Dhaka, Bangladesh, that has recruited cases and controls "frequency matched" by age and sex (in 5-year age bands) from 2011-2016.

### 4.3.2 Dietary assessment

Details of the dietary assessment methods have been provided in **Chapter 2**. Briefly, dietary information was obtained using a 145 item semi-quantitative FFQ. Participants were asked to choose from a list of nine consumption frequencies ranging from no intake to six or more portions per day. Food items in the FFQ were collated into groups based on nutrient content and culinary usage as shown in **Table 2.4** in **Chapter 2**. Median intakes of food groups with interquartile range were presented within consumers for cases and controls in **Table 2.5, Chapter 2**. The FFQ used in the BRAVE study has been adapted from a FFQ validated in Bangladesh.[7] In the validation study the correlations of macronutrients and common micronutrients comparing FFQ and 7 day food diary were ranging from 0·30 to 0·76.[8] Food groups were converted into quintiles. When it was not possible to convert food items into quintiles the non-consumers were treated as a separate group and quartiles were created between consumers. However, white meat, red meat, egg, yoghurt, boiled white rice and biryani were unable to be categorised in the values in 5 groups due to low intakes. Few foods from the FFQ were excluded in the analysis due to extremely low reported consumption.

Baseline characteristics and potential confounders of the controls (as they may represent the general population) were described as mean and standard deviations or percentages for quintile 1 and 5 of food group consumption were presented in **Table 3.4, Chapter 3**. Total energy intake per day (kcal/d) was calculated by multiplying the frequency of consumption of each FFQ item by the energy content of the standard portion size, divided by hundred. As summarised in **Chapter 2**, the underlying food composition data are primarily based on the publications from Bangladesh.[9] Where not available, the Indian food composition table was consulted.[10] For mixed dishes, recipe calculations were made using local Bangladeshi websites as references[11,12] and McCance and Widdowson's Composition of Foods[13], where nutrient values were unavailable from the food composition tables.

### 4.3.3 Statistical analyses

The present analyses were confined to 7066 cases and 8079 controls that had complete information on age, sex, smoking status, history of diabetes and history of hypertension.

*Association of food groups with acute myocardial infraction*

Multivariate logistic regression models were used to assess the association between different food groups and risk of AMI. The first quintile was used as reference group, and estimates were presented in odds ratios (ORs) and 95% confidence intervals (CI). For analyses involving more than two exposure categories, the floating absolute risk (FAR) method was used.[14] This method describes the uncertainty in risk without reference to another level, making the risk estimates more independent of the baseline reference category.

Progressive adjustments of the exposure-outcome associations were conducted to gain insight into the confounding effects of different factors in the BRAVE study.

Seven models were applied:

1) Model 1- The minimally adjusted model was adjusted for age and sex.

2) Model 2- additionally adjusted for smoking status (never, ex and current), physical activity (using cut offs of <600 and ≥ 600 Metabolic Equivalent of Task (METs)), annual income (income below the poverty line, low income, medium income and high income), education level (no schooling, primary, secondary, university/vocational) and occupation (business/professional, manual labour, non-manual labour and unemployed/student/retired).

3) Model 3- additionally adjusted for history of disease related variables: history of diabetes (yes or no), history of hypertension (yes or no) and family history of MI (yes or no)

4) Model 4 -additionally adjusted for total energy intake (kcal/day). Total energy intake (affected by physical activity, metabolic efficiency and bod size) is a potential confounder as it is associated with CHD risk and dietary intake. Thus, adjusting for total energy intake is important as failure to adjust for it may distort associations.

5) Model 5- Model 3+ additionally adjusted for intakes of all food groups (in quintiles) mentioned in **Table 2.4 in Chapter 2**.

6) Model 6- Model 4 + additionally adjusted for medications (anti hypertensives and anti-diabetics)

The “main” associations compared the age and sex model (Model 1) with Model 4.

*Mediators*

Additional sensitivity analyses were done to adjust for potential mediators:

7) Model 7- Model 4 + waist-to-hip ratio, LDL-C and HDL-C.

*Subgroup and sensitivity analyses*

To investigate if the associations were modified by age (<50 years or ≥50 years- as mean age was 50 years), sex (males, females), geographical location (urban/rural), smoking status (ex, never, current), BMI (<23 kg/$m^2$ or ≥23 kg/$m^2$- using WHO cut offs for Asians) and physical activity (<600 METs ≥ 600 METs), interaction terms were included between quintiles of food groups (ordered) and each of the indicated stratifying variables. These subgroups have been reported in previous literature.[15] Wald test was performed to get the p value for the interaction term across the quintiles of food consumption and each of the indicated variables. If p value was<0.05, this meant that the interaction between the categories of indicated variable was significant across quintiles of food groups.

To evaluate robustness of the findings, sensitivity analysis for Model 4 was done by excluding people with high and low energy intakes (<400 and >5000 kcal/day). These cut off have been taken from the previous largest study from South Asia, the PURE study.[5] This was used as a reference as no standard energy cut offs for a South Asian population are available to my knowledge.

In order to account for multiple testing Bonferroni correction was done as a sensitivity analysis for the main analyses. The results of which are presented in the footnotes of **Tables 4.1-4.4**.

P values <0.05 were considered to be significant. All statistical analyses were conducted on STATA 14.

## 4.4 RESULTS

### *4.4.1 Association of plant-based foods with risk of acute myocardial infarction*

In the age and sex adjusted Model 1, as compared to the lowest quintile, ORs for the highest quintile of different types of vegetables and fruits were inversely associated with the risk of MI. ORs were 0.64 (95 % CI 0.60-0.69) for green leafy and stem vegetables, 0.46 (95 % CI 0.41-0.52) for potatoes, 0.60 (95 % CI 0.56-0.65) for other vegetables, 0.58 (95 % CI 0.53-0.62) for vitamin C rich fruits and 0.41 (95 % CI 0.38-0.44) for other fruits (p trend <0.001 for all) **(Figure 4.1, Table 4.1)**. Following multivariable adjustment in the Model 4, ORs for highest quintiles of different types of vegetables and fruits were slightly attenuated, but remained inversely associated with the risk of MI. The ORs were 0.72 (95 % CI 0.67-0.78) for green leafy and stem vegetables, 0.66 (95 % CI 0.59-0.75) for potatoes, 0.69 (95 % CI 0.63-0.75) for other vegetables, 0.69 (95 % CI 0.63-0.76) for vitamin C fruits and 0.51 (95 % CI 0.46-0.56) for other fruits (p trend <0.001 for all) **(Figure 4.1, Table 4.1)**.

Supplementary analyses to mutually adjust for the intakes of all food groups in Model 5 and adjustment for potential mediators in Model 7 did not substantially alter the observed associations **(Table 4.1)**.

Overall, there was no difference in the overall direction or non-significance of the associations observed with different levels of adjustment for potential confounders and mediators **(Table 4.1)**.

*Subgroup and sensitivity analyses*

Similar associations were seen when plant-based foods were stratified by sex, location, smoking status, BMI and physical activity (p values >0.05) **(Figures 4.7-11)**. Moreover, the associations did not differ according to age groups for most plant-based foods. However, a stronger inverse association between other fruits and AMI was observed in younger age groups (≤50 years old) compared to older age groups (>50 years old): OR of 0.41 (95% CI 0.34-0.50) versus 0.58 (95% CI 0.49-0.68) when comparing highest vs lowest quintile (p trend 0.02) **(Figure 4.6)**. A similar strong association was also observed in younger age groups who consumed higher intakes of green leafy and stem vegetables, however the test for trend was non-significant.

Results from sensitivity analyses by excluding people with high and low energy intakes were consistent with the main results in Model 4 **(Figure 4.24)**.

### 4.4.2 Association of animal-based food groups with risk of acute myocardial infarction

In the age and sex adjusted Model 1 compared to the lowest quintile the ORs for the highest quintile of different animal-based food groups were 0.83 (95 % CI 0.77-0.89) for white meat, 0.95 (95 % CI 0.88-1.04) for red meat, 1.00 (0.94-1.08) for sweet water fish and 1.24 (95 % CI 1.15-1.33) for sea water fish. Whereas, ORs for highest versus lowest quintile of egg consumption were 0.96 (95 % CI 0.88-1.04), milk were 0.87 (95 % CI 0.80-0.95) and yoghurt were 0.63 (95 % CI 0.55-0.73) **(Figure 4.2, Tables 4.2-3)**.

After adjustment for several potential confounders in Model 4, white meat and red meat had no significant association with the risk of AMI ($p>0.05$). On the contrary, OR of 1.52 (95 % CI 1.39-1.66) were observed for sweet water fish and 1.39 (95 % CI 1.28-1.51) for sea water fish when comparing highest versus lowest quintile (p trend <0.001 for both). For both eggs (1.14 (95 % CI 1.05-1.25; p trend <0.001)) and milk (1.16 (95 % CI 1.06-1.27; p trend 0.14)) higher odds of AMI were observed only in the highest quintile versus the lowest group. The middle quintiles of egg consumption had a lower risk with MI. Only for yoghurt a lower odds of AMI was observed when consumption was higher (0.70 (95 % CI 0.60-0.82; p trend <0.001)) **(Figure 4.2, Tables 4.2-3)**.

Supplementary analyses to mutually adjust for the intakes of all food groups in Model 5 and adjustment for potential mediators in Model 7 did not materially alter the observed associations **(Tables 4.2-3)**. However, only for red meat a weak inverse association was observed after adjustment for Model 7 (p trend 0.02).

*Subgroup and sensitivity analysis*

When stratified by age, an increased association between highest intake of milk and AMI was observed in older age groups compared to younger age groups: OR of 1.37 (1.19-1.56) versus 0.87 (0.72-1.04) (p trend 0.001). For white meat, an inverse association was only observed in younger age groups, however the test for trend was non-significant **(Figure 4.12)**. Those in the highest intake of sea water fish and milk there seemed to be an increased association in rural areas but no association in urban areas, however test for interaction was non-significant **(Figure 4.14)**. Those who had higher BMI compared to lower BMI had no association with higher intakes of sea water fish but the test for trend was non-significant between the two categories of BMI **(Figure 4.16)**.

Except for the above mentioned, similar associations were seen when animal-based foods were stratified by age, sex, location, smoking status, BMI and physical activity (p values >0.05) **(Figures 4.12-17)**.

Results from sensitivity analyses by excluding people with high and low energy intakes were consistent with the main results in Model P **(Figure 4.24)**.

### 4.4.3 Association of other food groups with risk of acute myocardial infarction

In the age and sex adjusted Model 1, ORs for the highest versus lowest quintile of pulses 0.93 (95 % CI 0.86-1.00), boiled white rice 0.70 (95 % CI 0.64-0.76), biryani 1.14 (95 % CI 1.04-1.24) and for bread were 1.18 (95 % CI 1.10-1.27) **(Figure 4.3, Table 4.4)**. ORs for the highest versus lowest quintile of other food groups, after adjusting for the Model 4 for pulses were 1.02 (95 % CI 0.94-1.11; p-trend <0.001), boiled white rice 1.00 (95 % CI 0.91-1.09; P for tend 0.02), biryani 1.25 (95 % CI 1.13-1.39; p tend <0.01) and for bread were 0.98 (95 % CI 0.90-1.06; P for tend 0.04) **(Figure 4.3, Table 4.4)**.

In the additional analyses to mutually adjust for the intakes of all food groups in Model 5 and adjustment for potential mediators in Model 7 did not materially alter the observed associations when comparing highest versus lowest quintile of other food groups **(Table 4.4)**. However, only for boiled white rice after adjustment for food groups, highest versus lowest quintile of boiled was inversely associated with the risk of AMI (p trend <0.001).

*Subgroup and sensitivity analysis*

The associations did not differ according to age groups for all other food groups. However, only in the fourth quintile a stronger significant inverse association between pulses and AMI was observed in older age groups (>50 years old) compared to younger age groups (≤50 years old): OR of 0.74 (95% CI 0.63-0.88) versus 0.94 (95% CI 0.76-1.17) when comparing highest vs lowest quintiles (p trend 0.03) **(Figure 4.18)**. As for biryani there appeared to be an increased association only in older age groups, however the test for trend was non-significant (p trend 0.24). When stratified by sex, for quintile 3 of pulses intake a stronger inverse association was observed in females than in males (p trend 0.009) **(Figure 4.19)**. When stratified by smoking status had an increased association between highest intake of biryani and risk of AMI was observed only in current smokers (OR 1.42 95% CI 1.23-1.64) (p trend 0.01) **(Figure 4.21)**. For highest intake of bread intake, when stratified by smoking status an inverse association was observed only in ex-smokers (p trend 0.009).

Overall, other than the above mentioned, the analyses did not detect statistically significant interactions of other food groups with age, sex, location, smoking status, BMI and physical activity in relation to risk of AMI **(Figures 4.18-23)**.

In addition, results from sensitivity analyses by excluding people with high and low energy intakes were consistent with the main results in Model 4 **(Figure 4.24)**.

### 4.4.4 Association of spices/herbs with risk of acute myocardial infarction

ORs for the intake of ≥ 1 times/day vs. <1 time/day for cumin seeds were 0.71 (95 % CI 0.65-0.78), garlic were 0.74 (95 % CI 0.63-0.86), coriander powder were 0.78 (95 % CI 0.72-0.85), turmeric were 0.88 (95 % CI 0.69-1.12), ginger were 0.91 (95 % CI 0.82-1.00) and mixed spices were 0.98 (95 % CI 0.89-1.12) after adjusting for Model 4 **(Figure 4.4)**.

Further supplementary analyses to progressively adjust for potential confounders and mediators did not materially change the main findings **(Figure 4.5)**.

Results from sensitivity analyses by excluding people with high and low energy intakes were in line with the main results in Model 4 **(Figure 4.24)**.

## 4.5 DISCUSSION

### 4.5.1 Summary of main findings

In this large case-control study from Bangladesh, higher consumption of fruits, vegetables, yoghurt and certain spices (cumin, coriander and garlic) were associated with significantly lower risk of first AMI after multivariable adjustments (age, sex, smoking status, history of diabetes, history of hypertension, family history of MI, physical activity, education, income, occupation and total energy intake). Pulses and eggs consumption had an inverse association with the risk of AMI only in the middle quintiles. On the other hand, higher consumption of biryani, sweet water and sea water fish were associated with significantly higher risk of developing first MI. For white meat, red meat, bread and milk there was no significant association across quintiles when adjusted for the model 4. Overall, most of these associations were not affected by subgroup analyses by various baseline characteristics. However, higher intake of other fruits had a stronger inverse association in younger groups (<50 years). Whereas higher intakes of pulses appeared to be slightly stronger association among older age groups (≥50 years). For pulses intake a stronger inverse association was observed in middle quintile in females as compared to males. As for higher intakes of milk it had an increased association in older age groups but showed no association in younger age groups in relation to risk of MI. For biryani and bread consumption, when stratified by smoking status, an increased association was only observed in current smokers and an inverse association was observed only in ex-smokers respectively. Sensitivity analysis by excluding people with high and low energy intakes were consistent with the main results.

### 4.5.2 Plant-based food groups and acute myocardial infarction risk

The current analyses reported that plant-based foods (including different vegetables and fruits) had a strong inverse association with the risk of AMI after multivariate adjustments. For other fruits the associations appeared to be stronger in younger groups as compared to the older age group. The effect modification by age may be explained by differences in baseline risk between these two age groups. Comorbidities in old age group may be more than the younger age group people that may also explain the difference in risk. Younger people have generally healthier lifestyle behaviours as compared to older people. Another possibility is that pathophysiological changes may affect nutrient absorption and subsequent nutritional status in older population. For example, vitamin B12 deficiency is highly prevalent among adults 65 years or older because of poor diet and diminished

absorption associated with age.[16] Several studies from the west have reported inverse associations with varying magnitudes with the consumption of fruits and vegetables with CHD risk, however, there is limited evidence on how fruits and vegetables consumed affects AMI risk in low- and middle-income countries (LMICs) like Bangladesh. A recent meta-analysis of 95 cohort studies reported a 21% (OR 0.79, 95% CI 0.70-0.90) reduction in relative risk of CHD for up to 750–800 g/day for fruits and a 30% reduction (OR 0.70, 95% CI 0.65-0.74) in the relative risk up to 550–600 g/day for vegetables.[17] In addition, a prospective cohort study from China reported a 34% (OR 0.66, 95% CI 0.58-0.75) lower risk of incident coronary events with those who consumed fresh fruit daily as compared to those who never or rarely consumed.[15] The BRAVE study reported similar strong magnitudes of inverse association with different types of fruits and vegetables and risk of MI. It is important to note, however that these studies are not directly comparable as different types of fruits and vegetables are consumed in different parts of the world. In Bangladesh mostly fresh fruits are consumed, whereas vegetables and potatoes are consumed in the form of curries. The findings of the current study are consistent with the INTERHEART South Asian study that reported that consumption of >1 portion of fruits and vegetables a day decreases the risk of CHD by 35% (OR 0.65, 95% CI 0.53-0.81).[18] However, the INTERHEART study did not investigate the different types of fruits and vegetables and had relatively fewer number of participants from Bangladesh as compared to the BRAVE study.

The present analyses also reported strong inverse associations of higher potato consumption with AMI. There is scarce evidence on how potatoes effect the risk of CHD. However studies from west have reported that high consumption of potatoes consumed as french fries (chips) may be linked to obesity, Type II diabetes and total mortality.[19,20] In Bangladesh, however boiled potatoes are consumed in the form of curries and are often supplemented with other vegetables. It is important to note that although potatoes are high in starch, they also have high content of phytochemicals, potassium, vitamin C, folate and dietary fibre which may prevent atherosclerosis and explain the strong inverse associations.[21]

*Potential mechanisms*

There are several mechanisms that explain the beneficial effect of fruits and vegetables on CHD. Fruits and vegetables are rich in vitamins, anti-oxidants, flavonoids, potassium, dietary fibre, which may affect many biological pathways such as protecting endothelial function, regulating lipid metabolism, inhibiting platelets function, alleviating ischemia/reperfusion injury, suppressing thrombosis, reducing oxidative stress, and

attenuating inflammation to lower the risk of developing CHD.[17,22] There is evidence to suggest that the potential mechanisms by which plant based foods lower blood pressure is by improving the activity of protein kinase, increasing NOS expression and inhibiting $Ca^{2+}$ influx and $K^{+}$ induced contractions.[23] Furthermore, fruits and vegetables regulate lipid metabolism by lowering triglycerides and atherosclerotic plaque formation; act as antioxidants by scavenging free radicals (NO, superoxide etc.); improve endothelial function by decreasing artery intima-media thickness and act as anti-inflammatory agents by reducing TNF-α induced leukocytes.[22,23]

Evidence from various randomised clinical trials (RCTs) have also shown reduced risk of intermediate indicators (high blood pressure, carbohydrate metabolism, oxidised low density lipoprotein and plasma C-reactive protein) with fruits and vegetable consumption.[24–26] However, adjustment of low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and waist-to-hip ratio in the present study made little difference to the observed associations suggesting that there may be other biological mechanisms. Dietary fibre in fruits and vegetables have been shown to reduce cholesterol levels, blood pressure, inflammation and platelet aggregation, and improve vascular and immune function.[17,27,28] Furthermore, a recent meta-analysis has also shown inverse associations between fibre intake and CVD.[29] In addition, antioxidants in fruits and vegetables may neutralize reactive oxygen species and reduce DNA damage.[28] Moreover, fruit and vegetable intake has positive effects on reducing obesity and weight gain.[30,31] Although results are not entirely consistent; however, the associations observed in the present analyses appear to be independent of BMI. A high fruit and vegetable intake may also reduce chronic disease risk indirectly, by displacement of potential unhealthy foods high in saturated fat, trans fat, glycaemic load and sodium; however, most of the associations persisted in additional analyses when food groups were mutually adjusted.

### 4.5.3 *Animal-based food groups and acute myocardial infarction risk*

*White meat and red meat*

In model 4, the findings from this study reported that consumption of red meat had no significant association with the risk of AMI. The evidence of red meat with CHD risk has shown conflicting results from the west. The Health Professional's follow up study and the Nurses' Health study reported a higher risk of CVD mortality with higher red meat consumption.[32] Evidence from such studies led to the dietary recommendation of lowering the consumption of red meat and preferring lean meats.[33] In addition, a dose response meta-analysis reported that each additional daily 100 g of red meat had increased association with risk of CHD (Relative risk was 1.15; 95% CI 1.08-1.23, $I^2$ = 0%).[34]

Furthermore, it has been postulated that the unfavourable association of red meat with CHD may be driven by the saturated fatty acids (SFAs), cholesterol, high iron content and pro-inflammatory products that may affect the intermediate factors associated with CHD.[32,35,36]

Although there is evidence to suggest that red meat is associated with CHD risk, however, some studies from the west have challenged this, as association may vary depending on different types (processed, unprocessed) and sources of meat (beef, lamb, ham, pork) consumed.[37] For example, in the EPIC cohort, showed an increased association with all-cause and CVD mortality was found only for processed meat consumption and not for red meat or white meat.[38] Furthermore, a meta-analysis concluded that only processed red meat intake, not total red meat intake, was associated with 42% greater risk of CHD (RR per 50 g serving/ day = 1.42, 95% CI 1.07-1.89).[39] Processed meat contains 400% more sodium and 50% more nitrates than unprocessed meat, which may explain the strong association with CHD.[40] Inconsistent with much of the evidence related to red meat consumption and risk of CVD in observational studies, a recent meta-analysis of 24 RCTs concluded that total red meat intake of about 3.5 servings/week did not affect blood lipids, lipoprotein profiles or blood pressure which is.[41] The unexpected null association with red meat consumption and risk of AMI in the BRAVE study may also be explained because the meat consumed in this population is mostly unprocessed beef and lamb. Pork is not consumed due to religious reasons in Bangladesh. In addition, the extremely low range of intake (median intake is 6 g/day) in this study as compared to western studies, may not be high enough to do be detrimental for AMI risk.

A weak inverse association with white meat and AMI was observed in the minimally adjusted model however these associations became non-significant in the fully adjusted model, suggesting the role of confounding. There is scarce evidence on how white meat affects the risk of MI. The INTERHEART study reported that total meat (white and red meat) had no significant association with the risk of MI in the fully adjusted model which is consistent to what is observed in the present study.[3] In addition, a pooled analysis of cohort studies of 96,721 individuals from Asian countries (i.e., Bangladesh, mainland China, Japan, Korea, and Taiwan) also reported no association between red meat and white meat consumption with CVD, congruous to the findings of this study.[42]

*Sea water and sweet water fish*

This study showed a 52% (OR 1.52, 95 % CI 1.39-1.66) and 39% (OR 1.39, 95% CI 1.28-1.51) higher risk of AMI with higher consumption of sweet water and sea water fish respectively, after adjusting for the model 4. Although most studies have reported an inverse association with fish consumption and risk of CVD,[43–45] there are few studies that

have reported harmful effects. Fish are rich in long chained omega-3 fatty acids and may reduce the risk of CHD by reducing blood viscosity, insulin resistance, platelet aggregation, inflammation and by improving blood vessel function.[46] Though the protective effect of fish with CHD risk have plausible biological basis, there is still inconsistent evidence on how fish consumption affects the risk of CHD.

*Potential mechanisms*

The somewhat surprising link with the fish and CHD in this study may have little to do with fish itself, and could potentially explained by common local practices of adding potentially harmful toxic preservatives in fish (such as formalin),[47] addition of salt and a general "unhealthy cooking pattern" such a prolonged frying which can greatly alter a fish meal's nutrient composition e.g., increasing the n-6: n-3 ratio or energy density[48] and formation of advanced glycation end products (AGEs).[49] In addition, trans-fats in oils used for repeated frying may all increase the risk of CVD.[50] An evidence from a large cohort study from USA showed that consuming fried fish more than twice weekly increased the risk of CVD by 63% (HR 1.63, 95% CI 1.11-2.40), whereas non-fried fish was not associated with the risk of CVD, indicating associations may vary by preparation method.[51] Furthermore, unpublished results from the PURE study indicate that fish intake was inversely associated with CVD outcomes in South America, China, North America, and Europe (Risk Ratio (RR): 0.76–0.84) but had increased associated in South Asia (RR: 1.97).[52] Future studies need be conducted to collect more detailed information on the preparation methods to elucidate the associations observed with fish and AMI risk in South Asian countries including Bangladesh.

Bangladesh is a riverine country and fish constitute a large part of daily meal.[53] Over the years the rapid industrialization, unplanned urban expansion and population growth have contributed to massive amounts of domestic wastewater and untreated industrial effluents into rivers and coastal areas, becoming a major public health concern.[54] These contaminants can accumulate in aquatic ecosystems, can be readily taken up by aquatic organisms and have the potential to enter the food chains.[55] A study conducted in the coastal areas of Bangladesh showed that there were remarkably high concentrations of arsenic, copper and zinc in fish.[56] Moreover, when stratified by location in the present analysis, for highest quintile of sea water fish had an increased association was only observed in rural areas. It may be postulated that water bodies in rural areas are more contaminated with toxic metals and this may be deriving the association. A recent meta-analysis reported that exposure to arsenic even at low levels is an independent risk factor for CHD.[57] Clinical and experimental studies of arsenic exposure have reported the production of reactive oxygen species in endothelial cells, up regulation of inflammatory

signals, and higher blood pressure.[57] Therefore, further studies are required to investigate if the consumption of fish in Dhaka is contaminated with toxic metals such as arsenic.

*Dairy products*

The present study observed a higher risk of AMI only in the highest quintile of milk consumption (OR 1.12 95% CI 1.06-1.27), however the trend across quintiles was non-significant (p value 0.14). When stratified by age, older age group had increased association with the risk of AMI. This effect modification may be explained by the differences in baseline risk, with older people generally having an unhealthy lifestyle as compared to the younger group. On the contrary, a strong inverse association (OR 0.70 95% CI 0.60-0.82) with yoghurt consumption and risk of AMI was observed.

*Previous literature and potential mechanisms*

On one hand, dairy products provide essentials nutrients in varying quantitates such as calcium (for healthy bones and teeth), phosphorous (healthy bones), protein (for growth and repair), riboflavin (for healthy skin), zinc (for immune function), and potassium (for maintenance of health blood pressure).[58] Although traditional evidence suggested that dairy products rich in SFAs may increase the risk of CHD.[59] Resultantly this led to dietary recommendations to consume low fat dairy products and the increased availability of low fat dairy products in the markets. However, recent evidence has concluded that more than the quantity of fats it is the quality of fats that matter, and odd chained SFAs (15:0 and 17:0) present in dairy foods are cardio-protective.[60]

In a multi-ethnic cohort of 2837 US adults that investigated the association of biomarkers of dairy fat with CHD, it was found that plasma phospholipid 15:0 had an inverse association with CVD and its risk factors.[61] Similarly, it has also been reported by a meta-analysis that odd chained fatty acids related to dairy are inversely associated with the risk of CHD.[43] It is important to consider that dairy food products also contain other nutrients such as calcium, protein, vitamins and minerals that may have different physiological effects on the aetiology of CHD.[62] There are studies that have suggested that SFA such as lauric acid in dairy products can have anti-inflammatory effects.[63]

Furthermore, evidence from recent reviews and meta-analyses have concluded that there is inconsistent evidence to prove that dairy products including milk and yoghurt, whole fat or fat free increase the risk of CHD,[64–66] questioning the dietary guidelines that recommend intake of low fat dairy products. The no association of milk (had higher association only in the highest quintile) observed with CHD in this study may be explained by local practices of adulteration. A study conducted in rural areas in Bangladesh concluded that raw milk

had presence of preservatives such as formalin and sodium bicarbonate, which may dilute the potential beneficial effect of milk with CHD.[67]

The findings of a strong inverse association with yoghurt in this study are in contrast to a meta-analysis, which concluded that there was no significant association between yoghurt consumption and CHD.[68] However, no direct comparison could be made because of the different nature of the studies and also different types of yoghurt consumed in different countries. However, a study with data from Nurses' Health Study and the Health Professionals Follow-up Study concluded that hypertensive men and women who consumed ≥2 servings/week of yogurt, were at lower risk for developing CVD. It was postulated that the inverse association is driven by fermented dairy products that may lower CVD risk through effects on vascular stiffness as well as through direct effects on blood pressure.[69] During fermentation bioactive peptides, exopolysaccharides, and conjugated linoleic acid (CLA) are some of the beneficial compounds that are released.[70] Research has also shown that fermentation process increase the bioactivity of platelets activating factor (PAF) inhibitors. PAF are pro-inflammatory mediator that play a role in initiation and progression of atherosclerosis.[63] Additionally, fermentation has previously shown to lower LDL-C and total cholesterol in a trial and decrease carotid artery intima-media thickness in a study of elderly women.[71] However, further research is required to elucidate the correct mechanism.

*Eggs*

The study showed a higher risk of AMI in the highest quintile of egg consumption (having >1 egg a day) (OR 1.14 95% CI 1.05-1.25) but significantly lower risk of AMI in lower quintiles. The epidemiologic evidence relating egg-consumption to CHD is not entirely consistent. A medium egg contains ~225 mg cholesterol.[13] Although elevated serum cholesterol levels are considered to be a risk factor for CVD, however there are inconsistent results from studies on cholesterol and CVD.[72,73] A meta-analysis reported that consuming 1 egg/day increased plasma LDL cholesterol by 4.1 mg/dL, increased HDL cholesterol by 0.9 mg/dL, and had little effect on the overall ratio of LDL to HDL cholesterol which is a major risk factor of CHD.[74] The effect of egg on CHD cannot only be explained by cholesterol as egg also contains other nutrients, which may be cardio-protective. Egg is a good source of B vitamins, including choline, which may lower homocysteine concentrations.[75] Thus, the overall effect of egg consumption may reflect the interactions of cholesterol with other nutrients in egg. Hence, the Dietary Guidelines for Americans 2015 removed the prior recommendation to limit consumption of dietary cholesterol to 300 mg per day.[76] A meta-analysis of 9 prospective studies concluded that as compared to those who never consumed egg or ate egg less than once per week, individuals who ate egg once per day or more did not have significantly higher risks of CHD,[77] which negates

the apprehensions about the high cholesterol content of eggs that may be detrimental to CHD. It is important to consider that egg consumption may be replaced with fish and meat, therefore it is important to investigate the association of dietary patterns. Nevertheless, the range of egg consumption in this population are low to make valid conclusions due to less contrast. In addition, eggs might be hidden in other foods such as cakes, curries etc.

### 4.5.4 Association of other food groups and acute myocardial infarction risk

*Pulses*

The current analyses reported no significant association with higher intakes of pulses in the age and sex adjusted model. However, an inverse association was observed only in the middle quintiles after adjustment for additional covariates in model 4. When stratified by sex, a stronger inverse association was observed in the middle quintile in females as compared to men. The finding may be due to chance as the confidence interval of the association of females is large due to less power.

*Previous literature and potential mechanisms*

Pulses are rich sources of folate, fibre, potassium and magnesium and there is numerous evidence that these nutrients have been associated with lower risk CVD,[78] which explains the result in the middle quintiles (1-2 portions a day). In addition legume consumption has been shown to reduce blood pressure, total cholesterol and LDL-C, and triglycerides.[5] These results are in line with the recommendations to consume one serving of pulses a day.[79] In addition, evidence from a recent meta-analysis also concluded a non-linear dose-response association with legume intake; risk of CHD was lowered by approximately 10% with increasing intake of legumes up to 100 g/day.[34] However, no benefit for increasing intake was apparent above this value. However, this meta-analysis included ten studies which were mostly from western countries that may not be comparable to Bangladesh where the consumption of pulses is higher (median intake 77 g/day). In contrast, the PURE study reported an inverse association of legume intake (which included pulses) only with total mortality but not with the risk of MI and CVD in the fully adjusted model.[5] Although this study had few participants from Bangladesh (about 2700), the median intake of legume was 60 g/day which is comparable to the BRAVE study. In South Asia pulses contribute an important part of the diet and are often eaten as an alternative to meat (mainly fish). Nevertheless, mutually adjusting for all food groups did not substantially alter the observed associations. It was also observed that the inverse association of pulses with AMI in the middle quintiles was stronger in older age groups and in females. However,

these findings can be due to chance. Overall, the findings from this suggest that consumption of 1-2 portions of pulses per day may lower risk of AMI.

*Sources of carbohydrates: boiled white rice, biryani and bread*

In the model 4 highest consumption of boiled white rice had no significant association with AMI risk, but this risk became inversely associated when adjusted for all food groups. As reported in **Chapters 1 and 3** the consumption of rice is higher in Bangladesh as compared to western countries. Boiled white rice is a staple food in Bangladesh; a major source of their calorie intake and is often consumed with curries (vegetable and/or meat) and pulses. These results are in line with a large prospective study of about 84,000 participants from Japan, a population where boiled rice is consumed in higher amounts compared to Western populations.[80] In addition, rice provides essential nutrients vitamin B-6, folate, fibre, magnesium and may substitute unhealthy foods.[81] Furthermore, results from pooled analysis of 3 cohort studies from USA suggested that habitual consumption of white rice (≥5 servings/ week versus <1 serving/week) was not associated with the risk of CVD.[82] The pooled analysis also reported higher consumption of rice in Asians as compared to whites. Stratified analyses by ethnicity in this study also yielded null results between rice consumption and CVD risk. However, this study consisted largely of health professionals limiting the generalizability of the findings.

It is postulated that rice grown in contaminated waters is known to add to the arsenic burden in Bangladesh. A study based in rural areas of Bangladesh investigated the association of boiled white rice with urinary arsenic concentration and skin lesions and suggested that rice intake may be a major source of arsenic exposure.[83] However, the contamination depends on the geographical location, cultivation methods, arsenic concentration in the water used for irrigation and also method of cooking. Moreover, it is also very difficult to separate arsenic exposure coming from drinking water and other dietary factors. In addition, in contrast to the evidence on drinking arsenic contaminated water there is sparse evidence on how arsenic rice contaminated affects CHD. Further investigation is needed to evaluate the individual level arsenic content of rice in Dhaka which may be different than the rural areas of Bangladesh.

Associations, however, differed for highest intake of biryani (mixed rice dish with meat made in oil), with a significant association with AMI in the age and sex adjusted and model 4. Moreover, when stratified by age, a stronger increased association was observed in older age groups and when stratified by smoking status a higher association was observed only in current smokers. The stronger increased association in older age groups could be due to the differences in baseline health between old and young age groups as explained

before. The increased association of biryani specific to current smokers may be attributed to current smokers having higher risk of CHD. Ex-smokers have wide confidence interval due to power issue. Therefore, interpretation is going to be challenge. The effect modification potentially has a synergistic effect of biryani on top of the smoking behaviour which is also potentially detrimental. The overall increased association of biryani with AMI may be explained by the high salt content, addition of meat/fish and oil used in preparation of biryani. As for bread there was no association with risk of AMI in model 4. However, when stratified by smoking status, an inverse association was observed only in ex-smokers. However, as the numbers of people consuming bread are low, so these findings can be due to chance. There is scarce evidence on how bread affects the risk of MI. The INTERHEART study reported that grains (including chapatti, brown rice and pasta) were not associated with the risk of AMI in the fully adjusted model which is consisted with the findings of this study.[3]

### 4.5.5 Spices and acute myocardial infarction risk

Spices are an integral part of South Asian dishes and curries. In this study a lower risk of AMI was observed with those who consumed >1 times a day of cumin seeds, coriander and garlic as compared to those who consumed <1 times a day. Previous evidence from meta-analyses and reviews has suggested that these spices lower cholesterol, triglyceride, LDL-C and increase HDL-C levels.[84–86] In addition there is evidence that spices have bioactive agents that have shown beneficial effects on obesity, CVD and various cancers.[87] It is postulated that flavonoids and polyphenols in coriander are responsible for the hypoglycaemic and hypolipidemic effects.[88] Garlic contains allicin as the main active ingredient which has potential beneficial effects on CVD system. A study showed that allicin caused enhancement of antioxidant state by lowering of reactive oxygen species and increasing the production of glutathione.[89] Moreover, the dishes that the spices go into (vegetables, meat, rice, fish) are apart from the fish were inversely associated with AMI in this study. Evidence on how spices affect CHD risk is scarce. One large prospective study reported that habitual consumption of spicy foods was inversely associated with the risk of total mortality and mortality from CHD in China.[87]

### 4.5.6 Strengths and limitations

Strengths and limitations of this study merit consideration. This is the first large-scale study with detailed analysis of broad range of food groups including local foods with the risk of AMI in an indigenous South Asian population using case-control design. The analysis included about 14,000 cases and controls from Bangladesh which is greater than previously published studies. The study used a validated country specific FFQ to estimate intake of different types and subtypes of food groups, derived total energy intake using food composition table from Bangladesh, used standardised methods to collect information on AMI risk; and collected data on a large number of covariates which were adjusted for in the models. Alcohol consumption was not adjusted as a confounder as the majority of the participants were Muslims who do not drink alcohol due to religious reasons. Furthermore, the robustness of the findings was verified by a variety subgroup analyses showing results for the majority of the analyses. The study had about 50% people from urban areas and 50% from rural areas giving good representation of different areas in Bangladesh.

Nevertheless, the BRAVE study is observational in nature so from these results causality cannot be inferred. BRAVE is a hospital-based case-control study therefore is potentially open to selection bias, which may under-estimate or over-estimate the observed associations. However, as the BRAVE study hospital is the largest referral hospital in Bangladesh, it has good representation of AMI cases from all over the country. In addition, as data on diet was collected after cases experienced their first MI there may be possibility of reverse causation as they may change their diets and lifestyle due to pre-existing medical history (diabetes, hypertension). Although it is very challenging to remove such type of biases in a case-control study, the researchers of the study tried to minimize the biases in selection of controls by excluding individuals that had chronic conditions (such as malignancy, any infection, leprosy, inflammatory disorders, hepatitis or chronic kidney disease, recent history of surgery or were pregnant) and by recruiting cases with no history of CHD, which may have affected the hypotheses studied in BRAVE. Although the present analysis did adjust for potential confounders the possibility of residual confounding from unmeasured covariates be ruled out.

Residual confounding can be caused by unmeasured confounding factors, additional confounding factors that were not adjusted for and confounding factors that may not be measured precisely (e.g. socioeconomic factors). Although the analyses were well adjusted, self-reported measures, such as medical history, and a lack of data on relevant variables such as access to health care, contamination of food items, environmental pollution and nutritional deficiencies (such as iron and vitamin D), may have impacted the

observed associations. In addition, some of the significant findings in stratified analyses may be due to chance or multiple testing due to low consumption of food group in a particular subgroup and large number of analyses respectively.

In addition, it is important to note that the FFQ used for dietary assessment in this study has several limitations and it may not reflect true intake of participants. FFQ may be prone to recall bias as recalling a person's diet over the last year introduces error. In addition, reporting of dietary intake may depend on case-control status or other characteristics of participants such as age, sex, education, income, culture, location etc. Moreover, nutrition literacy about how much food is consumed, what is consumed and what are the portion sizes may also affect reporting of dietary data.

Measurement error is an inherent limitation of FFQ and can be random or systematic. Random error for example can occur if a page of FFQ is skipped or the FFQ is misread by the participants. As this error occurs at random or is unpredictable, this would lead to attenuation of the observed associations. On the other hand, differential under or over reporting of energy intake may lead to systematic error that can bias the observed associations. There is evidence that reporting of energy intake may be influenced by age, sex and BMI. Women and people with higher BMI are more likely to under report. Older participants may not be able to recall their diets and are more likely to underestimate their energy intake.[90] However, in this chapter the stratified analyses by BMI and exclusion of people with high and low energy intakes were in line with the main associations. It is also important to note, however that in this study the response rate of cases was slightly less than the controls as shown in **Table 2.6 of Chapter 2**. The data was missing at random and therefore may not have important implications on the observed associations. Nevertheless, the researchers tried to minimize the differential misclassification of diet by collecting information from cases as close as possible to the incidence of MI, in addition the dietary information was re-confirmed with the spouses or household members of the participants.

### 4.5.7 Implications

The current findings have several implications. This is a largest detailed study on wide range of dietary food groups and risk of AMI in a South Asian population. The study reported beneficial effects of fruits and vegetables with the risk of AMI. These results are consistent with a large body of evidence from western populations. However, contrary to what has been reported in western populations, findings from the study suggests that fish consumption has an increased risk with AMI in the Bangladeshi population. This result highlights that association of food groups with the risk of AMI may vary depending on the preparation methods and the environment the fish breeds in. In Bangladesh fish is contaminated with arsenic due to heavy water pollution and people consume fried fish which may explain the detrimental effects. In addition, the study also found that red meat was not associated with the risk of AMI, which is again in contrast to many studies from the west. The null association in Bangladesh could be attributed to consumption of low range of unprocessed form of red meat. The results also emphasise that biryani (rice fried in oil with fish or red meat) which is a locally consumed food item has an increased risk of AMI. Whereas, certain spices (being an integral part of South Asian cuisine) had inverse association with the risk of CHD.

These results highlight that dietary guidelines based on western populations may not be generalised to a South Asian population as heterogeneity in the estimates for some food items exists attributed to the type of food consumed, preparation or cooking practices, and possible contaminants which may vary by geographical location.

The study also has important policy implications for the government and other relevant stakeholders. The government should provide subsidies to agriculture sector to increase production of fruits and vegetables, such incentive can have stronger effects on low income groups, bridging health inequalities. In addition, mass media campaigns should be used to promote evidence based dietary guidelines. The government should recommend and enforce standards for food, making sure they are free of adulteration and contamination. Furthermore, health systems should be strengthened to give lifestyle interventions for high risk individuals (diabetes and hypertension patients).

## 4.6 Conclusion

In conclusion, the findings from the first detailed study from Bangladesh on diet and risk of AMI indicate that higher consumption of fruits, vegetables, yoghurt and certain spices are associated with a lower risk of AMI. By contrast, higher consumption of biryani, sweet water and sea water fish were associated with a higher risk of developing AMI. Additionally, white meat, red meat, bread and milk had no significant association with the risk of MI.

These findings, albeit based on observational evidence, were generally consistent with those conducted in the Western settings, with a few notable exceptions (such as red meat and fish) where the associations seemed to differ for AMI risk.

Nonetheless, the current results, based on a first-ever detailed investigation of food groups with the risk of AMI in Bangladesh, highlight the importance of further studies on diet-CHD risk in relatively under-studied non-Western settings, where the diet and their clinical consequences might differ importantly from those observed in the Western populations. Such work will have important implications to help shape local and regional dietary guidelines for effective CVD prevention.

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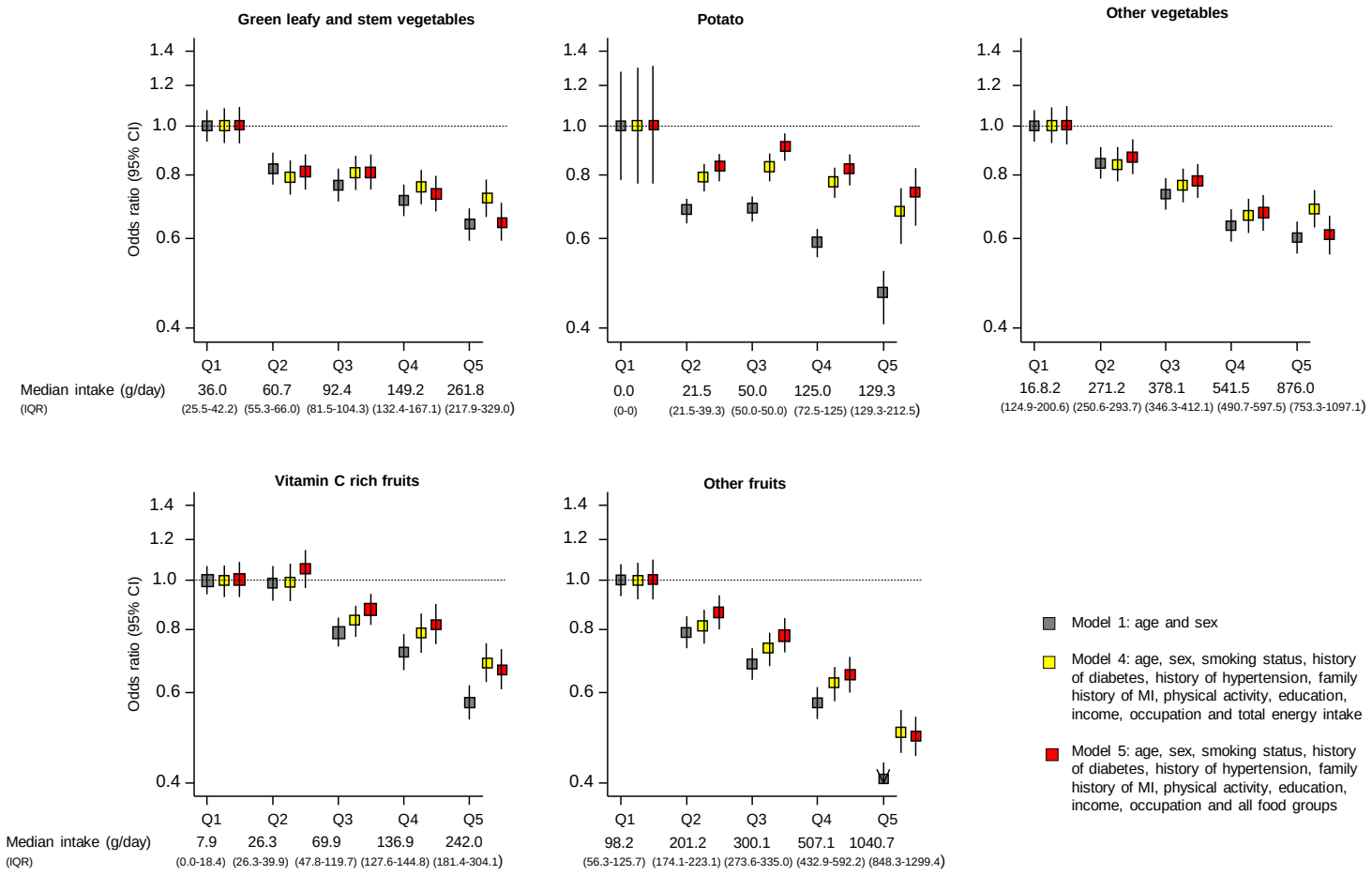
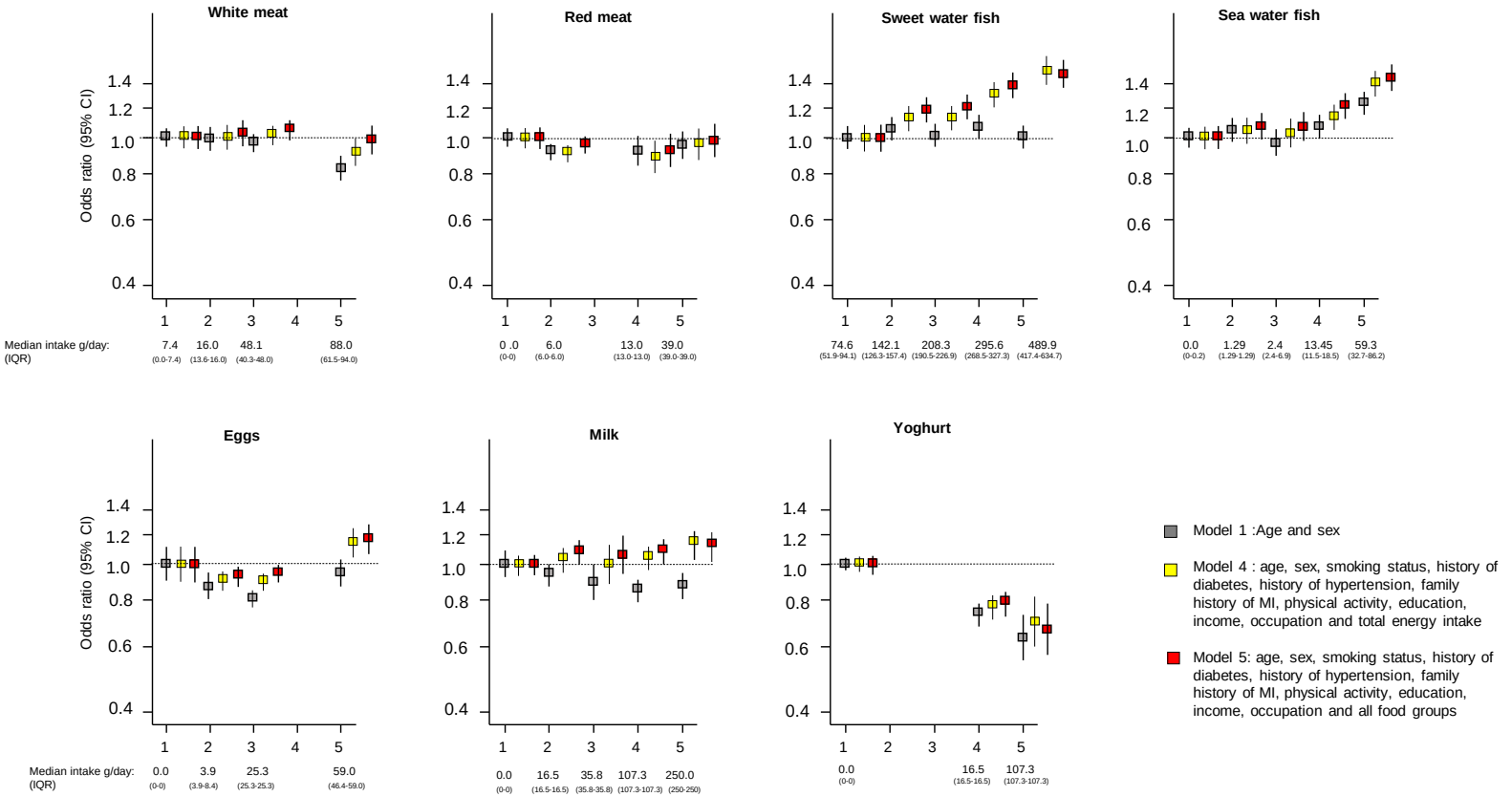
**Figure 4.1:** Plant based food groups and risk of myocardial infraction



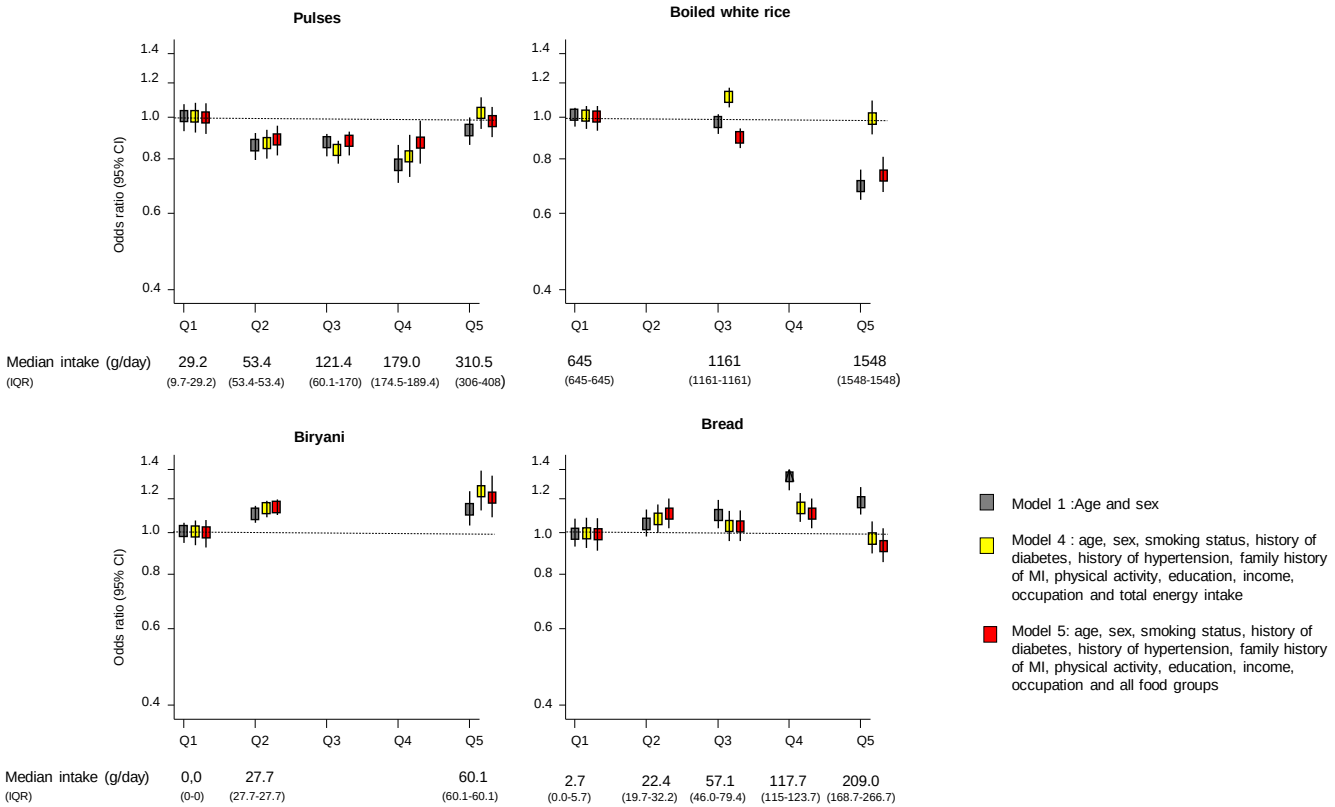

Q1 (reference group) to Q5 represent quintiles.
Green leafy and stem vegetables: spinach, amaranth stem, colocasia leaves, drumstick leaves and cabbage
Potatoes: potato and sweet potato
Other vegetables: all gourds, unripe papaya, tomato, cucumber, beans, water lily, carrot
Vitamin C rich fruits: grape fruit, orange and guava
Other fruits: banana, apple, pear, grapes, mango, jackfruit and others

**Figure 4.2**: Animal based food groups and risk of myocardial infraction



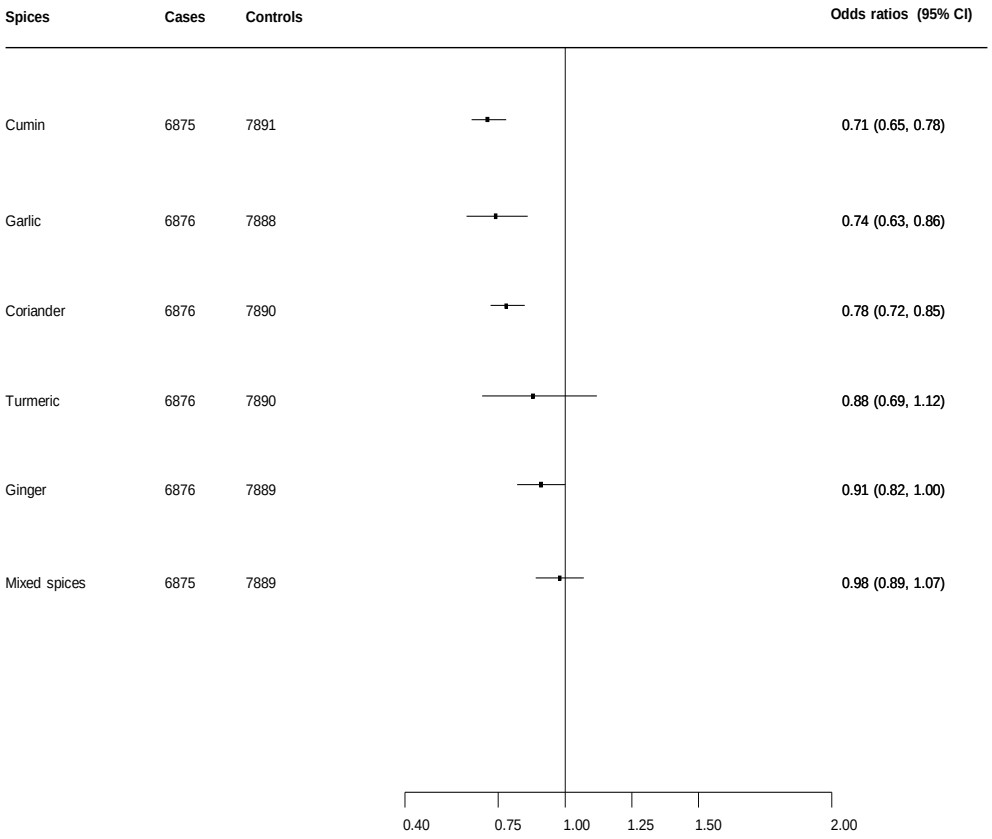

The black boxes represent odds ratios and the horizontal lines represent 95% confidence intervals calculated by floating absolute risks.
White meat: Chicken and duck
Red meat: Beef and goat meat
Sweet water fish: Rohu, catla, clown knife, hilsa, pangas, catfish, walking catfish, climbing perch, tilapua, tengra, barb, mola carplet, striped snake-head, spotted snake-head, byne, nola, silve carp, mirgel carp, koral, air and rissha
Sea water fish: Lobster, shrimps, pomfret, Bombay duck, croaker, rita, churi, hangori, pardey, giant sea perch and goby
In the case of white meat, red meat, eggs and yoghurt, I was unable to categorize data into 5 groups because most of the values lay at 1 point.

**Figure 4.3**: Other food groups and risk of myocardial infraction



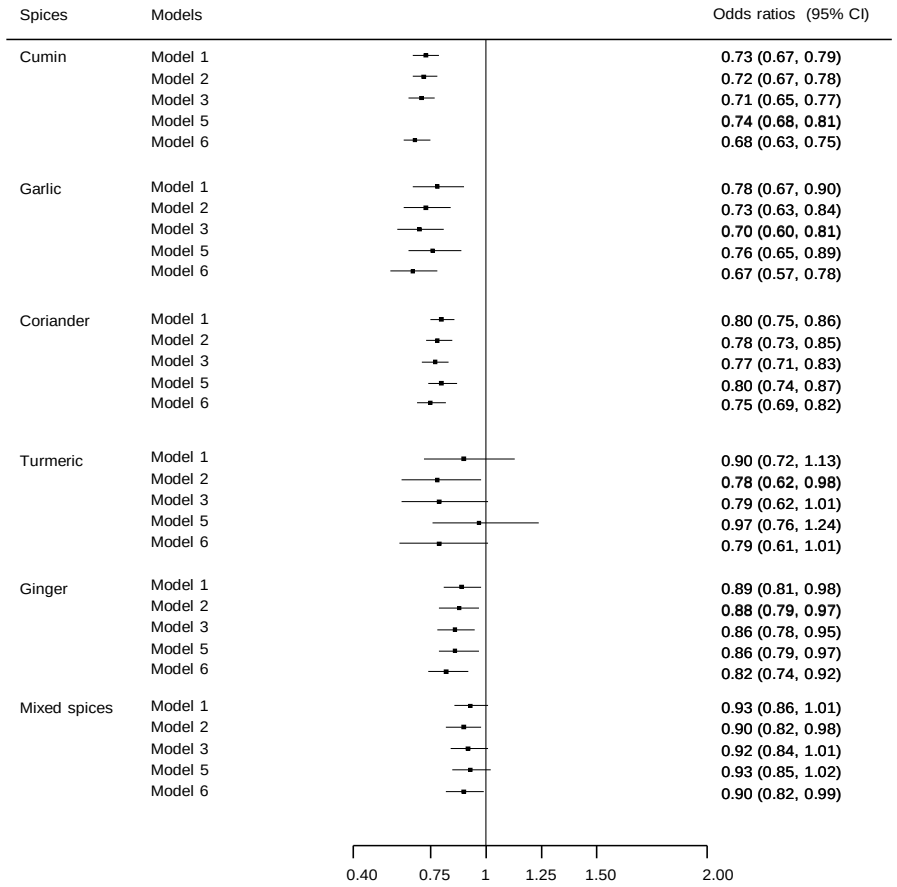

The black boxes represent odds ratios and the horizontal lines represent 95% confidence intervals calculated by floating absolute risks.
Pulses: mugger daal, buter daal, masroor daal
Bread: Ruti, paratha, chapatti and standard bread loaf
In the case of boiled white rice and biryani I was unable to categorize data into 5 groups because most of the values lay at 1 point.

**Figure 4.4:** Association of (≥ 1 times a day versus <1 time a day) of spices with myocardial infarction.




Adjusted for Model 4: age, sex, smoking status, history of diabetes, history of hypertension, family history of MI, physical activity, education, income, occupation and total energy intake.
For the analysis <1 time a day was taken as the reference category.
The black boxes represent odds ratios and the horizontal lines represent 95% confidence intervals calculated by floating absolute risks.

**Figure 4.5**: Association of (≥ 1 times a day versus <1 time a day) of spices with myocardial infarction after multivariate adjustments



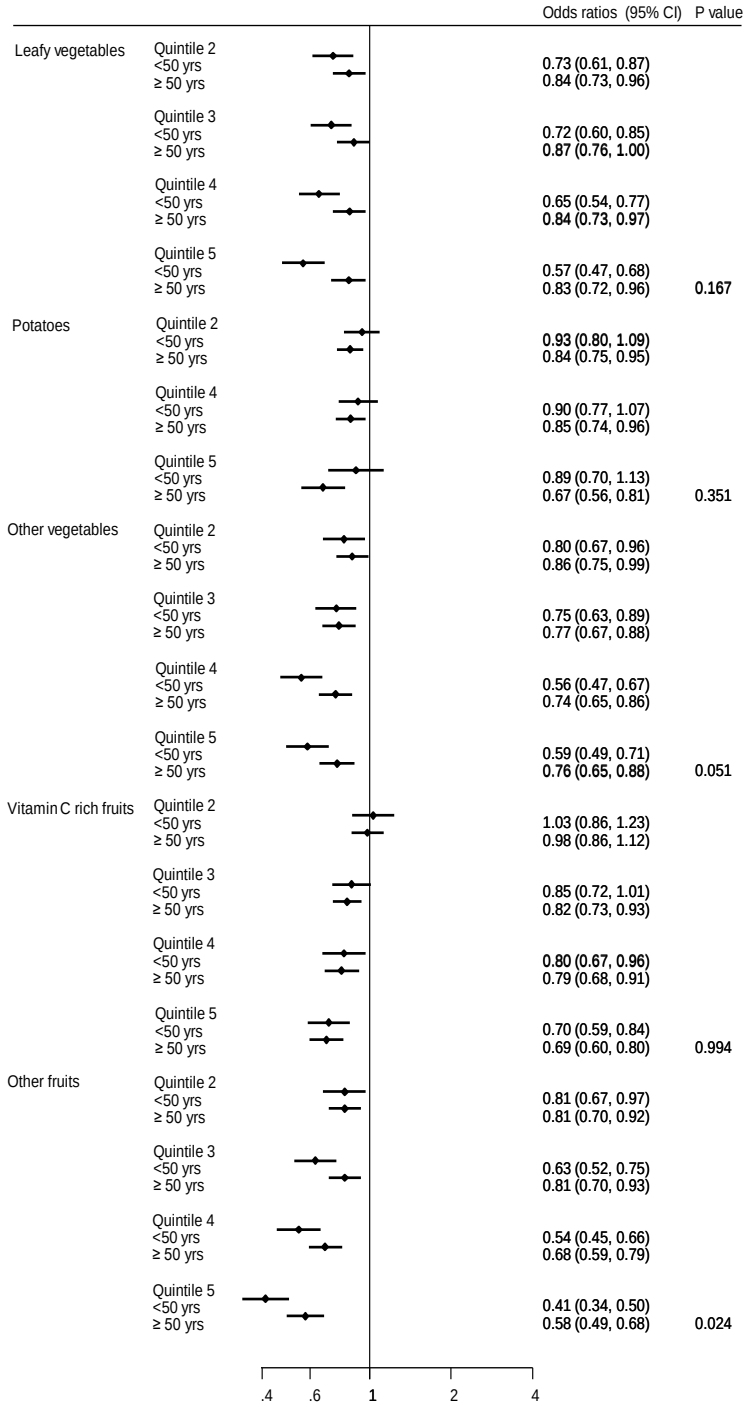

Model 1: age and sex adjusted
Model 2: Model 1+ smoking, physical activity, education, occupation and income

Model 3: Model 2 + history of disease related variables: history of diabetes, history of hypertension and family history of MI

Model 5: Model 3 + all food groups ( mutual adjustment)

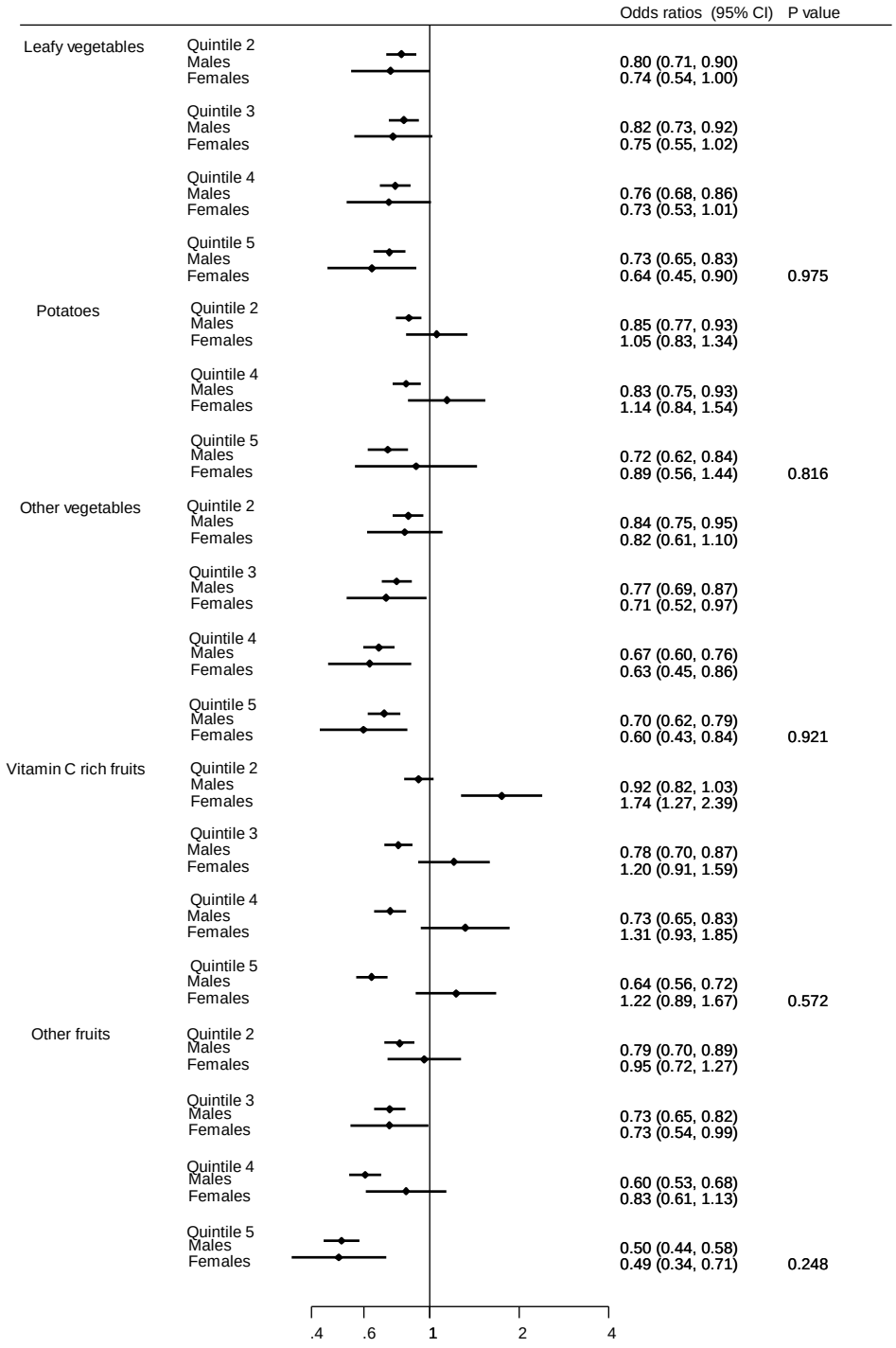
Model 6: Model 4 + waist to hip ratio + LDL-C and HDL-C

**Figure 4.6:** Association of plant-based food groups by age



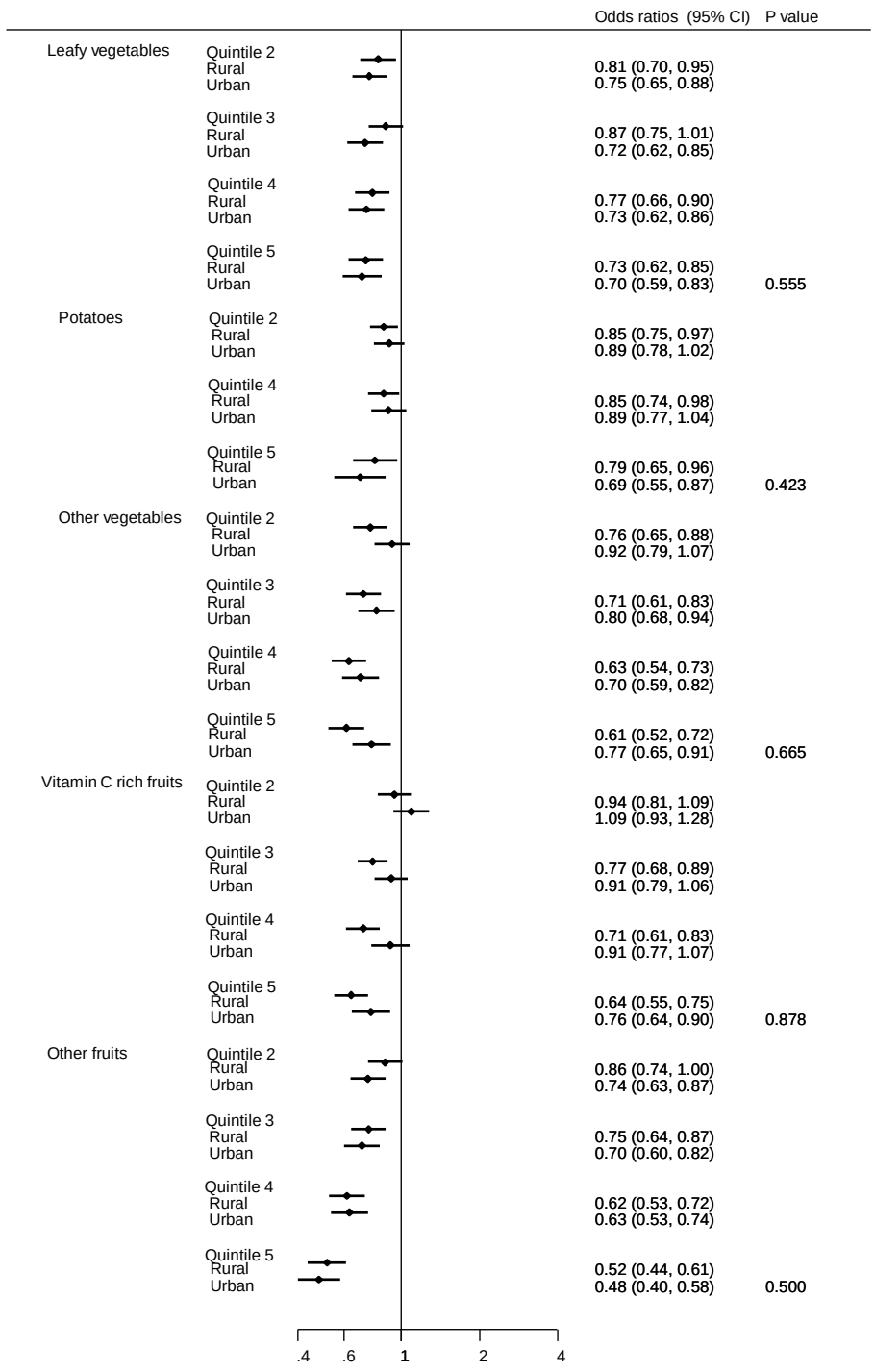

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.7:** Association of plant-based food groups by sex



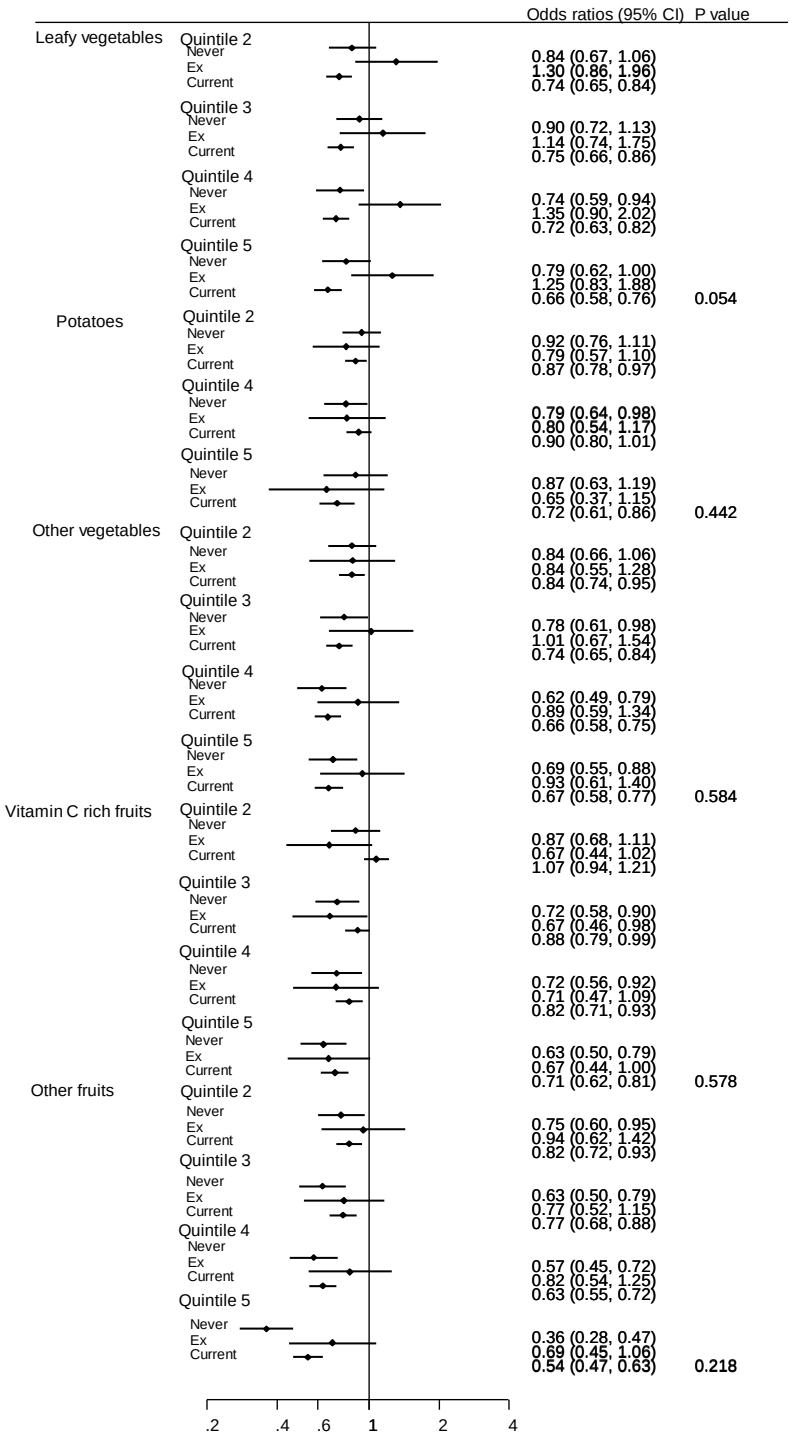

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.8:** Association of plant-based food groups by location



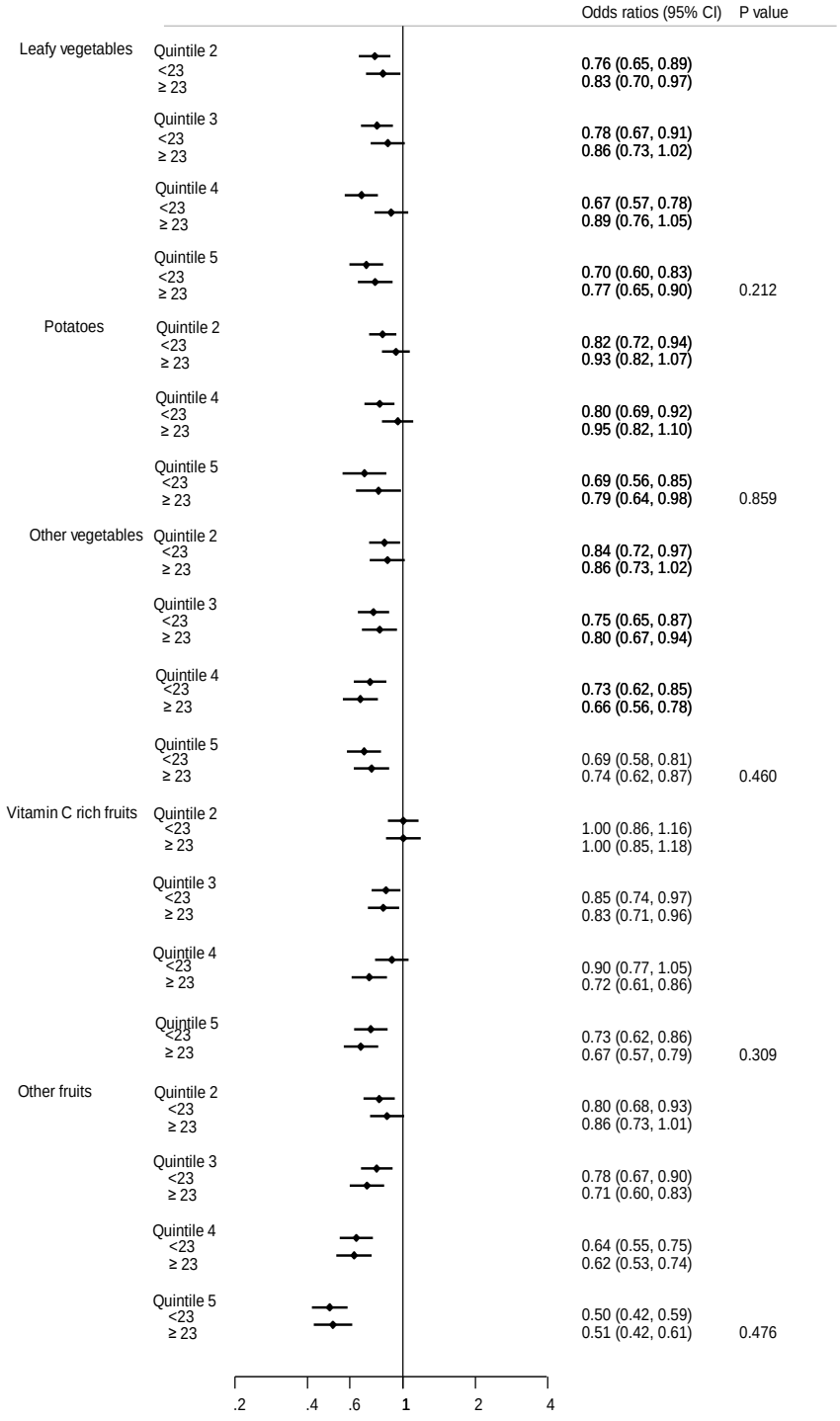

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.9:** Association of plant-based food groups by smoking



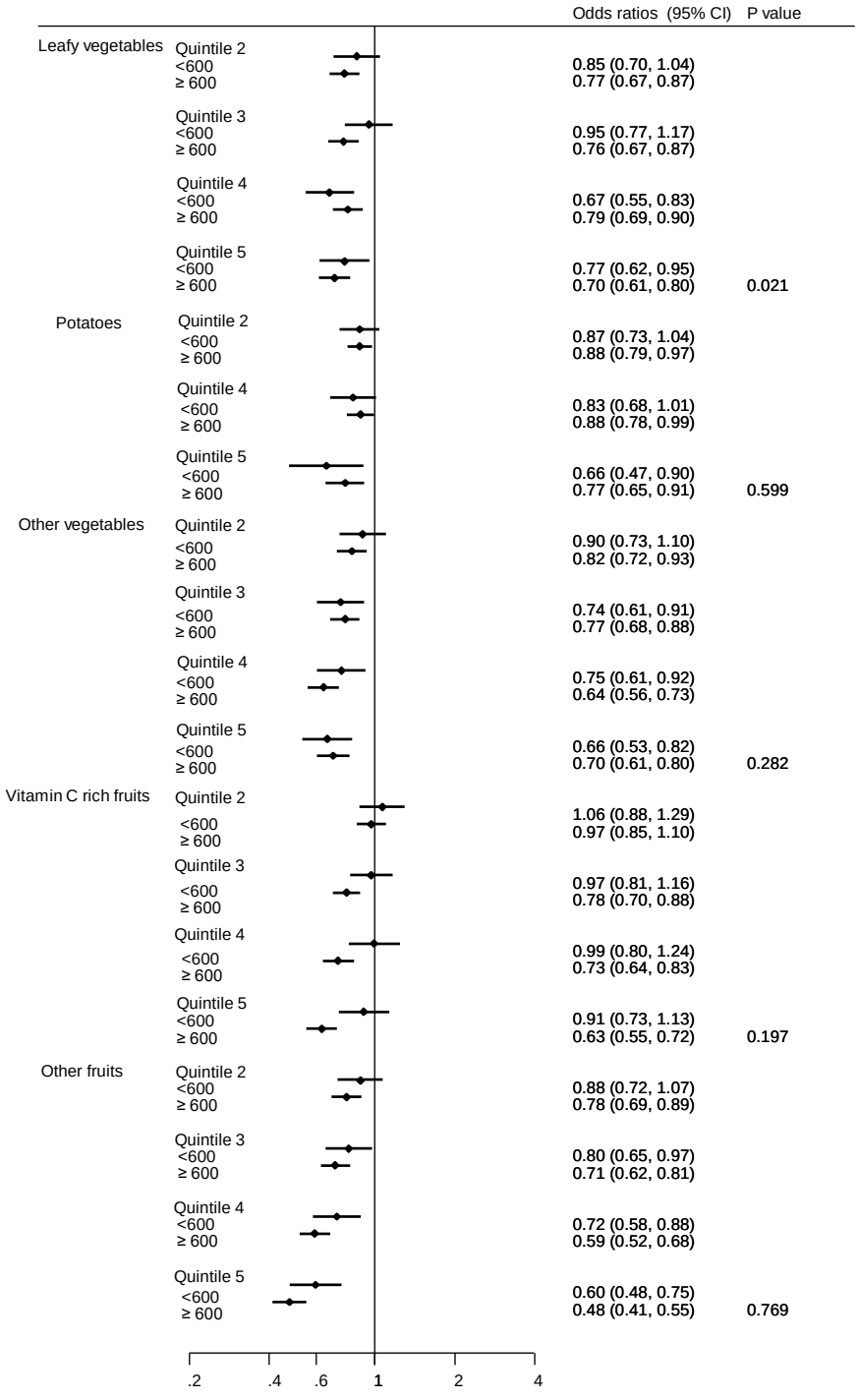

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.10:** Association of plant-based food groups by BMI



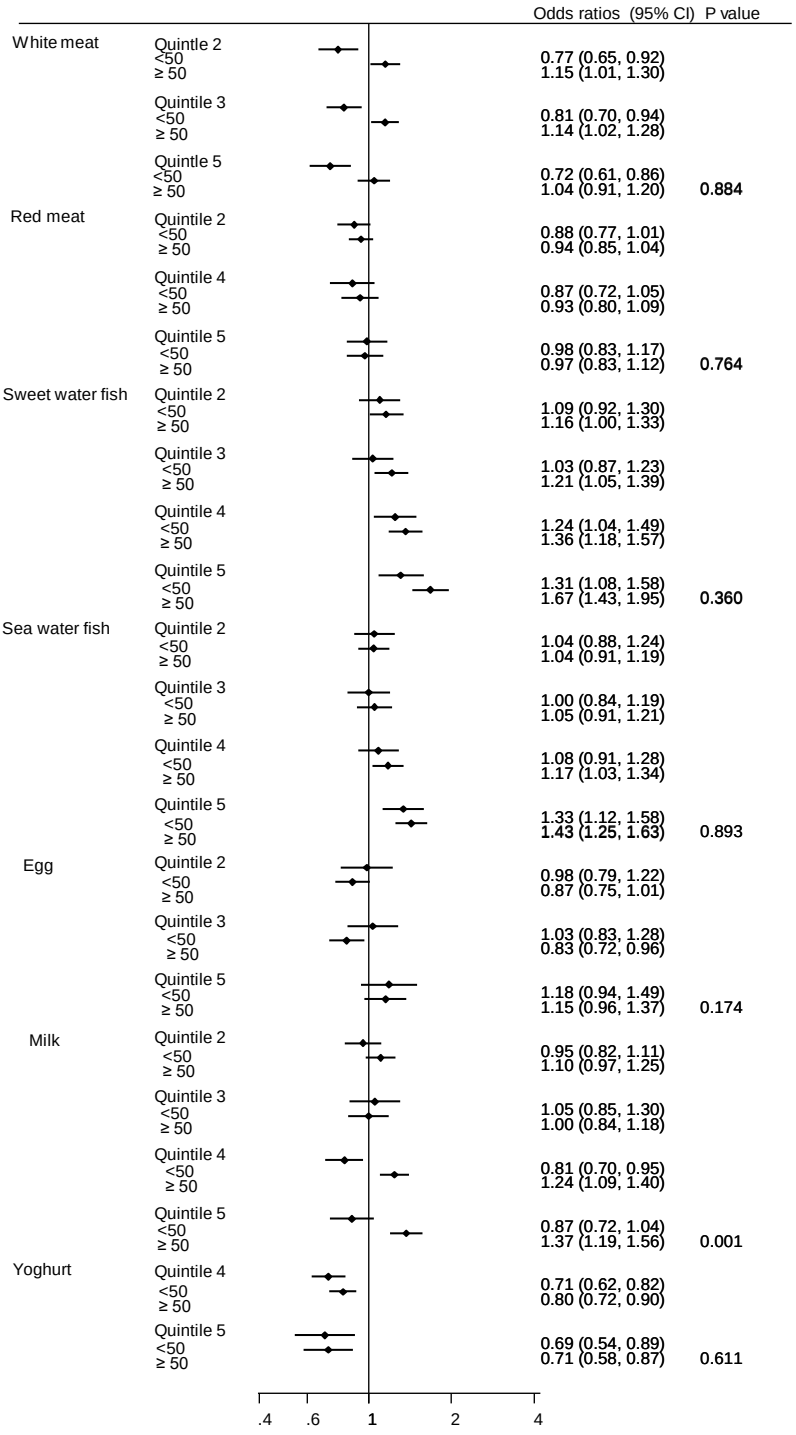

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.11:** Association of plant-based food groups by physical activity



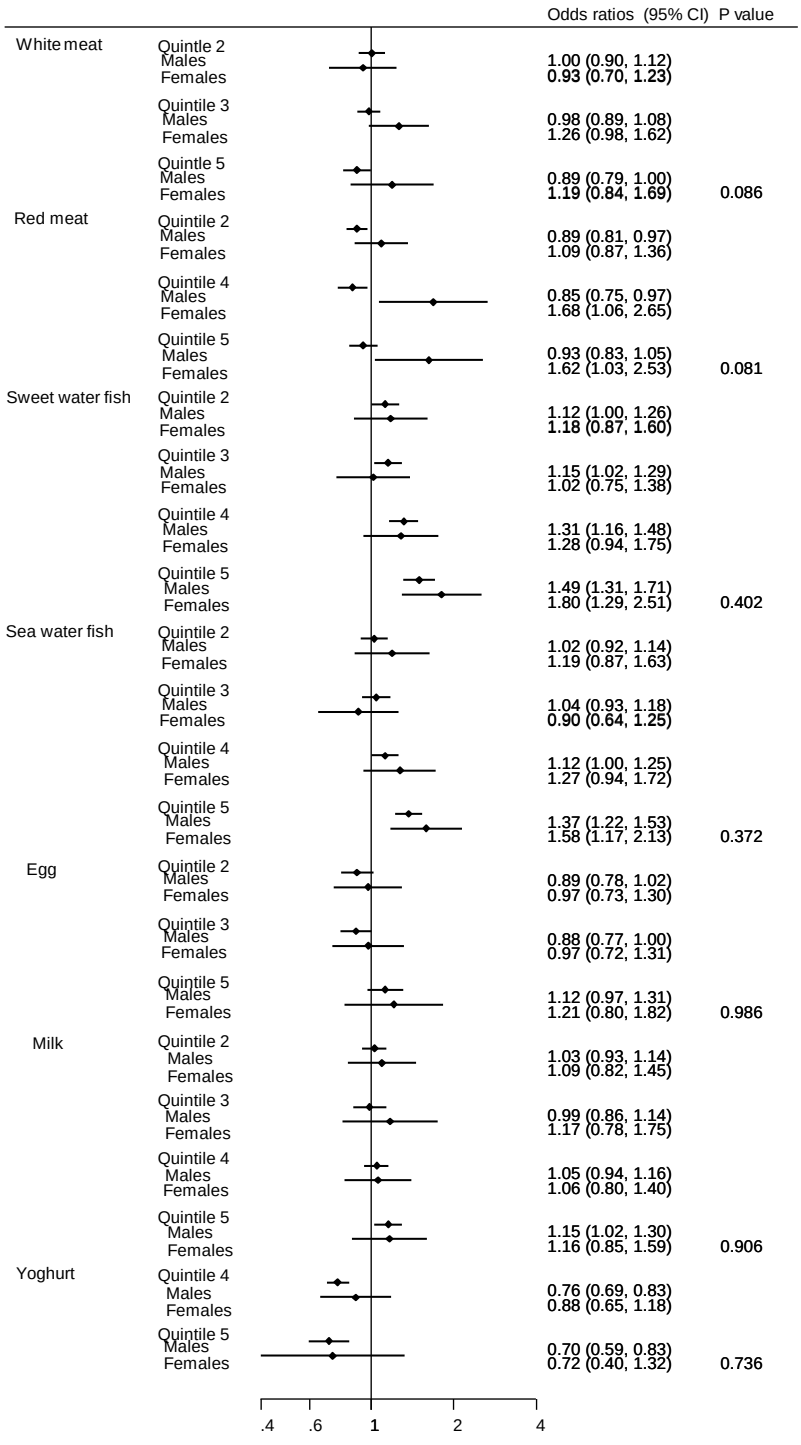

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.12:** Association of animal-based food groups by age



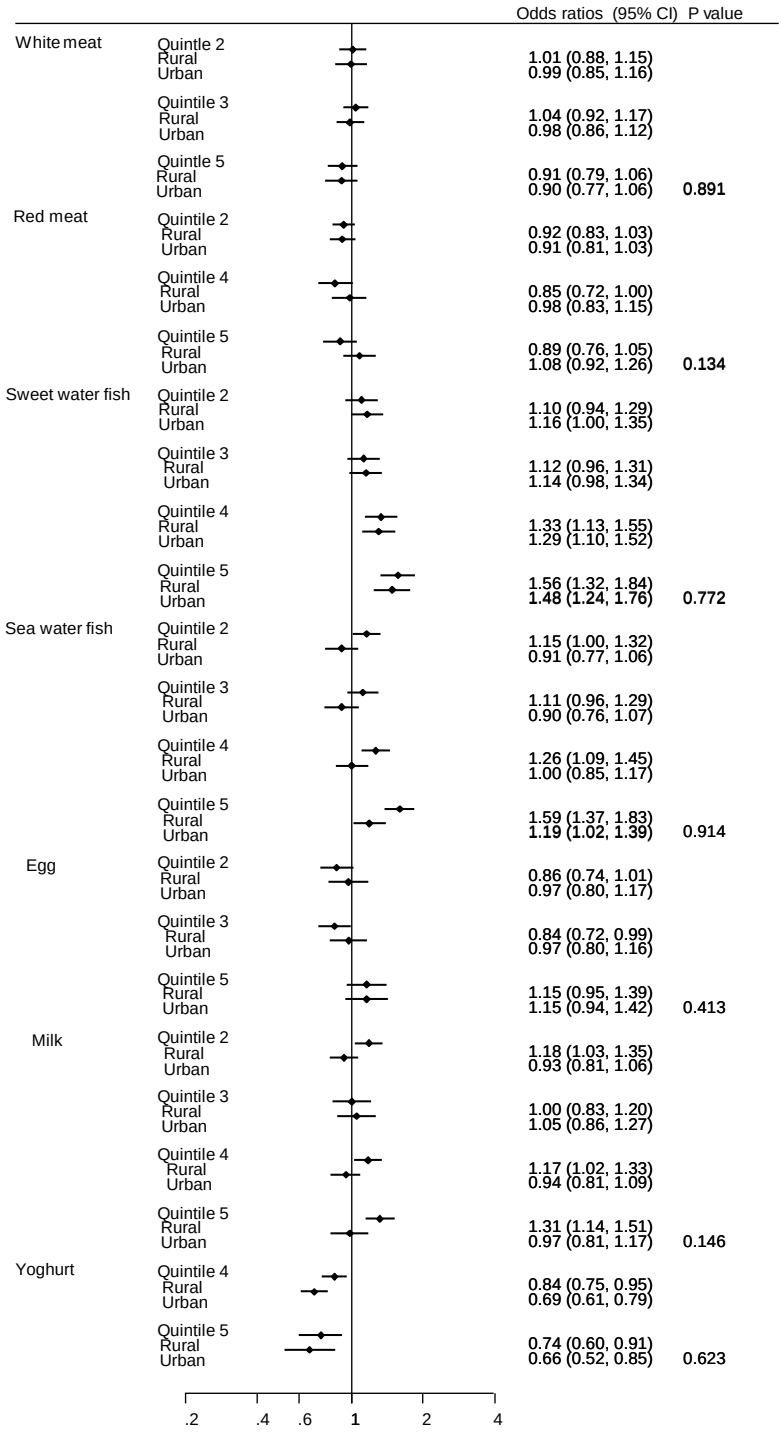

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.13:** Association of animal-based food groups by sex



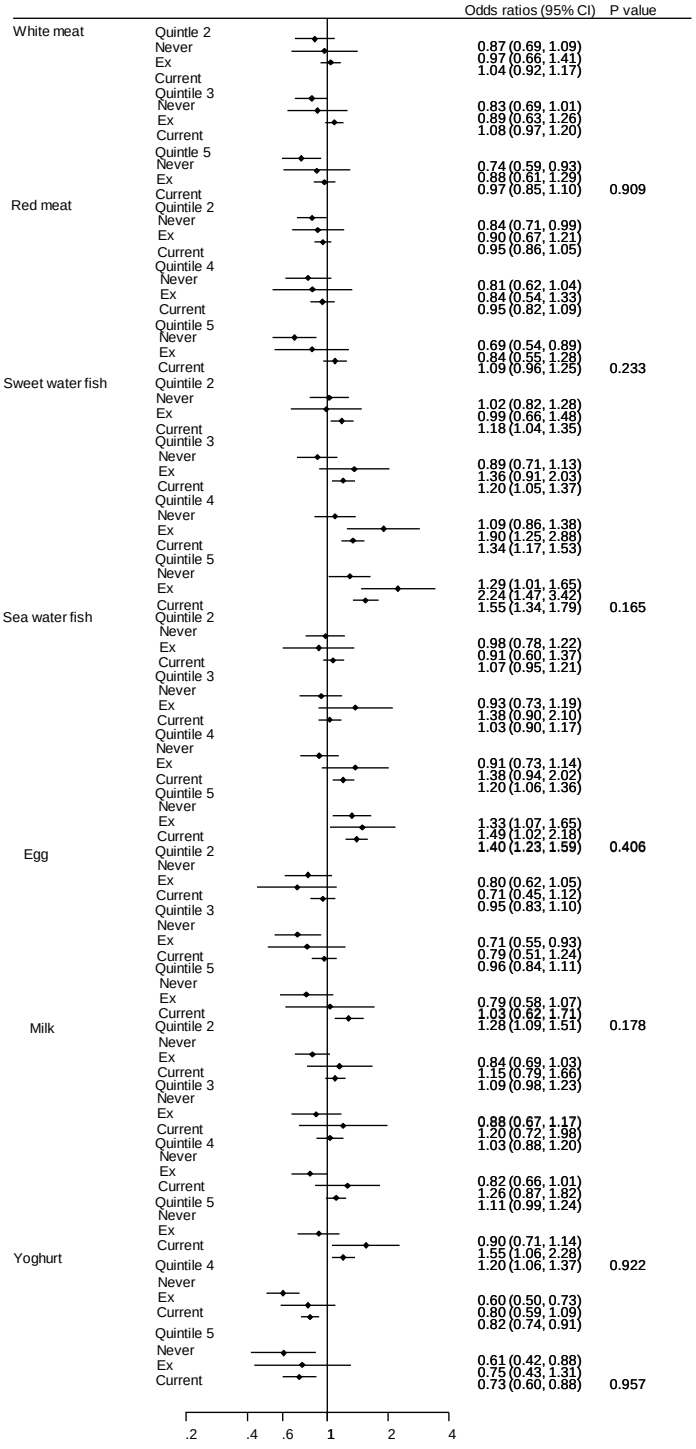

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.14:** Association of animal-based food groups by location




P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.15:** Association of animal-based food groups by smoking status




P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.16:** Association of animal-based food groups by BMI

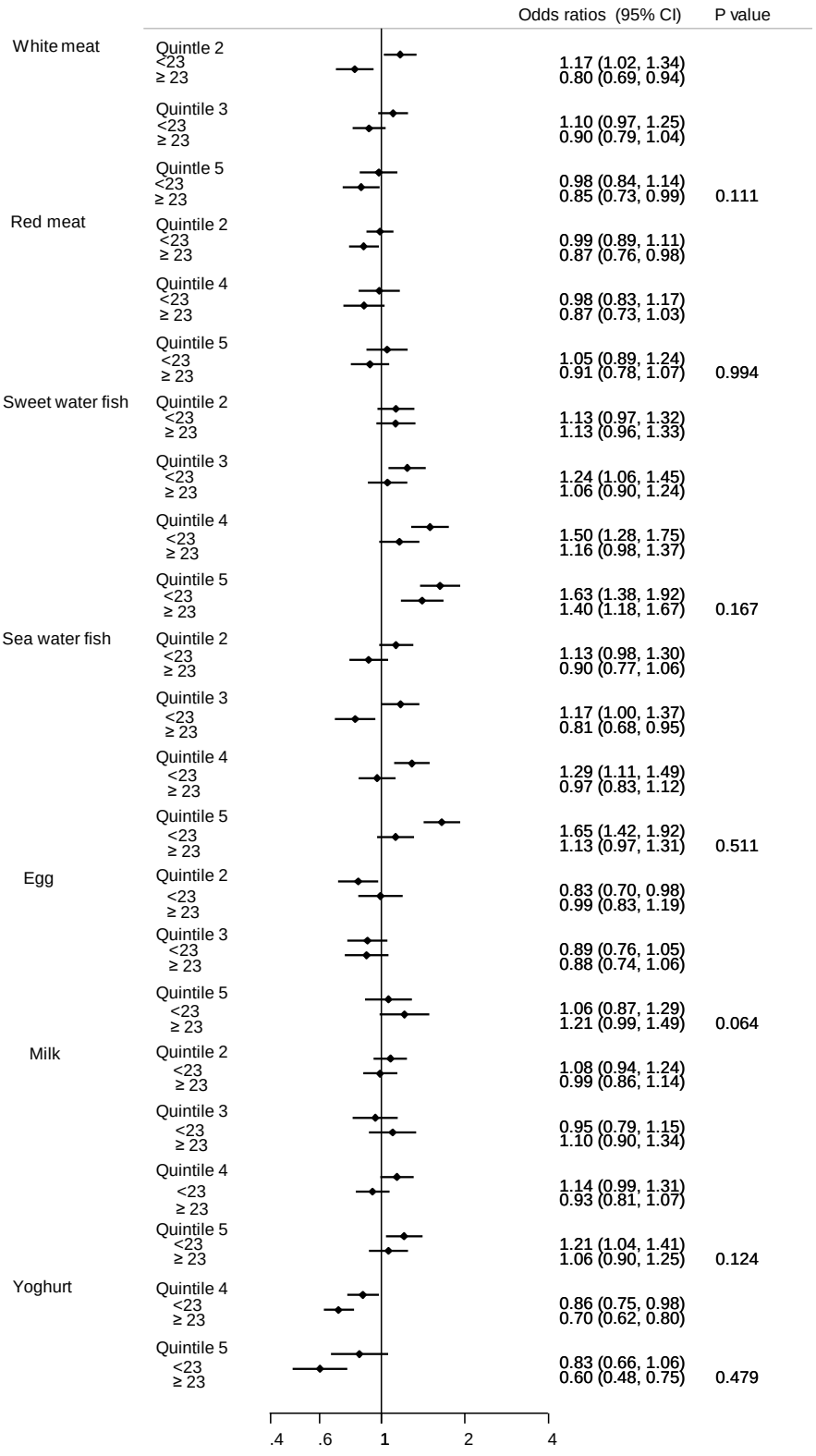
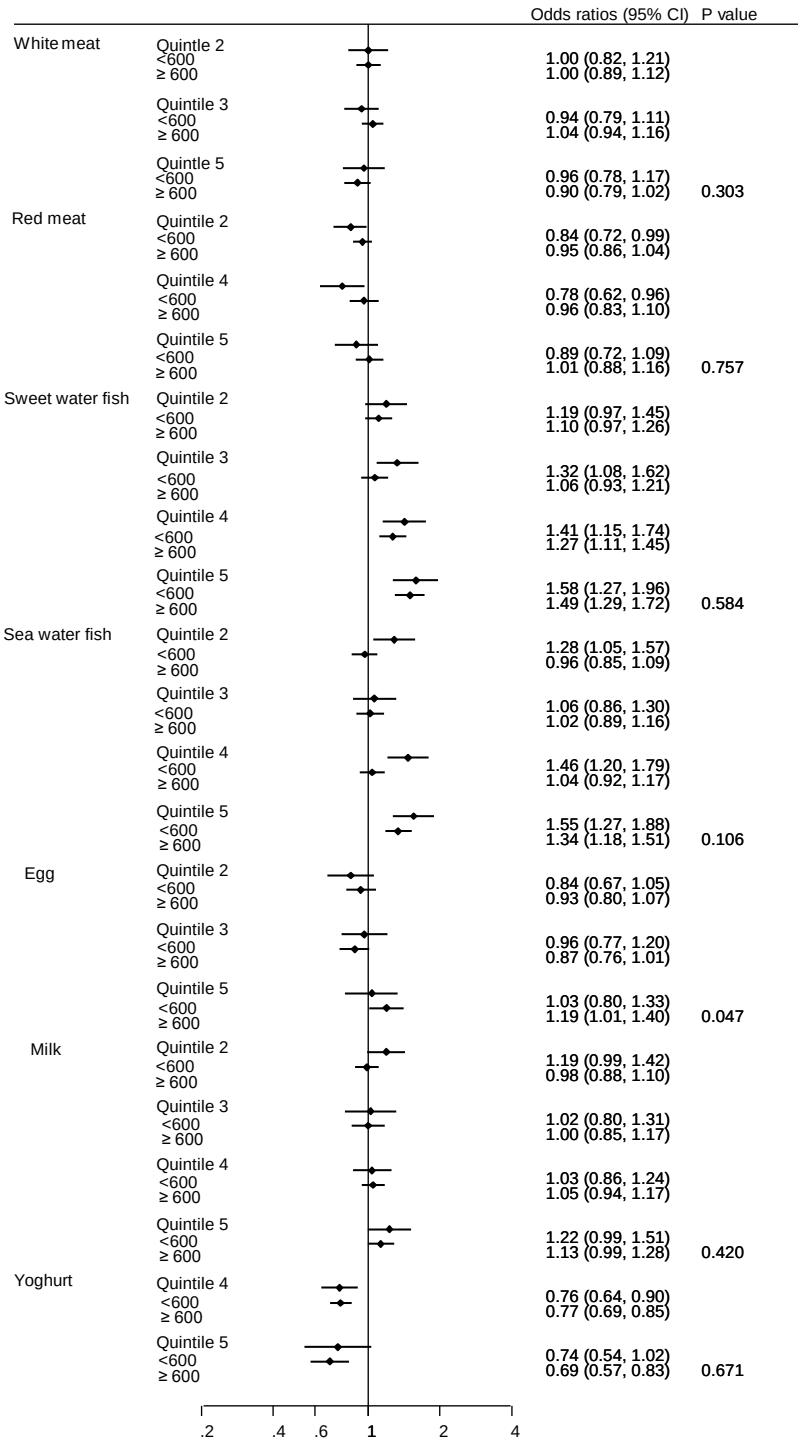
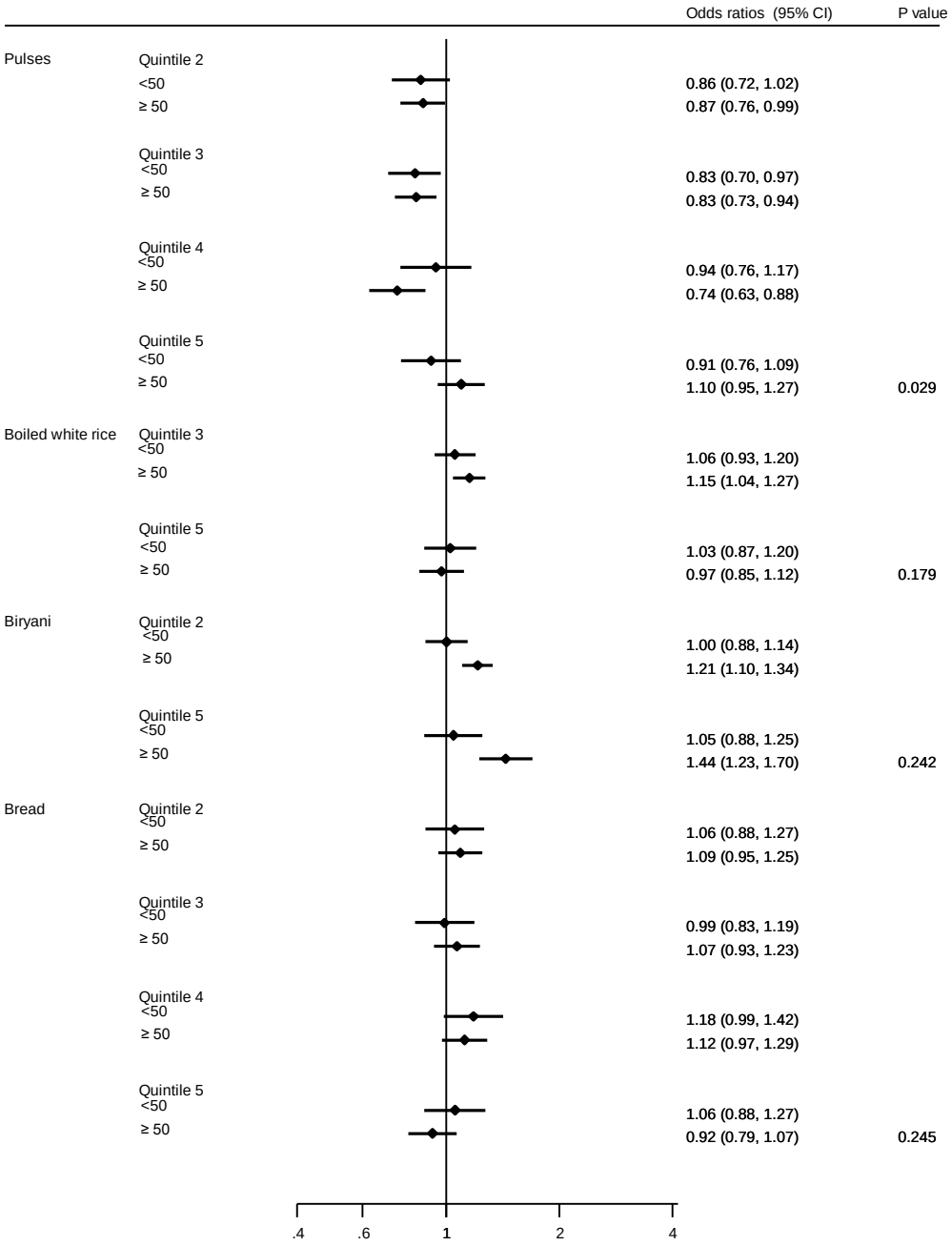


**Figure 4.17:** Association of animal-based food groups by physical activity



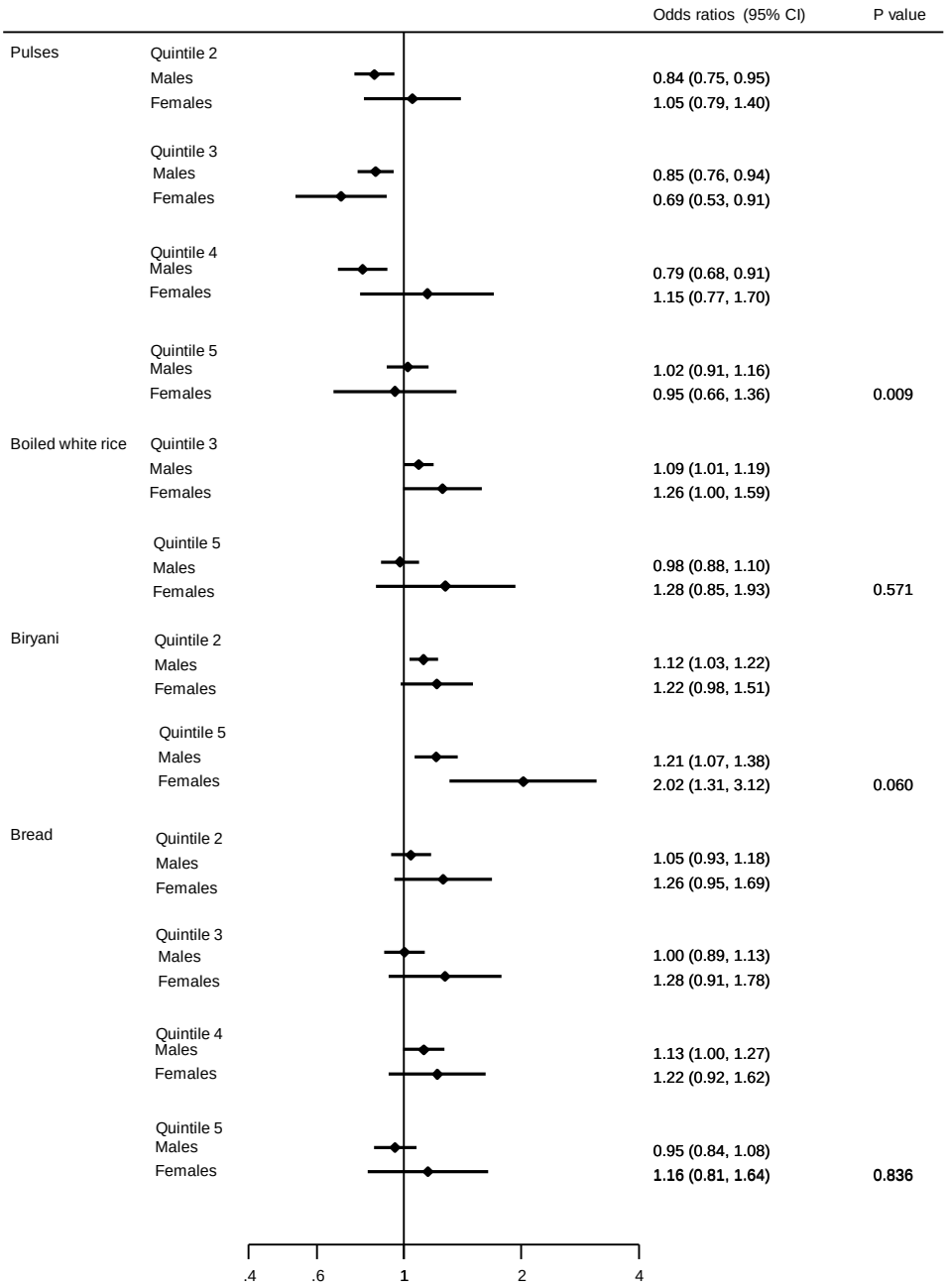

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.18:** Association of other food groups by age



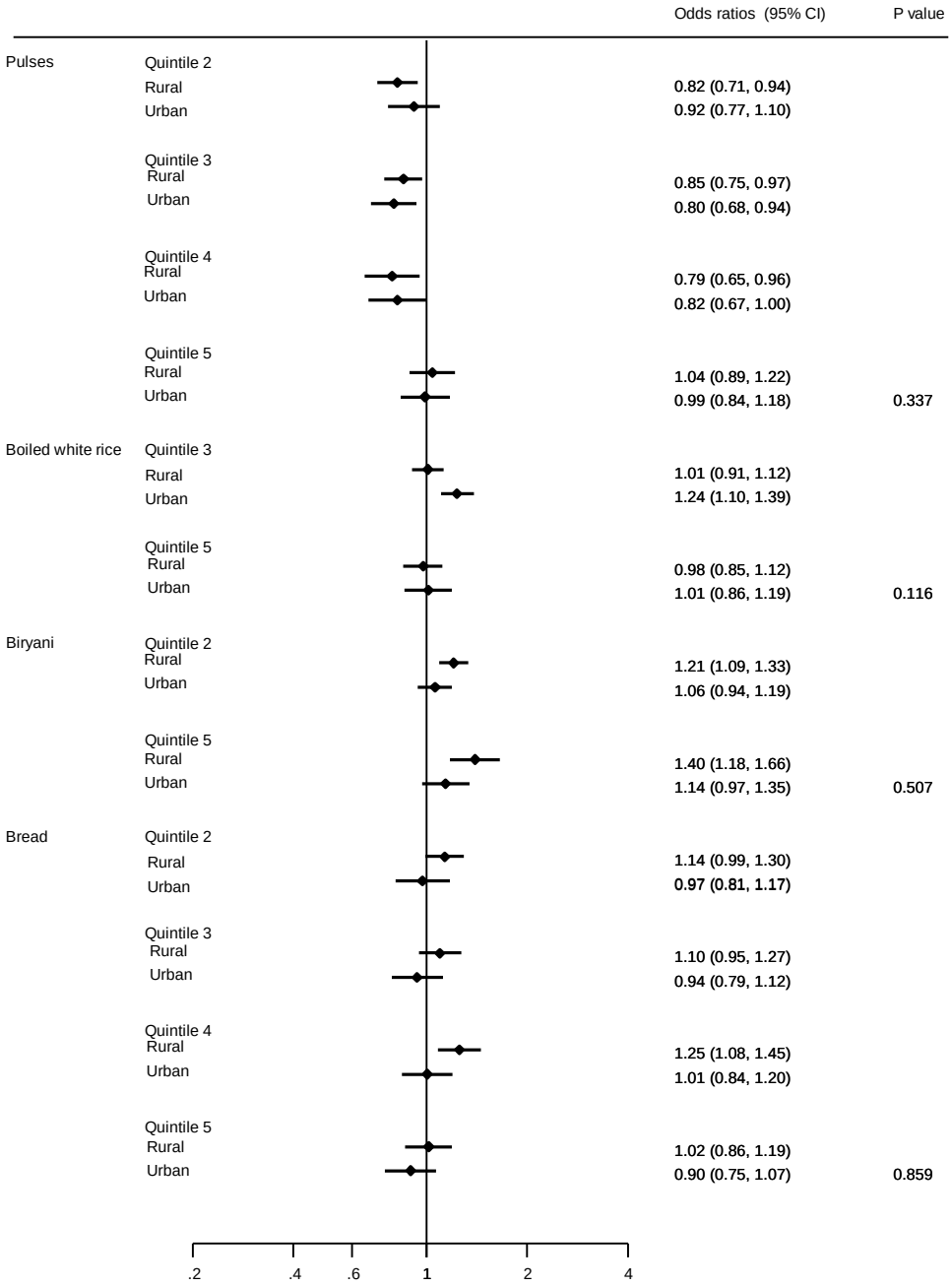

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.19:** Association of other food groups by sex



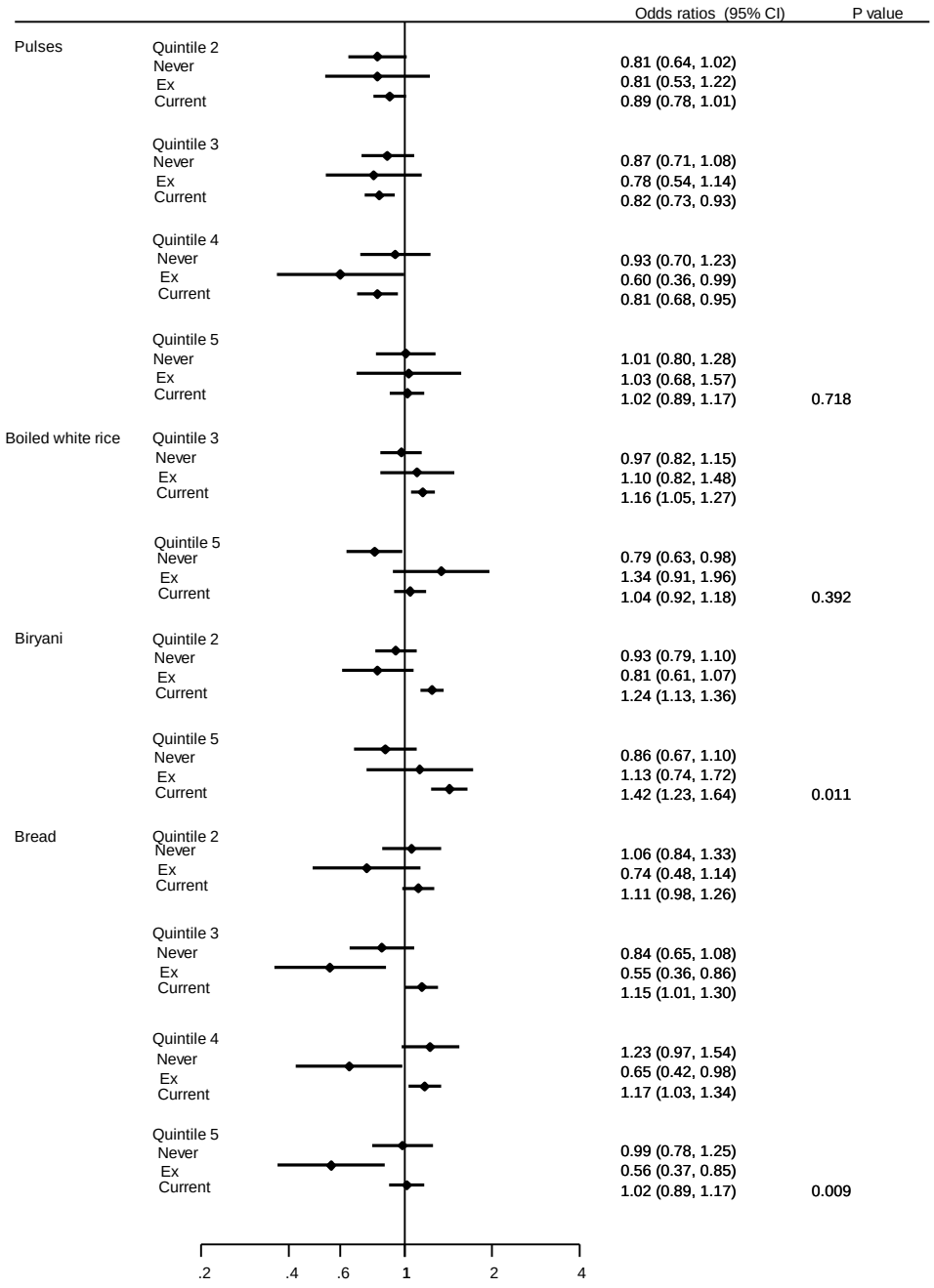

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.20:** Association of other food groups by location



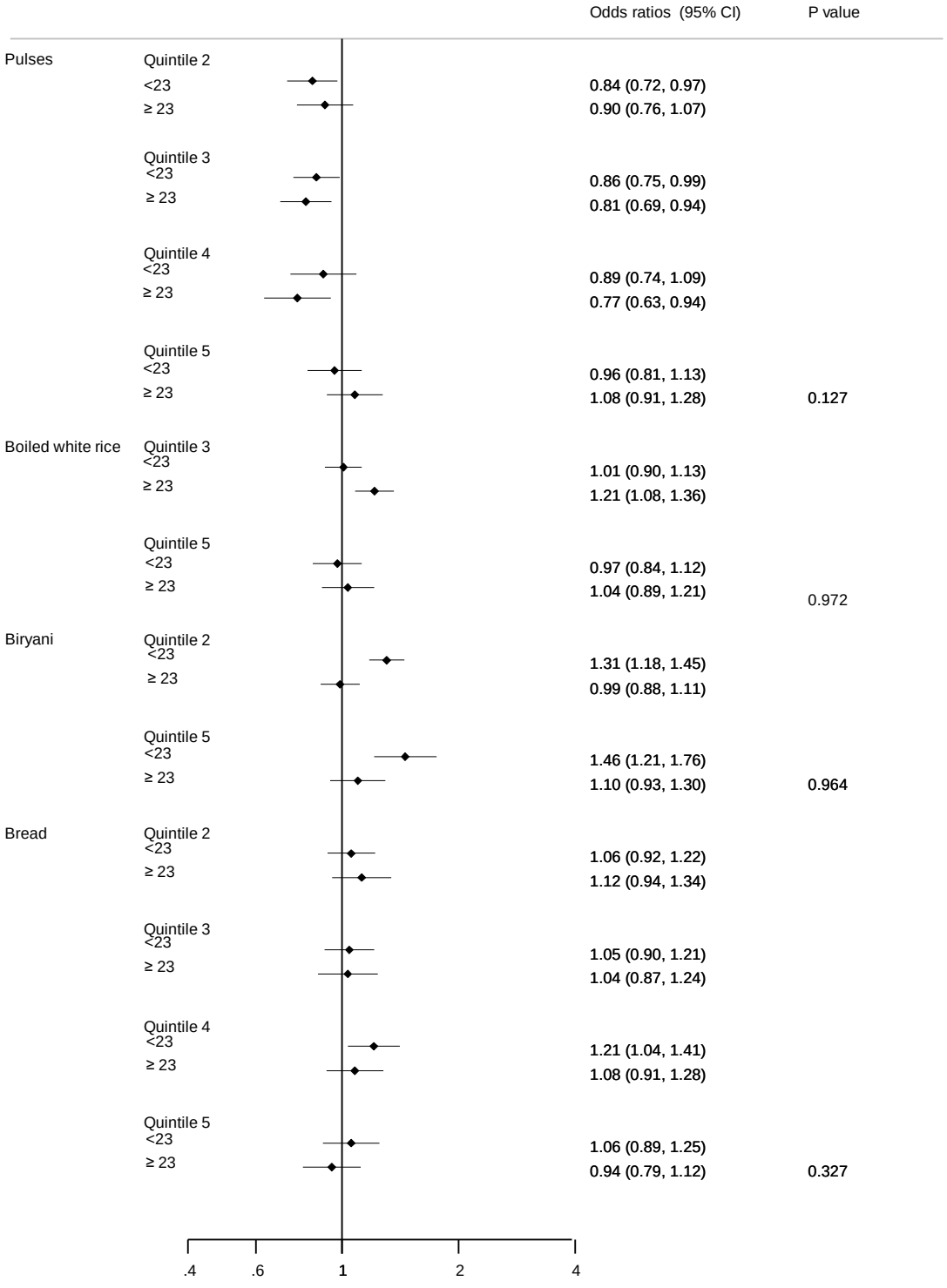

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.21:** Association of other food groups by smoking



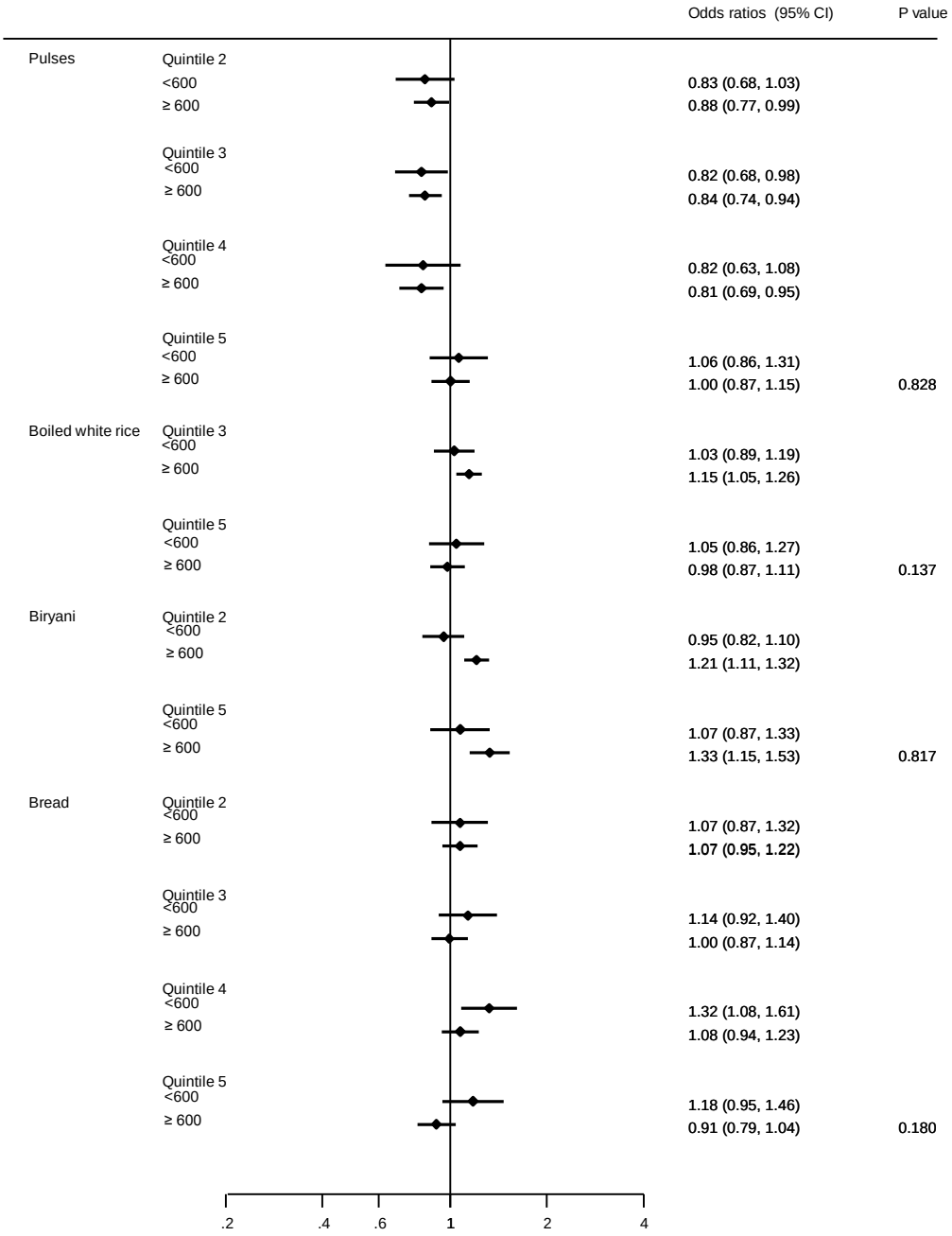

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.22**: Association of other food groups by BMI



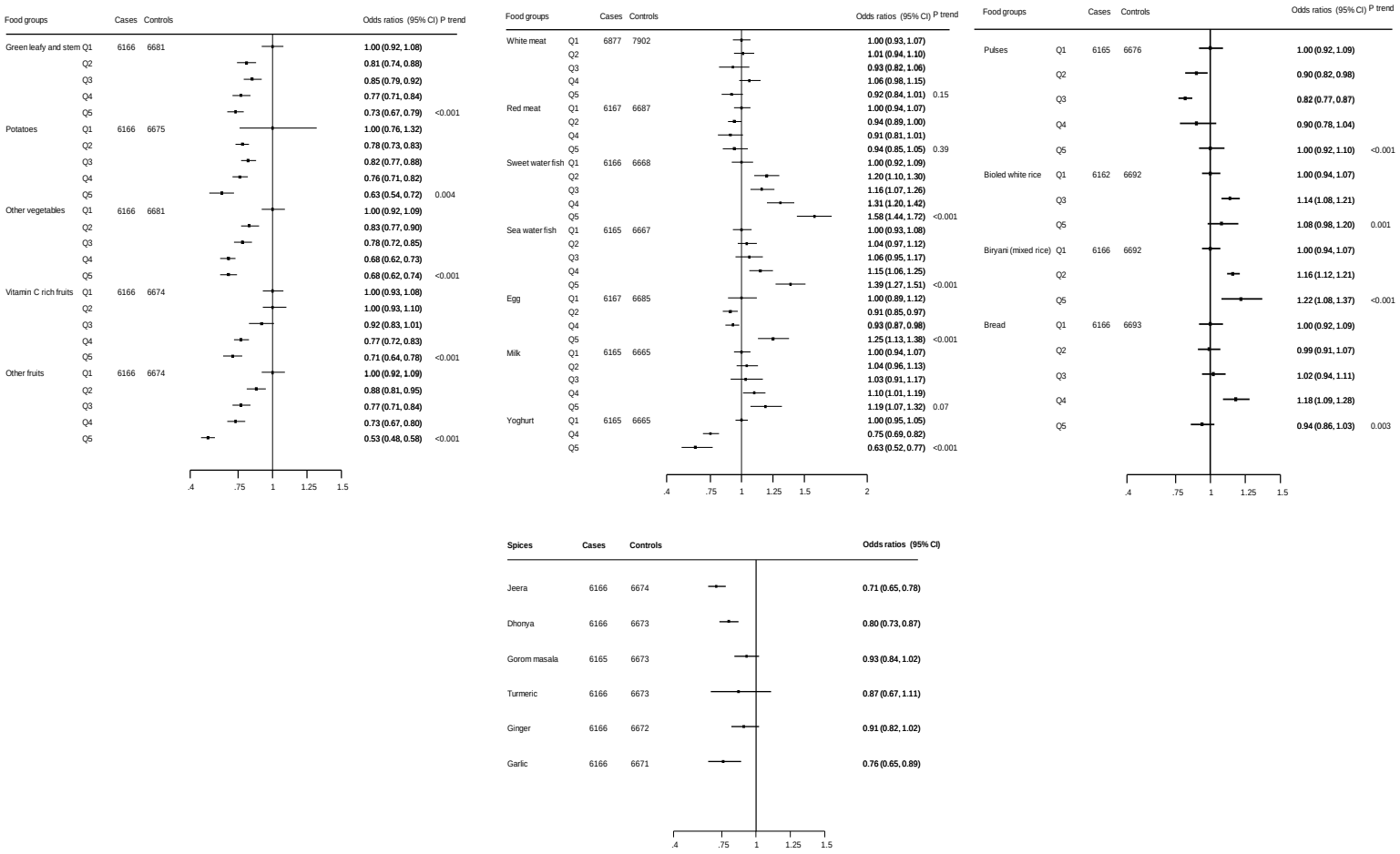

P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.23:** Association of other food groups by physical activity




P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Figure 4.24:** Association of food groups and myocardial infraction by excluding participants with high and low energy intake using cut offs from PURE study




P value is for interaction term for overall quintiles. Odds ratios were estimated using logistic regression models adjusted for Model 4.

**Table 4.1**: Association of plant-based foods with AMI after multivariate adjustments

| | Q2 | Q3 | Q4 | Q5 | P trend |
|---|---|---|---|---|---|
| **Green leafy and stem vegetables** | **60.7 g/day** | **92.4 g/day** | **149.2 g/day** | **261.8 g/day** | |
| Model 1 | 0.82 (0.77-0.89) | 0.77 (0.71-0.82) | 0.71 (0.66-0.77) | 0.64 (0.60-0.69) | <0.001 |
| Model 2 | 0.79 (0.74-0.85) | 0.78 (0.72-0.84) | 0.72 (0.68-0.78) | 0.65 (0.60-0.70) | <0.001 |
| Model 3 | 0.76 (0.71-0.83) | 0.76 (0.71-0.82) | 0.68 (0.63-0.74) | 0.60 (0.55-0.64) | <0.001 |
| Model 4 | 0.79 (0.73-0.85) | 0.80 (0.75-0.87) | 0.76 (0.70-0.82) | 0.72 (0.66-0.78) | <0.001 |
| Model 5 | 0.81 (0.75-0.88) | 0.81 (0.75-0.87) | 0.73 (0.68-0.79) | 0.64 (0.59-0.70) | <0.001 |
| Model 6 | 0.80 (0.73-0.85) | 0.81 (0.75-0.87) | 0.76 (0.70-0.82) | 0.72 (0.66-0.78) | <0.001 |
| Model 7 | 0.79 (0.73-0.86) | 0.83 (0.77-0.90) | 0.76 (0.70-0.82) | 0.73 (0.67-0.80) | <0.001 |
| **Potato** | **21.5 g/day** | **50 g/day** | **125 g/day** | **129.3 g/day** | |
| Model 1 | 0.68 (0.64-0.72) | 0.69 (0.65-0.73) | 0.59 (0.55-0.63) | 0.46 (0.41-0.52) | <0.001 |
| Model 2 | 0.69 (0.65-0.73) | 0.68 (0.65-0.73) | 0.60 (0.57-0.65) | 0.50 (0.44-0.56) | <0.001 |
| Model 3 | 0.79 (0.73-0.83) | 0.80 (0.75-0.85) | 0.72 (0.68-0.77) | 0.60 (0.53-0.68) | <0.001 |
| Model 4 | 0.79 (0.74-0.84) | 0.83 (0.78-0.88) | 0.77 (0.72-0.83) | 0.66 (0.59-0.75) | 0.01 |
| Model 5 | 0.83 (0.78-0.88) | 0.90 (0.85-0.96) | 0.82 (0.76-0.88) | 0.72 (0.63-0.82) | 0.01 |
| Model 6 | 0.79 (0.74-0.84) | 0.82 (0.77-0.88) | 0.77 (0.72-0.82) | 0.67 (0.58-0.75) | 0.002 |
| Model 7 | 0.77 (0.72-0.82) | 0.82 (0.77-0.87) | 0.75 (0.70-0.81) | 0.65 (0.57-0.74) | 0.01 |
| **Other vegetables** | **272.2 g/day** | **378.1 g/day** | **541.5 g/day** | **876.0 g/day** | |
| Model 1 | 0.84 (0.79-0.91) | 0.74 (0.68-0.79) | 0.64 (0.59-0.69) | 0.60 (0.56-0.65) | <0.001 |
| Model 2 | 0.85 (0.79-0.91) | 0.75 (0.70-0.81) | 0.66 (0.61-0.71) | 0.63 (0.58-0.68) | <0.001 |
| Model 3 | 0.80 (0.75-0.87) | 0.71 (0.66-0.77) | 0.60 (0.55-0.64) | 0.57 (0.52-0.61) | <0.001 |
| Model 4 | 0.84 (0.78-0.91) | 0.76 (0.71-0.82) | 0.67 (0.62-0.72) | 0.69 (0.63-0.75) | <0.001 |
| Model 5 | 0.87 (0.80-0.94) | 0.78 (0.82-0.84) | 0.67 (0.62-0.73) | 0.61 (0.56-0.67) | <0.001 |
| Model 6 | 0.84 (0.78-0.91) | 0.76 (0.71-0.83) | 0.67 (0.62-0.72) | 0.69 (0.63-0.75) | <0.001 |
| Model 7 | 0.83 (0.77-0.90) | 0.76 (0.70-0.82) | 0.67 (0.62-0.73) | 0.68 (0.62-0.74) | <0.001 |
| **Vitamin C rich fruits** | **26.3 g/day** | **69.9 g/day** | **136.9 g/day** | **242.0 g/day** | |
| Model 1 | 0.99 (0.91-1.07) | 0.79 (0.74-0.84) | 0.72 (0.66-0.78) | 0.58 (0.53-0.62) | <0.001 |
| Model 2 | 0.99 (0.91-1.07) | 0.81 (0.76-0.87) | 0.75 (0.69-0.81) | 0.62 (0.57-0.67) | <0.001 |
| Model 3 | 0.98 (0.90-1.06) | 0.79 (0.73-0.85) | 0.72 (0.66-0.79) | 0.58 (0.54-0.63) | <0.001 |
| Model 4 | 0.99 (0.92-1.08) | 0.83 (0.78-0.90) | 0.79 (0.72-0.86) | 0.69 (0.63-0.76) | <0.001 |
| Model 5 | 1.05 (0.96-1.14) | 0.87 (0.81-0.94) | 0.82 (0.74-0.90) | 0.67 (0.61-0.73) | <0.001 |
| Model 6 | 1.00 (0.91-1.08) | 0.84 (0.79-0.90) | 0.79 (0.72-0.86) | 0.69 (0.63-0.76) | <0.001 |
| Model 7 | 0.98 (0.90-1.07) | 0.81 (0.76-0.88) | 0.76 (0.70-0.84) | 0.66 (0.60-0.73) | <0.001 |

| **Other fruits** | **201.2 g/day** | **300.1 g/day** | **507.1 g/day** | **1040.7 g/day** | |
|---|---|---|---|---|---|
| Model 1 | 0.79 (0.73-0.85) | 0.68 (0.64-0.73) | 0.57 (0.53-0.62) | 0.41 (0.38-0.44) | <0.001 |
| Model 2 | 0.78 (0.72-0.84) | 0.70 (0.65-0.75) | 0.59 (0.55-0.64) | 0.42 (0.39-0.46) | <0.001 |
| Model 3 | 0.79 (0.73-0.85) | 0.70 (0.65-0.76) | 0.58 (0.54-0.63) | 0.43 (0.40-0.47) | <0.001 |
| Model 4 | 0.81 (0.75-0.88) | 0.74 (0.68-0.79) | 0.63 (0.58-0.68) | 0.51 (0.46-0.56) | <0.001 |
| Model 5 | 0.86 (0.80-0.93) | 0.78 (0.72-0.874) | 0.65 (0.60-0.70) | 0.49 (0.45-0.54) | <0.001 |
| Model 6 | 0.81 (0.75-0.88) | 0.74 (0.68-0.79) | 0.63 (0.59-0.68) | 0.51 (0.46-0.56) | <0.001 |
| Model 7 | 0.80 (0.74-0.87) | 0.71 (0.66-0.77) | 0.61 (0.56-0.66) | 0.50 (0.45-0.55) | <0.001 |

**Table 4.2:** Association of animal-based foods with AMI after multivariate adjustments

| | **Q2** | **Q3** | **Q4** | **Q5** | **P trend** |
|---|---|---|---|---|---|
| **White meat** | **16.0 g/day** | **48.0 g/day** | | **88.0 g/day** | |
| Model 1 | 0.99 (0.92-1.07) | 0.97 (0.91-1.02) | | 0.83 (0.77-0.89) | <0.001 |
| Model 2 | 0.99 (0.92-1.07) | 0.96 (0.91-1.02) | | 0.82 (0.75-0.88) | <0.001 |
| Model 3 | 0.97 (0.90-1.05) | 0.94 (0.88-1.00) | | 0.78 (0.72-0.84) | <0.001 |
| Model 4 | 1.00 (0.93-1.08) | 1.01 (0.95-1.08) | | 0.91 (0.84-0.99) | 0.24 |
| Model 5 | 1.03 (0.95-1.11) | 1.05 (0.98-1.12) | | 0.99 (0.90-1.08) | 0.64 |
| Model 6 | 1.00 (0.94-1.07) | 1.00 (0.95-1.07) | | 0.91 (0.84-1.00) | 0.23 |
| Model 7 | 0.97 (0.89-1.05) | 0.98 (0.92-1.04) | | 0.88 (0.80-0.96) | 0.13 |
| **Red meat** | **6.0 g/day** | **13.0 g/day** | | **39.0 g/day** | |
| Model 1 | 0.92 (0.87-0.96) | 0.92 (0.84-1.01) | | 0.95 (0.88-1.04) | 0.13 |
| Model 2 | 0.87 (0.83-0.92) | 0.81 (0.73-0.89) | | 0.80 (0.73-0.87) | <0.001 |
| Model 3 | 0.89 (0.84-0.93) | 0.83 (0.75-0.92) | | 0.83 (0.76-0.91) | <0.001 |
| Model 4 | 0.92 (0.87-0.97) | 0.90 (0.82-1.00) | | 0.98 (0.89-1.08) | 0.14 |
| Model 5 | 0.96 (0.91-1.01) | 0.93 (0.84-1.03) | | 0.99 (0.90-1.09) | 0.63 |
| Model 6 | 0.92 (0.87-0.97) | 0.90 (0.81-1.00) | | 0.97 (0.88-1.07) | 0.12 |
| Model 7 | 0.89 (0.84-0.94) | 0.85 (0.76-0.94) | | 0.88 (0.80-0.98) | 0.02 |
| **Sweet water fish*** | **142.1 g/day** | **208.3 g/day** | **295.6/ g/day** | **489.9 g/day** | |
| Model 1 | 1.06 (0.98-1.14) | 1.01 (0.94-1.09) | 1.07 (1.00-1.15) | 1.00 (0.94-1.08) | 0.58 |
| Model 2 | 1.08 (1.00-1.17) | 1.02 (0.94-1.10) | 1.09 (1.01-1.17) | 1.02 (0.95-1.10) | 0.39 |
| Model 3 | 1.06 (0.98-1.14) | 1.00 (0.92-1.07) | 1.05 (0.97-1.13) | 0.99 (0.91-1.07) | 0.64 |
| Model 4 | 1.13 (1.05-1.22) | 1.13 (1.05-1.22) | 1.31 (1.21-1.42) | 1.52 (1.39-1.66) | <0.001 |
| Model 5 | 1.19 (1.10-1.28) | 1.21 (1.12-1.31) | 1.38 (1.28-1.50) | 1.49 (1.37-1.62) | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| Model 6 | 1.13 (1.05-1.22) | 1.13 (1.05-1.22) | 1.31 (1.21-1.42) | 1.52 (1.39-1.67) | <0.001 |
| Model 7 | 1.09 (1.00-1.19) | 1.12 (1.04-1.22) | 1.31 (1.21-1.42) | 1.52 (1.38-1.67) | <0.001 |
| **Sea water fish*** | **1.3 g/day** | **2.4 g/day** | **13.5 g/day** | **59.3 g/day** | |
| Model 1 | 1.05 (0.97-1.13) | 0.97 (0.89-1.05) | 1.07 (0.99-1.15) | 1.24 (1.15-1.33) | <0.001 |
| Model 2 | 1.05 (0.94-1.15) | 0.94 (0.84-1.05) | 1.07 (0.97-1.18) | 1.19 (1.08-1.32) | <0.001 |
| Model 3 | 1.02 (0.94-1.10) | 0.93 (0.85-1.01) | 1.04 (0.96-1.12) | 1.14 (1.06-1.23) | 0.01 |
| Model 4 | 1.04 (0.96-1.13) | 1.03 (0.95-1.12) | 1.14 (1.05-1.28) | 1.39 (1.28-1.51) | <0.001 |
| Model 5 | 1.07 (0.99-1.17) | 1.07 (0.98-1.17) | 1.22 (1.12-1.32) | 1.45 (1.33-1.57) | <0.001 |
| Model 6 | 1.04 (0.96-1.13) | 1.03 (0.94-1.12) | 1.14 (1.05-1.23) | 1.39 (1.28-1.51) | <0.001 |
| Model 7 | 1.02 (0.94-1.11) | 0.98 (0.89-1.08) | 1.09 (1.00-1.18) | 1.33 (1.22-1.45) | <0.001 |

Association of total fish (sweet water and sea water) after adjusting for Model 4 was OR 1.64 95% CI 1.50-1.80, p value <0.001 when fifth quintile was compared to first quintile.

**Table 4.3:** Association of animal-based foods with AMI after multivariate adjustments

| | **Q2** | **Q3** | **Q4** | **Q5** | **P trend** |
|---|---|---|---|---|---|
| **Egg** | **3.9 g/day** | **25.3 g/day** | | **59.0 g/day** | |
| Model 1 | 0.82 (0.77-0.86) | 0.76 (0.72-0.80) | | 0.96 (0.88-1.04) | <0.001 |
| Model 2 | 0.86 (0.81-0.91) | 0.80 (0.76-0.85) | | 0.94 (0.87-1.03) | <0.001 |
| Model 3 | 0.90 (0.85-0.95) | 0.85 (0.80-0.90) | | 0.99 (0.91-1.08) | 0.004 |
| Model 4 | 0.90 (0.85-0.96) | 0.90 (0.85-0.95) | | 1.14 (1.05-1.25) | <0.001 |
| Model 5 | 0.93 (0.87-0.99) | 0.94 (0.89-1.00) | | 1.17 (1.07-1.28) | <0.001 |
| Model 6 | 0.90 (0.85-0.95) | 0.89 (0.84-0.94) | | 1.14 (1.04-1.24) | <0.001 |
| Model 7 | 0.89 (0.83-0.94) | 0.87 (0.83-0.93) | | 1.13 (1.03-1.24) | <0.001 |
| **Milk** | **16.5 g/day** | **35.8 g/day** | **107.3 g/day** | **250.0 g/day** | |
| Model 1 | 0.93 (0.87-1.00) | 0.89 (0.80-1.00) | 0.85 (0.79-0.91) | 0.87 (0.80-0.95) | 0.003 |
| Model 2 | 0.99 (0.92-1.06) | 0.94 (0.83-1.05) | 0.90 (0.83-0.96) | 0.94 (0.86-1.02) | 0.17 |
| Model 3 | 1.02 (0.94-1.09) | 0.97 (0.86-1.09) | 0.94 (0.88-1.02) | 0.97 (0.89-1.05) | 0.68 |
| Model 4 | 1.04 (0.96-1.17) | 1.00 (0.90-1.13) | 1.05 (0.97-1.12) | 1.12 (1.06-1.27) | 0.14 |
| Model 5 | 1.08 (1.00-1.17) | 1.06 (0.94-1.20) | 1.08 (1.00-1.17) | 1.13 (1.03-1.24) | 0.22 |
| Model 6 | 1.04 (0.96-1.15) | 1.00 (0.89-1.12) | 1.04 (0.97-1.13) | 1.16 (1.06-1.27) | 0.14 |
| Model 7 | 1.05 (0.97-1.13) | 1.06 (0.94-1.20) | 1.07 (0.98-1.15) | 1.18 (1.07-1.30) | 0.09 |
| **Yoghurt** | | | **16.5 g/day** | **107.3 g/day** | |
| Model 1 | | | 0.73 | 0.63 | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| | | | (0.68-0.78) | (0.55-0.73) | |
| Model 2 | | | 0.74 (0.69-0.80) | 0.62 (0.53-0.72) | <0.001 |
| Model 3 | | | 0.71 (0.66-0.77) | 0.59 (0.51-0.69) | <0.001 |
| Model 4 | | | 0.76 (0.71-0.83) | 0.70 (0.60-0.82) | <0.001 |
| Model 5 | | | 0.79 (0.73-0.85) | 0.67 (0.58-0.79) | <0.001 |
| Model 6 | | | 0.77 ( 0.71-0.83) | 0.70 (0.60-0.82) | <0.001 |
| Model 7 | | | 0.74 (0.69-0.81) | 0.68 (0.58-0.80) | <0.001 |

**Table 4.4:** Association of other food groups with AMI after multivariate adjustments

| | **Q2** | **Q3** | **Q4** | **Q5** | **P trend** |
|---|---|---|---|---|---|
| **Pulses** | **53.4 g/day** | **121.4 g/day** | **179.0 g/day** | **310.5 g/day** | |
| Model 1 | 0.86 (0.80-0.92) | 0.86 (0.81-0.91) | 0.78 (0.70-0.86) | 0.93 (0.86-1.00) | <0.001 |
| Model 2 | 0.85 (0.79-0.92) | 0.81 (0.76-0.86) | 0.75 (0.68-0.84) | 0.85 (0.79-0.92) | <0.001 |
| Model 3 | 0.83 (0.77-0.90) | 0.78 (0.73-0.83) | 0.70 (0.63-0.78) | 0.81 (0.75-0.87) | <0.001 |
| Model 4 | 0.87 (0.80-0.94) | 0.83 (0.78-0.88) | 0.82 (0.73-0.91) | 1.02 (0.94-1.11) | <0.001 |
| Model 5 | 0.89 (0.82-0.96) | 0.88 (0.82-0.93) | 0.88 (0.79-0.99) | 0.98 (0.90-1.06) | 0.04 |
| Model 6 | 0.87 (0.80-0.94) | 0.83 (0.78-0.88) | 0.82 0.73-0.91) | 1.03 (0.94-1.12) | <0.001 |
| Model 7 | 0.85 (0.92-1.09) | 0.79 (0.74-0.85) | 0.77 (0.68-0.86) | 0.96 (0.88-1.05) | <0.001 |
| **Boiled white rice** | | **1161 g/day** | | **1548 g/day** | |
| Model 1 | | 0.97 (0.92-1.02) | | 0.70 (0.64-0.76) | <0.001 |
| Model 2 | | 0.93 (0.88-0.98) | | 0.74 (0.68-0.81) | <0.001 |
| Model 3 | | 1.00 (0.95-1.06) | | 0.83 (0.76-0.90) | <0.001 |
| Model 4 | | 1.11 (1.06-1.17) | | 1.00 (0.91-1.09) | 0.02 |
| Model 5 | | 0.90 (0.85-0.95) | | 0.74 (0.67-0.82) | <0.001 |
| Model 6 | | 1.11 (1.05-1.17) | | 1.00 (0.94-1.09) | 0.02 |
| Model 7 | | 1.11 (1.05-1.17) | | 1.06 (0.96-1.16) | 0.05 |
| **Biryani** | **27.7 g/day** | | | **60.1 g/day** | |
| Model 1 | 1.10 (1.06-1.15) | | | 1.14 (1.04-1.24) | 0.008 |
| Model 2 | 1.08 (1.03-1.12) | | | 1.02 (0.93-1.13) | 0.12 |
| Model 3 | 1.04 (1.00-1.09) | | | 0.99 (0.90-1.09) | 0.42 |
| Model 4 | 1.14 (1.09-1.18) | | | 1.25 (1.13-1.39) | <0.001 |
| Model 5 | 1.15 (1.11-1.20) | | | 1.22 (1.09-1.36) | 0.001 |
| Model 6 | 1.14 (1.09-1.18) | | | 1.25 (1.13-1.40) | <0.001 |
| Model 7 | 1.10 | | | 1.16 | 0.03 |

| | | | | | |
|---|---|---|---|---|---|
| | (1.05-1.15) | | | (1.04-1.29) | |
| **Bread** | **22.4 g/day** | **57.1 g/day** | **117.7 g/day** | **209.0 g/day** | |
| Model 1 | 1.05 (0.98-1.13) | 1.10 (1.03-1.19) | 1.35 (1.26—1.45) | 1.18 (1.10-1.27) | <0.001 |
| Model 2 | 1.07 (1.00-1.16) | 1.05 (0.98-1.14) | 1.28 (1.19-1.38) | 1.16 (1.08-1.26) | <0.001 |
| Model 3 | 1.04 (0.97-1.12) | 0.99 (0.91-1.07) | 1.09 (1.00-1.17) | 0.87 (0.80-0.94) | 0.001 |
| Model 4 | 1.08 (1.00-1.16) | 1.04 (0.96-1.12) | 1.15 (1.06-1.24) | 0.98 (0.90-1.06) | 0.04 |
| Model 5 | 1.12 (1.03-1.21) | 1.04 (0.96-1.13) | 1.12 (1.03-1.21) | 0.94 (0.86-1.03) | 0.02 |
| Model 6 | 1.08 (1.00-1.17) | 1.04 (0.96-1.12) | 1.15 (1.07-1.24) | 0.98 (0.90-1.07) | 0.03 |
| Model 7 | 1.04 (0.96-1.13) | 0.99 (0.91-1.07) | 1.06 (0.98-1.15) | 0.93 (0.85-1.01) | 0.17 |

After Bonferroni corrections (p value = 0.05/112= <0.001). The associations remained significant for green leafy and stem vegetables, other vegetables, vitamin C rich fruits, other fruits, sweet water fish, sea water fish, eggs, yoghurt, pulses and biryani in Model 4.

**footnotes table 4.1-4:**

Model 1: adjusted for age and sex

Model 2: Model 1+ smoking status, physical activity, occupation, education and income

Model 3: Model 2 + history of diabetes, history of hypertension, family history of MI

Model 4: Model 3 plus total energy intake.

Model 5: Model 3 + all food groups and spices

Model 6: Model 4 + medications (anti-hypertensives and anti-diabetics)

Model 7: Model 4 + waist-to-hip ratio, LDL-C and HDL-C

# Chapter 5: Derivation of the dietary patterns, their correlates and risk of acute myocardial infarction in Bangladesh

## 5.1 Chapter summary

This chapter examines the association between dietary patterns and risk of acute myocardial infraction (AMI) from the BRAVE case-control study in Bangladesh. Principal component analysis (PCA) was used to identify dietary patterns derived from data collected through food frequency questionnaire (FFQ) from cases and controls. The resulting dietary patterns were converted into quintiles. Logistic regression was used to investigate the association of dietary patterns with the risk of AMI adjusting for potential confounders and mediators. Three dietary patterns were identified: (1) the “energy dense food pattern”, characterised mainly by higher intake of fried and savoury snacks, biryani, red meat, sugar-sweetened beverages (SSB) and sweets; (2) the “vegetable pattern” was characterised by leafy and other vegetables, pulses and sweet-water fish, and (3) the “fruits and dairy pattern” was characterised by citrus and other fruits, milk, yoghurt and sweet water fish.

The results from this analyses indicate that: (1) “energy dense food pattern” had no significant association with the risk of AMI after multivariate adjustments; (2) “vegetable pattern” and “fruit and dairy pattern” were associated with a nearly linear significant inverse association of AMI after multivariate adjustments; (3) there were modest effect of potential confounding when adjusted for different covariates; (4) all associations were largely unaffected by subgroup analyses according to various individual characteristics; (5) when stratified by age and education levels, for “vegetable pattern” an inverse association was only observed in younger age groups and in those who had primary and secondary education; (6) a syronger association between highest quintile of “fruits and dairy pattern” and AMI was observed in high income level group as compared to income levels below poverty line and (7) various sensitivity analyses had only minimal impact on overall findings.

## 5.2 BACKGROUND

As discussed in **Chapter 1** dietary pattern analysis is a relatively new approach to study diet-disease associations.[1] Traditionally the evidence on diet and disease associations was focused on nutrients, however the food we consume is made up of a myriad of nutrients that may act synergistically or antagonistically to affect the risk of CHD.[2] Many studies have also investigated how food groups affect the risk of CHD.[3] It is important to note, however, diet is a complex exposure, we consume foods as part of meals which consists of combination of other food groups; therefore it is more informative to study the overall dietary pattern and its association with CHD risk.[4] In this context, dietary pattern analysis offers a more holistic approach to study diet-disease associations, allowing assessment of possible interactions between food groups. It is postulated that findings of dietary patterns analysis is better amenable to nutritional guidelines and to be used in public health practice.[5] In addition, dietary guidelines based on cohesive dietary patterns may be easier for public to understand than to follow guidelines on individual nutrients.[6]

There are several ways to investigate dietary patterns or habits in the population. Some investigators have used a hypothesis driven “a priori” approach, which uses available scientific evidence to generate scores and indices that measure adherence to current dietary recommendations.[7,8] Whereas, others have used empirically driven “a posteriori” approach, that uses statistical data reduction methods such as PCA or cluster analysis to derive dietary patterns.[9] Data driven methods identify dietary habits of participants in the study. In addition to these methods, a hybrid method called Reduced Rank Regression (RRR) has also been used by some studies. This method uses prior knowledge to define response variables (intermediate variables or biomarkers of CHD) and then determines the combination of food groups that may explain how these response variables are associated with the disease.[10,11] The next section describes the different methods used to derive dietary patterns in a population.

### 5.2.1 "A priori" defined scores and indices to define dietary patterns

Score based approaches are based on dietary recommendations and assess degree of adherence to healthy diets, examples include the Healthy Eating Index (HEI)[12], Alternative Healthy Eating Index (AHEI),[13] Mediterranean diet score[14] and the Dietary Approaches to Stop Hypertension (DASH) diet.[15] In the HEI, individuals are scored on 12 components and then are ranked within the population; a higher score indicates better adherence.[16] However, this has been shown to be ineffective in predicting the risk of chronic disease so an AHEI was constructed.[13] This takes into account different types of fats, carbohydrates and sources of protein.

One widely used diet score is the Mediterranean diet score which incorporates the traditional eating habits of people from countries bordering the Mediterranean Sea. This score is largely based on high intake of fruits, vegetables, legumes, fish, whole grains, nuts, and olive oil, moderate intakes of alcohol and low intakes of meat and dairy products.[17] Mediterranean diet score has shown to lower the risk of cardiovascular disease (CVD) in randomised controlled trials (RCTs). In the Lyon Heart trial cases of first MI randomized to Mediterranean diet showed a lower risk of second CVD event after 4 years of follow up.[18] Later in the PREDIMED trial[17] about 7500 high risk participants were randomly assigned to one of the three groups; Mediterranean diet 1 supplemented with extra-virgin oil, Mediterranean diet 2 supplemented with mixed nuts and a control group who were given advice to reduce dietary fat. After 5 years of follow-up there was 30% reduced risk of CVD with Mediterranean diet 1 and 2 as compared to the control group, with significant improvements in blood pressure, lipids and oxidative stress.[17] These studies underscore the importance of dietary patterns in relation to CVD risk.

The advantages of using "a priori" defined scores are that they are easily reproducible and comparable. However, there are many limitations of using this method to derive dietary patterns. First, subjectivity is introduced in the selection of food groups to match the dietary guidelines.[19,20] Second, the guidelines are general and may not be specific to the outcome/disease of interest. Third, dietary guidelines often change so these may not be truly optimal for health. Fourth, it is also important to consider that different populations may have different dietary guidelines, therefore a dietary score based on one population may not be applicable to another population. Fifth, dietary indices and scores may miss critical components in the diet for example the Mediterranean diet score missed sugar intake while calculating the score. Sixth, summation of equally weighted dietary scores may mean that each score is equally important and additively related to health.[21] Lastly, dietary habits change over time and scores may not reflect that.

### 5.2.2 Data driven "a posteriori" approaches to define Dietary patterns

Empirically driven "a posteriori" methods use statistical data reduction methods such as PCA and cluster analysis to derive dietary patterns.[9] Typical dietary patterns defined by these method are healthy or prudent dietary patterns (mainly fruits, vegetables, fish, whole grains and poultry) and unhealthy or western dietary patterns (mainly meat, processed meat, refined grains, sweets, sugar drinks and fried foods).[9,22,23]

(1) Principal Component Analysis

The most common factor analysis method used in nutritional epidemiology is PCA, a form of exploratory factor analysis.[9] The primary advantage of using such approach is that the healthy eating pattern is not defined as priori and is based on statistical methods using the dietary data collected in the study. PCA reduces large number of variables into small number of variables. The new variables (or patterns) are created from the combinations of the original variables based on correlations between them. This method allows for the variation in food intake in the population, allowing observation of interactions between food groups.[20]

The study of dietary patterns using PCA offers a broader view of food and nutrient consumption and overcomes the methodological limitations related to the study of single nutrients or foods.[24] However, there are many limitations of this method. It has been criticised for the subjective way in which the food groups are categorised before applying PCA. In addition, decisions such as standardization, the number of factors chosen, the analytical approach used (use of rotation), the value of the factor loading chosen to describe the factors and naming of the dietary patterns may influence the results. Furthermore, although the original aim of PCA is to reduce detailed information into smaller number of interpretable variables that have the characteristic of explaining high variability in the target behaviour. However, variance explained by dietary data available in literature is usually small. This small amount is attributed to the multidimensionality of diet.[25]

(2) Cluster analysis

Cluster analysis is another data driven method which groups together participants in mutually exclusive groups based on their dietary habits.[21,26] In other words, cluster analysis examines whether or not there are groups in the population that are markedly different from one another, and if so, what characterises their diets. This method puts individuals in mutually exclusive groups based on similarities between individuals. Large clusters represent general behaviours shared by many individuals and small clusters represent very specific behaviours shared by few individuals.[27] Limitations include subjective decisions about how many clusters to choose, how many people in each group,

interpretation and labelling of clusters. Cluster analysis is sensitive to outliers and it has reduced statistical power as compared to PCA.[9]

(3) The Reduced Rank Regression

RRR is a combination of both data driven and knowledge based approaches.[10] It is similar to PCA, but it derives patterns that are predictive of intermediary (response) variables associated with disease.[10,28] It uses disease specific response variables such as biomarkers based on prior scientific knowledge to determine combinations of food groups or dietary patterns associated with the known disease risk factor. For example a study used response variables such as cholesterol and lipoprotein(a) as a predictor of future MI, coronary death, and angina pectoris.[29] Strengths of this approach include evaluation of biological pathways between diet and disease through response variables. However, its use depends on the availability of data on response variables.

In summary, there are different methods that have been used to derive dietary patterns, each with its strengths and limitations. Although several studies have investigated the association of dietary patterns with CHD, however there is scarce evidence from Bangladesh.[30] The primary objective of the current analyses is to assess the association of dietary patterns with the risk of AMI, incorporating adjustments for potential confounding and mediators using the BRAVE case-control study. For this thesis I used PCA to identify dietary patterns in Bangladesh. There is no gold standard statistical method to derive dietary patterns, different approaches answer different questions, so the choice of method depends on the research question is. The rationale of using PCA is mentioned in the discussion section of this chapter in detail. Briefly, this approach has an advantage of not making any prior assumptions about the eating habits of the population. It uses correlations between food groups to identify underlying dietary patterns. The present study differs from previous studies in South Asia in important ways. First, it is largest dietary study of CHD in a South Asian population (Bangladesh). Second, it characterises the shapes of association of dietary patterns with CHD adjusting for potential confounders and mediators. Finally, it does several subgroup analyses and sensitivity analyses to reinforce the observed associations.

The specific aims of this chapter are to: (1) derive the dietary patterns in BRAVE study using PCA in cases and controls; (2) study the cross-sectional correlates dietary patterns with several baseline characteristics; (3) investigate the association of dietary patterns with risk of AMI adjusting for potential confounders and mediators and (4) the potential effect modification by age, sex, location, smoking status, BMI, physical activity and education level.

## 5.3 METHODS

### 5.3.1 Participants

Details of study selection, data collection and harmonisation procedures have been described in **Chapter 2**.

### 5.3.2 Derivation of dietary patterns

PCA is a data driven technique that reduces the dimension of the data and groups correlated variables. Dietary patterns derived by PCA reflect foods consumed by participants with a high degree of inter-correlation.[24] PCA was conducted using the food groups in grams or millilitres per day defined in **Table 2.4 Chapter 2,** in cases and controls without normalising the intake or standardising the intake to a set daily energy intake. Spices were not included in the PCA as they are part of cooked food and act as condiments. To achieve better interpretability, an orthogonal rotation (the varimax option in STATA 14) was used to derive dietary patterns uncorrelated to one another. This method does not change the basic aspects of the analysis and is used only to make the results more interpretable. To determine the number of meaningful diet patterns, conventional criteria for PCA including eigenvalue, the scree test, and the interpretation criterion were considered.[31] To determine what dietary patterns to retain typically an eigenvalue of ≥ 1 is considered meaningful. However, in this analysis the elbow of the scree plot (a graphic representation of eigenvalues >1.4) was considered to limit the meaningful factors to three as demonstrated in **Appendix 6**. Each food group received a factor loading, making it possible to identify the food groups most correlated with each diet pattern. Component loadings >0.25 were presented in bold and considered meaningful. For every subject factor scores were calculated on each of the three retained factors by summing the consumption (g/day) multiplied by factor loadings across all food items. The dietary patterns were named depending on the highest factor loadings.

### 5.3.3 Correlates of dietary patterns

Resulting dietary patterns were converted into quintiles. Contribution of food groups to Quintile 1 vs Quintile 5 of dietary patterns were presented in median intakes with inter-quartile range in controls only. This was done to study the intakes of food groups within each dietary pattern. Baseline characteristics of controls were presented comparing Quintile 1 vs Quintile 5 of the distribution of the dietary patterns with continuous variables reported as means and standard deviations, and categorical variables as percentages.

### 5.3.4 Association of dietary patterns with acute myocardial infraction

Multivariable adjusted logistic regression was performed to evaluate associations between dietary patterns and risk of AMI.

Six models were applied:

1) Model 1- The minimally adjusted model was adjusted for age and sex.

2) Model 2- additionally adjusted for smoking status (never, ex and current), physical activity (using cut offs of <600 and ≥ 600 Metabolic Equivalent of Task (METs)), annual income (income below the poverty line, low income, medium income and high income), education level (no schooling, primary, secondary, university/vocational) and occupation (business/professional, manual labour, non-manual labour and unemployed/student/retired).

3) Model 3- additionally adjusted for history of disease related variables: history of diabetes (yes or no), history of hypertension (yes or no) and family history of MI (yes or no)

4) Model 4 or the primary model -additionally adjusted for total energy intake (kcal/day).

5) Model 5- Model 4 + additionally adjusted for medications (anti hypertensives and anti-diabetics)

The "main" associations compared the age and sex model (Model 1) with the Model 4.

### 5.3.5 Mediators

Waist-to-hip ratio, blood LDL-C and HDL-C were not included in the models as these are considered potential mediators rather than confounders. Additional sensitivity analyses were also done to adjust for these potential mediators in Model 4:

6) Model 6- Model 4 + waist-to-hip ratio, LDL-C and HDL-C

For all analyses the floating absolute risk (FAR) method was used.[21] FAR is used to compare risks in two groups, even if neither is used as a baseline group. This tries to eliminate the bias in selecting an arbitrary reference group when comparing two groups. Wald test was used to get p value for linear trend.

### 5.3.6 Subgroup and sensitivity analyses

To assess potential effect modification between exposures and baseline characteristics a covariate was included for the effect modifier and for the interaction term between exposure and effect modifier in the Model 4. Subgroup analyses were performed for age (<50 years or ≥50 years), sex (males, females), location (urban/rural), smoking status (ex, never, current), BMI (<23 kg/$m^2$ or ≥23 kg/$m^2$) physical activity (<600 METs or ≥ 600 METs), education level (no schooling, primary, secondary, university/vocational) and income levels (income below the poverty line, low income, medium income and high income) The Wald test was performed to get the p value for the interaction term across the quintiles of food consumption and these variables. These have been assessed in previous studies.

Sensitivity analyses were run in which I (1) excluded individuals with energy cut offs from the PURE study (<400 and >5000 kcal/day);[32] (2) adjusted energy by density method and used those as input variables in PCA (3) computed the matrix of loadings in controls only and inferred scores for cases and controls and (4) split population in half to see if the dietary patterns generated were similar. This was done to ensure that the resulting patterns were valid and could be replicated, random split sample method was used in STATA to divide the sample in half.

As a sensitivity analysis, p values were corrected for multiple testing using Bonferroni correction which is described in the footnotes of the **Table 5.4**.

All statistical analyses were performed using STATA v.14 (Statacorp).

## 5.4 RESULTS

### 5.4.1 Description of dietary patterns and their correlates

*Dietary pattern description*

**Table 5.1** shows the factor-loading matrix, which lists the correlations between the food items on the three major dietary patterns identified. The dietary patterns were named based on the high loading of foods in the dietary pattern. The “energy dense food pattern” was characterised by higher correlation of fried snacks (0.43) and savoury snacks (0.39), biryani (0.39), red meat (0.38), SSB (0.36) and sweets (0.25). The “vegetable pattern” was characterised mainly by leafy vegetables (0.60), other vegetables (0.60), pulses (0.29) and sweet water fish (0.28). The “fruits and dairy pattern” was characterised by vitamin C rich fruits (0.46), milk (0.46), other fruits (0.45), yoghurt (0.39) and sweet water fish (0.26). The variance explained by the three patterns was about 33% collectively.

*Contribution of food groups to dietary patterns in BRAVE controls*

**Table 5.2** shows the median intakes of food groups in Quintile 1 vs Quintile 5 of the three dietary patterns. In the highest quintile of “energy dense food pattern” the median intake of fried snacks was 51.8 g/day, savoury snacks was 60.0 g/day, biryani was 27.7 g/day, red meat was 13.0 g/day, SSB was 35.8 g/day and sweets was 30.7 g/day. In the highest quintile of “vegetable pattern” median intake of leafy vegetables was 257.0 g/day, other vegetables was 856.1 g/day and pulses was 179.0 g/day. In the highest quintile of “fruits and dairy pattern” intake of vitamin C rich fruits was 171.2 g/day, milk was 107.3 g/day, other fruits was 924.8 g/day and yoghurt was 16.5 g/day. Sweet water fish had approximately similar intakes in the “vegetable” and “fruits and dairy patterns”. The intakes of deep fried and savoury snacks were low in the “vegetable” and “fruits and diary pattern” as compared to the energy dense food pattern when comparing highest quintile.

*Association of dietary patterns with baseline characteristics in controls*

**Table 5.3** compares the general characteristics of controls in quintile 1 and quintile 5 of each dietary pattern. Controls in the highest quintile (quintile 5) as compared to the lowest quintile (quintile 1) of “energy dense food pattern” tended to be younger (46.9 (10.1) vs 55.4 (9.8)), more educated, had higher annual income, less likely to be currently smoking (61.2% vs 66.3%), slightly less physically active, had marginally higher percentage of history of diabetes and higher family history of MI and were mostly residing in urban areas. Those in the highest quintile of “vegetable pattern” were younger (50.9 (9.8) vs 52.8 (10.7)), more educated, had higher annual income (less percentage of participants below poverty line), marginally less likely to be current smokers, more physically active, had

higher percentage of history of diabetes, hypertension and family history of MI and were mostly residing in rural areas. Controls in the highest quintile of “fruits and dairy pattern” were slightly younger, more educated, had higher annual income, less likely to be currently smoking (60.2% vs 66%), more physically active (83.3% vs 71.1%), had higher family history of MI and were mostly residing in urban areas. There were no substantial differences in history of diabetes and hypertension in those in the highest versus lowest quintile of “fruits and dairy pattern”.

### 5.4.2 Association of energy dense food pattern with risk of acute myocardial infraction

In the age and sex adjusted model, the "energy dense food pattern" was inversely associated with the risk of AMI but the association was weak. Odds ratios (ORs) were 0.90 (95 % CI 0.84-0.97) for quintile 2, 0.94 (95 % CI 0.87-1.01) for quintile 3, 0.85 (95 % CI 0.79-0.92) for quintile 4 and 0.86 (95 % CI 0.80-0.93) for quintile 5 (p for trend 0.03) **(Figure 5.1, Table 5.4)**. After adjustment for additional covariates in Model 4, the ORs for highest quintile of "energy dense food pattern" were 0.90 (95% CI 0.83-0.97) for quintile 2, 0.97 (95% CI 0.90-1.05) for quintile 3, 0.95 (95% CI 0.88-1.03) for quintile 4 and 1.05 (95% CI 0.95-1.16) for quintile 5 (p trend 0.10) **(Figure 5.1, Table 5.4)**.

Further supplementary analyses to additionally adjust for potential mediators in Model 6 slightly attenuated the observed associations **(Figure 5.2)**.

Overall adjustment with Models 1-3 showed an inverse association of "energy dense food pattern" with the risk of AMI. However, after adjustment for energy intake in Model 4 the associations became non-significant **(Table 5.4)**.

*Subgroup analyses*

When stratified by smoking status, current smokers in the highest quintile of "energy dense food pattern" appeared to have a higher association with the risk of AMI. However, the test for trend was non-significant **(Figure 5.11)**. There were no significant interactions observed between energy dense food pattern and age, sex, location, BMI, physical activity, education level and income levels after adjusting for Model 4 **(Figures 5.7-10, 5.12-14)** (p value >0.05 for all)**.**

### *5.4.3 Association of vegetable pattern with risk of acute myocardial infraction*

In the age and sex adjusted Model 1, as compared to the lowest quintile, ORs for the highest quintile of "vegetable pattern" were inversely associated with the risk of AMI. ORs were 0.81 (95 % CI 0.75-0.87) for quintile 2, 0.82 (95 % CI 0.76-0.88) for quintile 3, 0.72 (95 % CI 0.67-0.78) for quintile 4 and 0.63 (95 % CI 0.59-0.68) quintile 5 (p for trend <0.001) **(Figure 5.1, Table 5.4)**. The associations were slightly attenuated after multivariate adjustments in Model 4. In Model 4, compared to the lowest quintile the adjusted ORs were 0.81 (95% CI 0.73-0.90) for quintile 2, 0.88 (95% CI 0.78-0.98) for quintile 3, 0.80 (95% CI 0.72-0.90) for quintile 4 and 0.77 (95% CI 0.68-0.88) for quintile 5 (p-trend <0.001) **(Figure 5.1, Table 5.4)**.

Further supplementary analyses to additionally adjust for potential mediators in Model 6 did not alter the observed associations **(Figure 5.2, Table 5.4)**.

Overall, there was no difference in the overall direction or non-significance of the associations observed with different levels of adjustment for potential confounders and mediators **(Table 5.4)**.

*Subgroup analyses*

When stratified by age, strong inverse association between highest quintile of vegetable pattern and AMI was only observed in younger age groups (<50 years old) compared to older age groups: OR of 0.61 (0.51-0.74) versus 0.89 (0.78-1.04) (p trend 0.01) **(Figure 5.7)**. When stratified by education level, there appeared to be a stronger association only in those who had primary and secondary education (p trend 0.01) **(Figure 5.13)**. No significant interactions were seen when vegetable pattern was stratified by sex, location, smoking status, BMI, physical activity and income levels after adjusting for Model 4 (p values >0.05) **(Figures 5.8-12 and 5.14)**.

### 5.4.4 Association of fruits and dairy pattern with risk of acute myocardial infraction

An inverse association was also observed between the "fruits and dairy pattern" and risk of AMI in age and sex adjusted Model 1 **(Figure 5.1, Table 5.4)**. ORs were 0.92 (95 % CI 0.85-0.98) for quintile 2, 0.74 (95 % CI 0.69-0.80) for quintile 3, 0.68 (95 % CI 0.63-0.73) for quintile 4 and 0.55 (95 % CI 0.51-0.59) quintile 5 (p for trend <0.001). These associations were markedly attenuated after multivariate adjustments Model 4. In Model 4, compared to the lowest quintile the adjusted ORs were 0.99 (95% CI 0.91-1.06) for quintile 2, 0.85 (95% CI 0.79-0.91) for quintile 3, 0.84 (95% CI 0.78-0.91) for quintile 4 and 0.76 (95% CI 0.69-0.84) for quintile 5 (p for trend <0.001) **(Figure 5.1, Table 5.4)**.

Further supplementary analyses to additionally adjust for potential mediators in Model 6 did not alter the observed associations **(Figure 5.2)**

Overall, there was no difference in the overall direction or non-significance of the associations observed with different levels of adjustment for potential confounders and mediators **(Table 5.4)**.

*Subgroup analyses*

A stronger association between highest quintile of "fruits and dairy pattern" and AMI was observed in high income level group as compared to income levels below poverty line **(Figure 5.14)**. Although it was observed that there was a strong inverse association between highest quintile of "fruits and dairy pattern" and AMI only in younger age groups (<50 years old) compared to older age groups (≥50 year old), but the interaction was non-significant. Furthermore, those who had high level of education appeared to show a stronger association, however the test for trend was non-significant. Overall, as shown in

**Figures 5.7-13** there were no significant interactions between age, sex, location, BMI, smoking status, physical activity and education level across quintiles after adjusting for Model 4 (p value >0.05 for all).

### 5.4.5 Sensitivity analyses

Similar associations were observed when a sensitivity analysis was conducted by excluding people with high and low energy intakes using cut offs from the PURE study **(Figure 5.3)**. This led to exclusion of 622 observations. As participants who reported extreme energy intakes were about 4% so therefore, they were not excluded from all main analyses as we were unsure if it is true intake or implausible energy intake. Sensitivity analysis by using energy adjusted food variables as input variables in PCA **(Figure 5.4)** has no substantial effect on the "vegetable" and "fruits and dairy pattern". Although the "energy dense food pattern" showed a weak inverse association in quintiles 2 and 4 only, overall there was no significant association. Computing factor loadings for controls only and using the scores for cases and controls also yielded similar results to the main Model 4 **(Figure 5.5)**. Lastly, when the study population was randomly split into half, again similar associations were observed **(Figure 5.6)**.

## 5.5 DISCUSSION

### 5.5.1 Summary of main findings

This chapter reported the association between dietary patterns and risk of AMI in the BRAVE study. Three dietary patterns were identified in the Bangladeshi population; the "energy dense food pattern" was characterised by higher correlation of fried snacks and savoury snacks, biryani, red meat, SSB and sweets; the "vegetable pattern" was characterised mainly by leafy vegetables, other vegetables, pulses and sweet water fish and the "fruits and dairy pattern" was characterised by vitamin C rich fruits, milk, other fruits, yoghurt and sweet water fish. Dietary patterns were derived using PCA, a method that represents the total dietary intake, accounting for interactions between nutrients and other components within the food groups.[52]

As for median intakes of food groups constituting the dietary patterns in the highest quintile, the "energy dense food pattern" had the highest median intakes of fried and savoury snacks, SSB and red meat as compared to the other two patterns. As expected, the "vegetable pattern" had the highest median intake of green leafy and other vegetables. Whereas the fruits and dairy pattern had the highest median intakes of fruits and dairy products as compared to other patterns. The intake of boiled white rice was similar in all patterns. As for sweet water fish the median intakes were broadly similar in the "vegetable" and "fruits and dairy pattern".

The present study found that overall higher scores on the three dietary patterns identified in this study were associated with higher socio-economic status and healthy behaviours. Those in the higher scores were younger, more educated, had higher annual income, less likely to be current smokers, and were more physically active. Higher scores on the "energy dense food pattern" and "fruits and dairy pattern" was observed in urban areas whereas "the vegetable pattern" was higher in rural areas. This may highlight that those in urban areas people have more access to energy dense foods than in rural areas. As Bangladesh is an agrarian based economy, people residing in rural areas may depend on vegetables for their diet.

As for associations with AMI, it was observed that largely independent of potential confounders and mediators, the "energy dense food pattern" had no significant association with risk of AMI. On the contrary the "vegetable pattern" and "fruits and dairy pattern" were associated with significantly lower risk of AMI. These associations were largely consistent across various subgroups of participants defined by baseline characteristics. However, when stratified by age and education level, inverse association with vegetable pattern was observed in younger age groups and those who had primary and secondary education. For "fruits and dairy" pattern a stronger inverse association was observed for

high income level group. The main results did not change appreciably after doing several sensitivity analyses.

### 5.5.2 Comparison with past literature and potential mechanisms

Dietary pattern analysis evaluates the cumulative effect of dietary patterns (combination of different food groups) and diseases; people eat meals that consist of complex combinations of nutrients present in different food groups that may act synergistically or interactively. Several studies of mostly western populations have assessed the association between dietary patterns and risk of CHD,[31,33–35] with scarce evidence from Bangladesh. Majority of the studies from the west have identified two distinct dietary patterns; prudent or healthy pattern (characterised by high intakes of fruits, vegetables, fish, whole grains and poultry) and the western or unhealthy pattern (characterised by intakes of meat, processed meat, refined grains, sweets, sugar drinks and fried foods).[23] Most studies have shown an inverse association with a healthy or prudent pattern, but the magnitude of the benefit varied considerably in different studies. In contrast, a weak association has been reported for the potential unhealthy or western pattern.[23]

"*Energy dense food pattern"*

The present analyses reported a weak increased association between highest intake of "energy dense food pattern" (mainly consisting of fried snacks and savoury snacks, biryani, red meat, SSB and sweets) and risk of AMI when after multivariate adjustments, however the test of trend was non-significant. When stratified by smoking status, an increased association with highest quintile of "energy dense food pattern" was only seen in current smokers. However, the test of trend was non-significant. This can be because the smokers may snack on fried foods while smoking or this could be a chance finding. This is concurrent to the finding that an increased association between highest intake of biryani and risk of AMI was observed only in current smokers **(Chapter 4)**. The deleterious manifestation of "energy dense pattern" with current smokers may be attributed to the earlier manifestation of AMI in smokers as compared to non-smokers and ex-smokers.

There is equivocal evidence on how the "western" dietary patterns affect the risk of CHD, which could be partly explained by the different composition of western or unhealthy dietary patterns in different populations. Although not directly comparable to BRAVE study, pooled results from a meta-analysis of nine cohort studies investigating the association between western/unhealthy patterns and risk of CHD also showed no significant associations.[23] However, the "energy dense food pattern" is different to what has been reported in western populations. In western populations the dietary pattern mainly consists of quantitatively higher loading of unprocessed meat, processed meat, refined

grains, sweets, sugar drinks and fried foods.[23] The “energy dense food pattern” in this study has an overlap with the western pattern reported by the INTERHEART study that was characterised by high loading of fried foods, salty snacks, eggs, and meat.[6] Consistent to the results of this study, the INTERHEART case-control “western pattern” was not associated with risk of AMI in South Asia but had a significant association with risk of AMI (Quartile 1 vs Quartile 4) in China, Southeast Asia and Central Europe.[6] However, it is important to consider that the INTERHEART had fewer participants from Bangladesh as compared to the BRAVE study and it did not report percentage of variance explained by the derived dietary patterns, questioning the precision of the results.

It is postulated that the unexpected lack of increased association with the “energy dense food pattern” may be due to the presence of mixture of foods in this dietary pattern. This pattern consisted of some legume based light snacks which may not be detrimental for the risk of MI. There is evidence to suggest that out of all foods, snacks are more likely to be underreported and this under-reporting can vary markedly in different groups in the population.[36] As snacks have substantial effect on energy intake and this may bias the associations towards null. It is also important to note that the range of consumption of fried snacks, sweets, SSB and red meat were very low in this dietary pattern and thus may not be detrimental for the risk of AMI. A study which compared data on diet quality in 187 countries also reported that Bangladesh ranked high on less consumption of unhealthy dietary pattern based on less consumption of seven unhealthy items as compared to the majority of the countries. However, it ranked low on adherence to a healthy dietary pattern based on greater consumption of ten more healthy items.[37] This suggests that in Bangladesh generally less potentially unhealthy foods are consumed.

*"Vegetable pattern" and "Fruits and Dairy pattern"*

The present analyses showed a protective role of the "vegetable pattern" (mainly consisting of green leafy and stem and other vegetables, sweet water fish and pulses) and "fruits and dairy pattern" (mainly consisting of vitamin C rich fruits, other fruits, milk, yoghurt and sweet water fish) in age and sex adjusted models. After adjustment for fully adjusted model the "vegetable pattern" showed 23% decrease in the risk of AMI (OR 0.77, 95% CI 0.68-0.88) and "fruits and dairy pattern showed 22% decrease in the risk of CHD (OR 0.76, 95% CI0.69-0.84). Although it is unlikely to find exact dietary patterns in other populations due to diversity of dietary habits in different populations and due to differences in methods used to identify patterns. Nevertheless, the dietary patterns identified in the BRAVE study are broadly similar to what has been reported before. The "vegetable and "fruits and dairy patterns" have an overlap with the prudent pattern which has been reported in studies in South Asia and the rest of the world.[6,23,38]

Specifically, the INTERHEART case-control study of 52 countries identified a prudent dietary pattern that was highly loaded on vegetables and fruits. They observed a strong inverse association comparing quartile 1 with quartile 4 (OR 0.70, 95% CI 0.61-0.80, p trend <0.001) after adjusting for all of the INTERHEART risk factors.[6] The US Health Professionals study also identified a dietary pattern highly loaded on green leafy vegetables, yellow vegetables, other vegetables, fruits, legumes and fish and observed a strong inverse association with CHD (OR 0.70, 95% CI: 0.56-0.86, p trend <0.001).[39] Similarly, in the EPIC- Spain cohort study of about 41,000 participants the evolved Mediterranean pattern characterised by high intakes of vegetables, fruits, olive oil whole grains and fish was associated with lower risk of CHD.[35] A recent meta-analysis of 11 cohort studies comparing highest versus lowest category of prudent dietary pattern with CHD also showed a significantly lower risk of CHD (OR 0.83, 95% CI 0.75-0.92, $I^2$ =44%).[23] In addition, consistent with the evidence of the current study results from 3 case-control studies (including the INTERHEART study) investigating prudent dietary patterns with risk with AMI showed an inverse association (OR 0.72, 95% CI, 0.63-0.80, $I^2$ 0.0%), similar strengths of association were observed with the vegetable pattern and fruit and dairy pattern.[23] Numerous mechanisms can explain the lower risk of AMI with the "vegetable pattern" and "fruit and dairy pattern". The two patterns are pre-dominated with vegetables and fruits, which are rich in vitamins, antioxidants, phytochemicals that are known to lower the risk of CVD.[40] The details of the mechanisms have been discussed in **Chapter 4**. Briefly, Fruits and vegetables are rich in vitamins, anti-oxidants, flavonoids, potassium, dietary fibre, which may affect many biological pathways such as protecting endothelial function, regulating lipid metabolism, inhibiting platelets function, alleviating ischemia/reperfusion injury, suppressing thrombosis, reducing oxidative stress, and

attenuating inflammation to lower the risk of developing CHD.[40,41] Additionally, evidence from various randomised clinical trials (RCTs) have shown reduced risk of intermediate indicators (high blood pressure, carbohydrate metabolism, oxidised low density lipoprotein and plasma C-reactive protein) with fruits and vegetable consumption.[42–44]

When stratified by age the inverse association with "vegetable pattern" was only seen in younger age groups in the present analysis. One possibility is that pathophysiological changes associated with old age may affect nutrient absorption and subsequent nutritional status in older population due to difference in metabolism. In addition, older people are more susceptible to AMI and may have co-morbidities that may explain the observed associations. Moreover, the differences could also be explained by residual confounding as young people have healthier lifestyles than older people. Additionally, there could also be a possibility that younger group consumes different quality of vegetables than the older group. When stratified by education levels with "vegetable pattern", an inverse association was only seen in those who had primary and secondary education levels. This may mean that educated people have more access to the foods present in the pattern or this could be a chance finding. In **Chapter 3**, BRAVE controls in the highest quintile (as compared to the lowest quintile) of total vegetable consumption tended to be more educated. When "fruits and dairy" pattern was stratified by income levels there was a stronger inverse association with higher income levels. This could be that people with higher incomes can afford relatively expensive foods such as fruits and dairy products as compared to people with low incomes. In **Chapter 3**, it was observed that controls in the highest quintile of total fruits consumption tended to have higher income levels as compared to controls in the lowest quintile. In addition, it may be postulated that people with high incomes have generally healthier lifestyles than those with low incomes. There is also measured and unmeasured confounding by higher socio-economic status as they have better health awareness and better access to healthcare which may explain the observed associations.

Although there is an overlap between the two patterns identified in the BRAVE study and the prudent dietary pattern but unlike the prudent dietary pattern the "vegetable pattern" in this study did not have a high loading of fruits and the "fruits and dairy pattern" did not have high loading of vegetables. Again, emphasising that it is difficult to find exact dietary patterns from different populations. It is also important to note, that these two patterns in the BRAVE study also included sweet water fish (main source of animal protein of a typical Bangladeshi diet). Although, a higher association with sweet water fish and risk of AMI was observed in **Chapter 4**, the loading of this food group is low in these two dietary patterns, and therefore the protective effect of vegetables/fruits may have counterbalanced the potential negative effect of sweet water fish. This signifies the need

for investigating dietary patterns as the associations for individual foods may be different when combined with other foods as part of a meal.

There are not many studies from Bangladesh that have investigated the association of dietary patterns with AMI risk. To my knowledge there is only one previous prospective cohort study (Health Effects of Arsenic Study- HEALS) of about 11,000 participants based in Araihazar Bangladesh that yielded a “gourd and root vegetable” dietary pattern that heavily relied on a variety of gourds, radishes, pumpkin, sweet potato, and spinach.[30] However, unlike the BRAVE study this dietary pattern was not associated with the risk of death from heart disease. However, it is possible that due to small number of events for heart disease (n=32), generating precise and reliable estimates was difficult in this study.

### 5.5.3 Strengths, Limitations and methodological considerations

*Choice of method to derive dietary patterns*

As aforementioned, there are many methods available to characterise food groups into dietary patterns such as “a priori” based approach using score/indices and posteriori-based approaches using data-driven techniques such as factor analysis and cluster analysis, and an approach that is a combination of both, RRR. No method has been regarded as the best approach as different methods answer different questions. Nevertheless, the majority of the studies from the west have investigated the association of dietary patterns and CVD using PCA.[23] In addition, one previous study from Bangladesh investigating dietary patterns and health outcomes has also used PCA to derive dietary patterns.[30] Although PCA and cluster analysis take alternate approaches to identify dietary patterns some studies have compared these two methods. These studies have demonstrated similarities in the identified dietary patterns.[45,46]

For the present analysis on the largest study on dietary patterns and risk of AMI in a Bangladeshi population, PCA was chosen to identify the patterns. This is because scores and indices are based on dietary guidelines and since there are no evidence based and reliable dietary guidelines from Bangladesh, scores may not be the best approach. In addition, scores and indices based on other populations such as the Mediterranean score may not be applicable to Bangladesh where the dietary patterns are more diverse than the Mediterranean countries. Although scores and indices reflect a particular eating patterns, they do not overcome the problem of multi-collinearity of various food groups, which is not the limitation of PCA.[33] In the present study, frequent intake of green leafy vegetables was positively correlated with frequent intakes of other vegetables; further, frequent intakes of biryani was positively correlated with frequent intakes of red meat and frequent intake of boiled white rice was negatively correlated with intake of white bread as reported in **Table 3.5 Chapter 3**. Hence, PCA uses inter-correlations between food groups within a diet to derive dietary patterns.[33]

As for data driven techniques other than PCA, cluster analysis is limited by low statistical power and is highly influenced by outliers.[9,47] In addition, the results from cluster analyses are less meaningful than those obtained from PCA. Whereas, RRR is more useful in identifying intermediate response variables/biomarkers that affect the disease outcome. The knowledge of these biomarkers depends on prior scientific knowledge. Therefore, PCA was used to avoid such decisions in a relatively understudied population of Bangladesh.

Nevertheless, there are several limitations of PCA. The subjective nature of PCA may influence the dietary patterns derived.[9,21,26] Decisions about the dietary items included in the analysis, grouping the dietary data, treatment of dietary data in grams/day or

frequencies or percentage of energy or standardization, the number of factors chosen, the analytical approach used (use of rotation), the value of the factor loading chosen to describe the factors and also naming of the dietary patterns may influence the results.[48] I am now going to discuss how I made these decisions to overcome some of the limitations while doing PCA.

*Determining number of food groups*

The number of foods selected as input variables in PCA is important. If there are too few foods added to the PCA it may not capture the differences in food consumption, on the other hand too many foods added to PCA may lead to odd combinations with little influence (for example if there are a few high consumers and many non-consumers of unusual foods, or when foods that are generally eaten together are broken down into subgroups).[21] I based food groupings on culinary use and nutrient content. These food groups used are comparable to those mentioned in a national nutrition survey conducted in Bangladesh.[49]

*Energy adjusted or unadjusted input variables*

There is little evidence on studies that have used energy adjusted food groups as input variables in PCA because individuals with higher energy intakes may have unduly large effects on the patterns. However, most studies have adjusted for energy intake after conducting PCA. A study compared energy adjusted and unadjusted input variables and suggested that there are no substantial differences between them.[50] I also did similar sensitivity analyses and found that there were no substantial differences in energy adjusted (density method) and unadjusted input variables used in conducting PCA.

*Determining number and names of derived of Dietary patterns*

Determining the number of components/dietary patterns to be included is a crucial step in PCA. Most studies base the number of derived factors based on eigen values, elbow of the scree plot and interpretability of factors. To decide the number of dietary patterns to be retained normally eigenvalues >1 are used as a cut-off **(Appendix 6)**, which significates that the dietary patterns explains more of the variance in the correlations than is explained by a single variable.[9,21,50,51] In the present study, I initially identified five components with eigenvalues exceeding 1. However, some of them were not interpretable, therefore I used the elbow of the scree-plot to derived three distinct dietary patterns (eigen value >1.4).[33] Most previous studies have derived two to three patterns [6,30,33,35] which is consistent with

this study. As mentioned, subjectivity is also introduced while naming the dietary patterns. I named the dietary patterns based on the highest factor loadings of food groups in the respective patterns.

*Variance explained*

The three derived dietary patterns explained 33% of variance which agrees with previous studies. However, it is important to note that the number of food items included in the PCA affects the variance explained. More food items present in the analyses decreases the variance.[25] A study reported that increasing the variance explained by the derived patterns did not appear to improve estimates of risk.[25]

*Reproducibly and generalisability*

Another challenge of PCA is the reproducibility and generalisability in other populations. The derived dietary patterns explain the variability of diet in a particular population and therefore it is unlikely that exactly the same dietary patterns will be found in a different population.[19] In addition, different studies have used different methods and therefore it is very challenging to compare dietary patterns in different studies. This is expected as dietary patterns are country specific and therefore different countries will have different dietary habits. However, there is some overlap of dietary patterns across different studies as most have identified prudent and western dietary patterns and this may imply a small possibility of reproducibility. In addition, when I did sensitivity analysis by randomly splitting data into half and observed similar dietary patterns in the two halves.

*Misclassification of food groups*

As discussed in **Chapter 4**, FFQ does not measure absolute intake which can lead to misclassification of food groups due to under and/or over-reporting by participants. FFQ can therefore affect the total energy intake estimate. This misclassification bias if occurs at random can attenuate the associations. However, if the misclassification is different across different groups then bias can go in either direction. In addition, it is important to note people change their dietary habits over time due to food availability, disease, pregnancy etc. The FFQ does not capture change in diet over time. When sensitivity analysis was done by excluding people who were low and high energy reporters with the aim to increase validity in dietary exposure by reducing misclassification due to misreporting of food groups, the results were in line with the main associations. Furthermore, the identified dietary patterns and their association with AMI risk were nevertheless largely consistent in this study when data were stratified by age, sex,

location, BMI, smoking status, physical activity and education levels. However, I cannot exclude factors which might be unmeasured causing residual bias.

*Residual confounding:*

Residual confounding is defined as "the distortion that remains after controlling for confounding in the design and/or analysis of a study".[52] Residual confounding can be caused by unmeasured factors, additional factors that were not adjusted for and factors that may not be measured precisely (e.g. socioeconomic factors).[52] Although the analyses were adequately adjusted for all conventional CHD risk factors as well as other potential confounders such as medical history. There is lack of data on additional factors which could be relevant (such as health systems related factors, contamination of food items, environmental pollution (indoor air pollution while preparing food, water contamination-water used for food preparation) and concomitant nutritional deficiencies (such as iron and vitamin D), which may have impacted the observed associations. In addition, there may be errors in self-reported measures such as medical history, socio-economic status and physical activity that may also affect the observed associations.

### 5.5.4 Implications

Findings from the analyses may have potential public health importance. The study derived three dietary patterns from an understudied population in Bangladesh using PCA. The study highlighted that "vegetable pattern" and "fruits and dairy pattern" reduce the risk of CHD. Dietary pattern analysis provides a more holistic approach to study diet-disease associations. Foods are not eaten as isolated items but as part of meals, therefore it is important that future dietary guidelines are based on dietary patterns than individual foods and nutrients.[5,6] Furthermore, diets focusing on single nutrients have had negative consequences that have led to the emergence of nutrient-defined diets such as the low-fat and low-carbohydrate diets.[5] The trend of low-fat diets did not talk about the replacement nutrient and resultantly led to excess intake of refined carbohydrates and added sugar, which can increase the risk of CHD.[5] Therefore, findings of dietary pattern analysis are more relevant to public health practice and more accessible for the general public to understand and comply to.[6] This study advocates that people should be encouraged to consume fruits, dairy and vegetables based dietary patterns to reduce the risk of CHD. The study also found that "energy dense pattern" had no significant association with the risk of CHD. However, the results should be interpreted carefully as this pattern had low loadings of potentially unhealthy foods.

The chapter also had implications for future epidemiological research. It is important to carry out PCA in other cities in Bangladesh to determine if the dietary patterns differ in

urban and rural areas of Bangladesh. In addition, a large prospective cohort study may be carried out that is more robust than a case-control study to confirm the observed associations. Once the observations have been confirmed the evidence from dietary pattern analysis can be used to form dietary guidelines specific to this population.

### 5.5.5 Conclusion

In this current chapter, evaluating the associations between dietary patterns and risk of AMI in Bangladesh revealed that the “vegetable pattern” and “fruits and dairy” pattern was inversely associated with risk of AMI largely independent of potential confounders and mediators. However, the “energy dense food pattern” had no significant association with the risk of AMI. These findings further underscore the importance of conducting dietary pattern analysis to study diet-disease associations, given the relative strength and limitation of this method. If confirmed in future studies, public health preventive guidelines, based on locally-appropriate dietary patterns, could help reduce the CHD burden in Bangladesh and in similar settings.

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**Figure 5.1:** Dietary patterns and risk of AMI after various multivariate adjustments

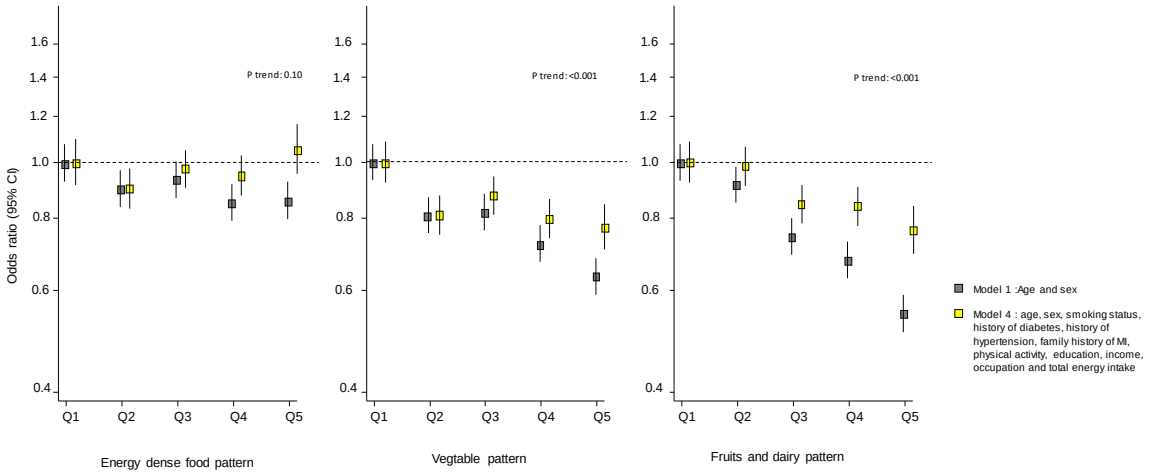



**Figure 5.2:** Dietary patterns and risk of AMI after adjustment of potential mediators

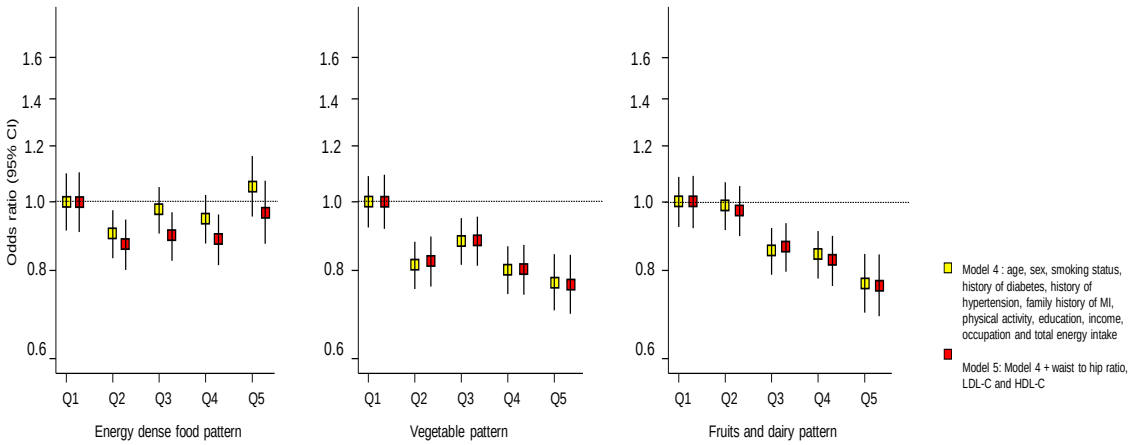


**Figure 5.3:** Association of dietary patterns and AMI by excluding participants with high and low energy intake using cut offs from PURE study

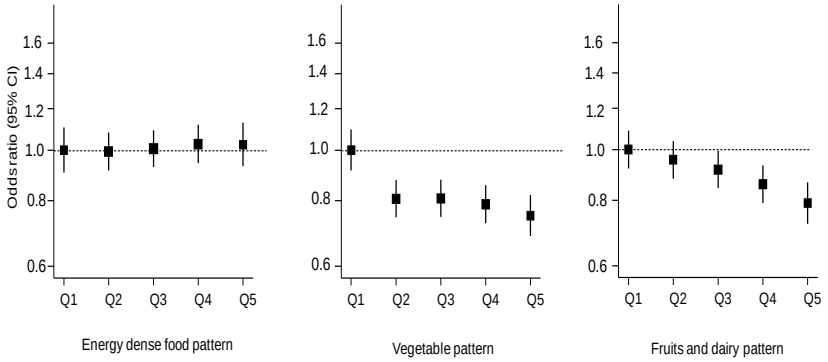
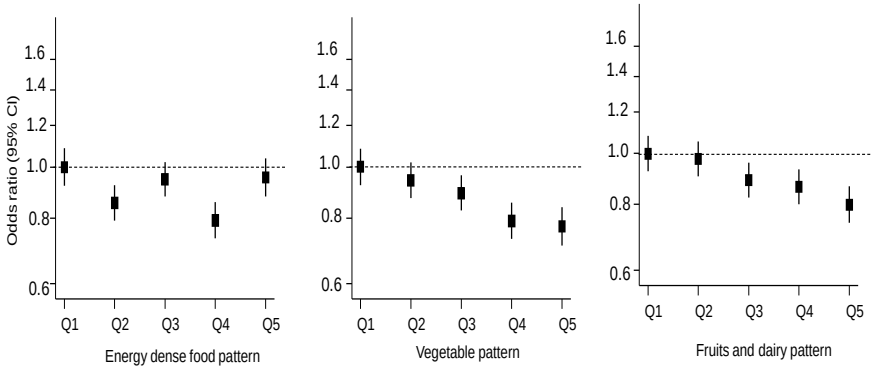



**Figure 5.4:** Association of dietary patterns and AMI by using energy adjusted variables as input variables




Odds ratios were estimated using logistic regression models adjusted for Model 4 (age, sex, smoking status, history of diabetes, history of hypertension, family history of MI, physical activity, education, income, occupation and total energy intake).

**Figure 5.5:** Association of dietary patterns and AMI by creating quintiles in controls and
using those for cases

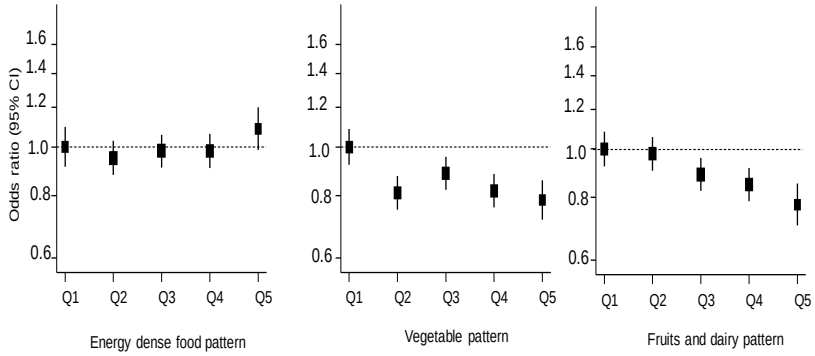
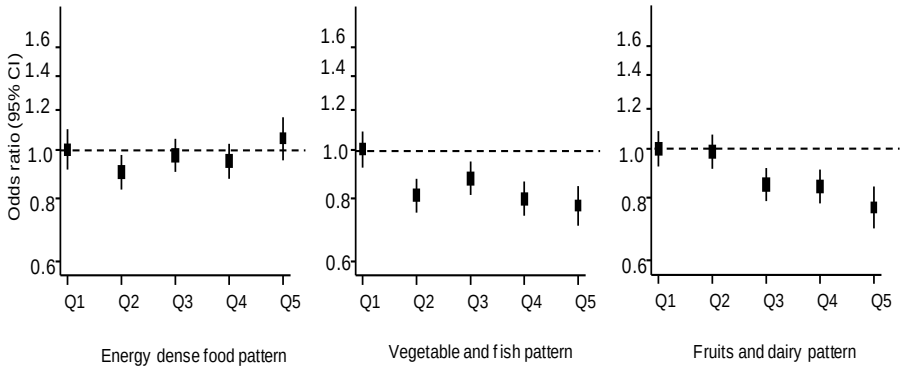



**Figure 5.6:** Association of dietary patterns and AMI by half splitting the data




Odds ratios were estimated using logistic regression models adjusted for Model 4 (age, sex, smoking status, history of diabetes, history of hypertension, family history of MI, physical activity, education, income, occupation and total energy intake).

**Figure 5.7 :** Association of dietary patterns by age

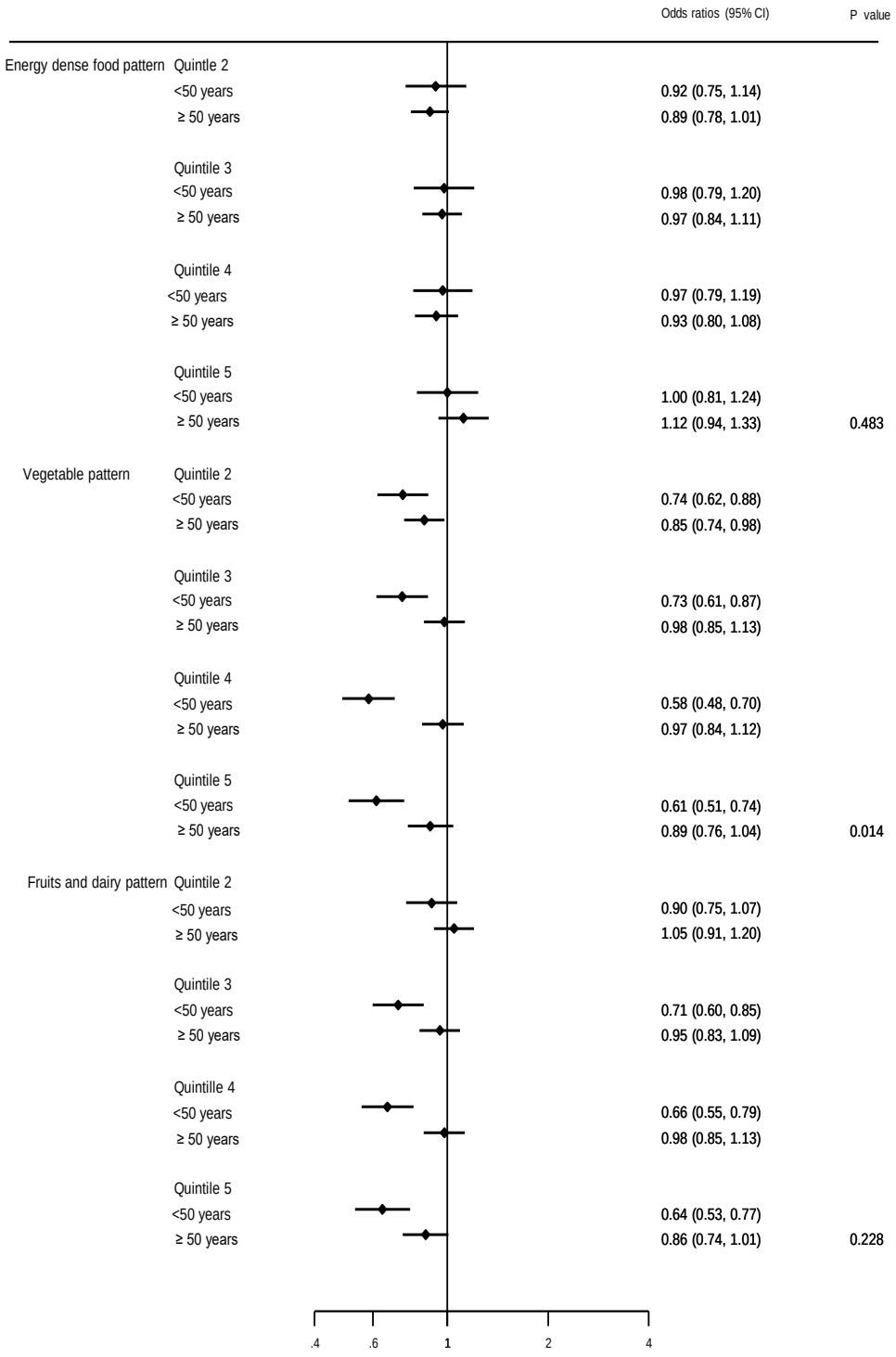
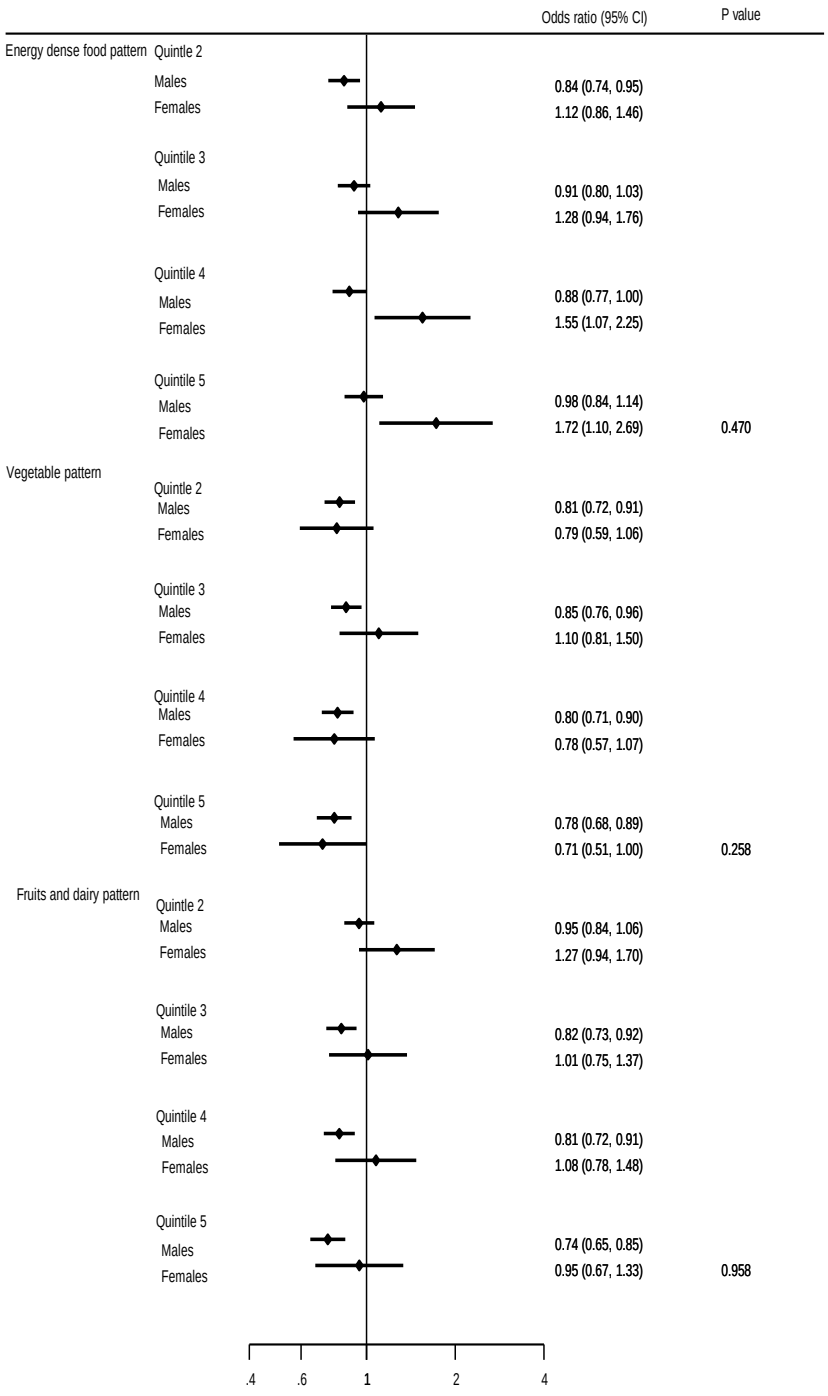
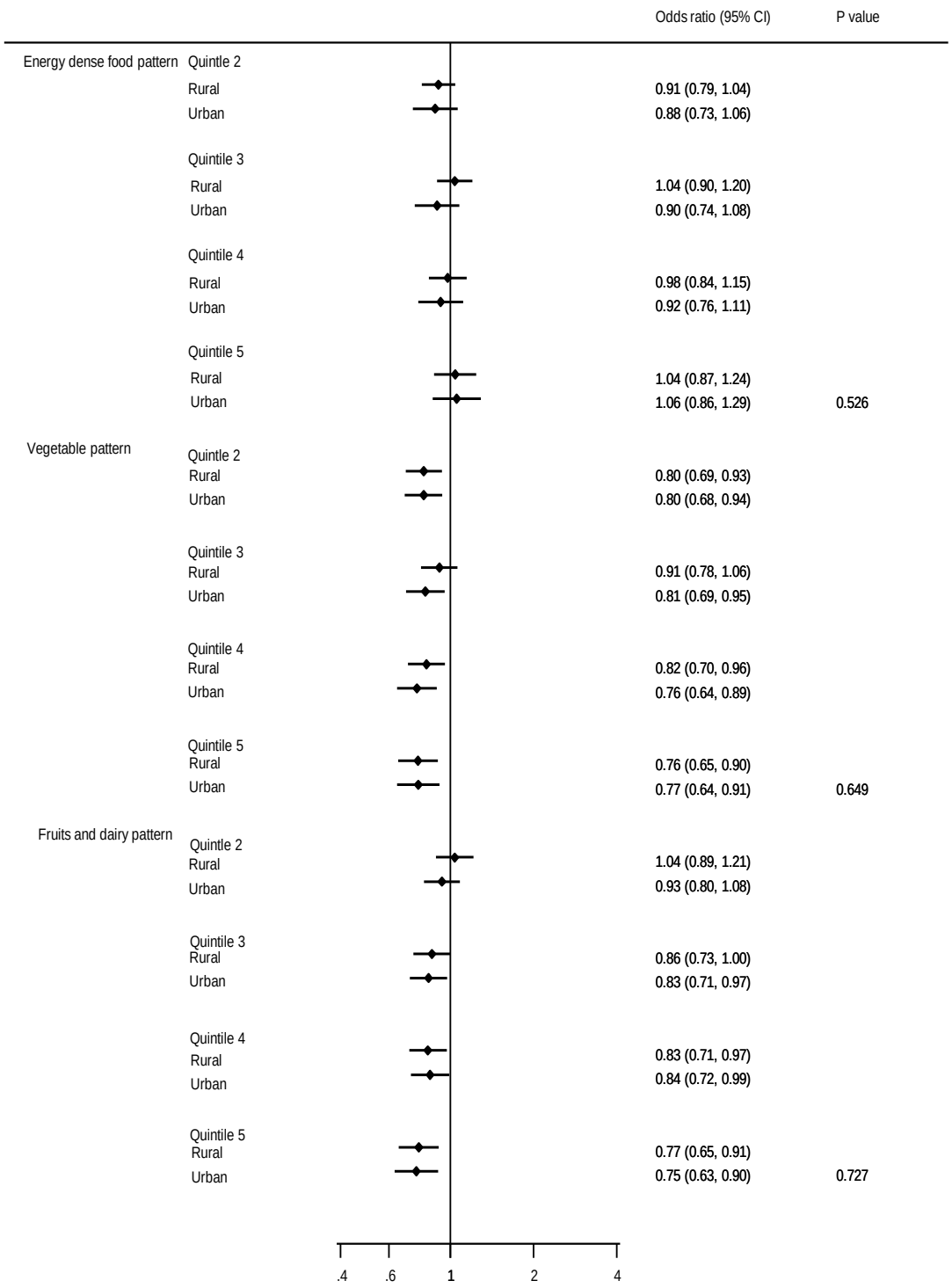


**Figure 5.8:** Association of dietary patterns by sex



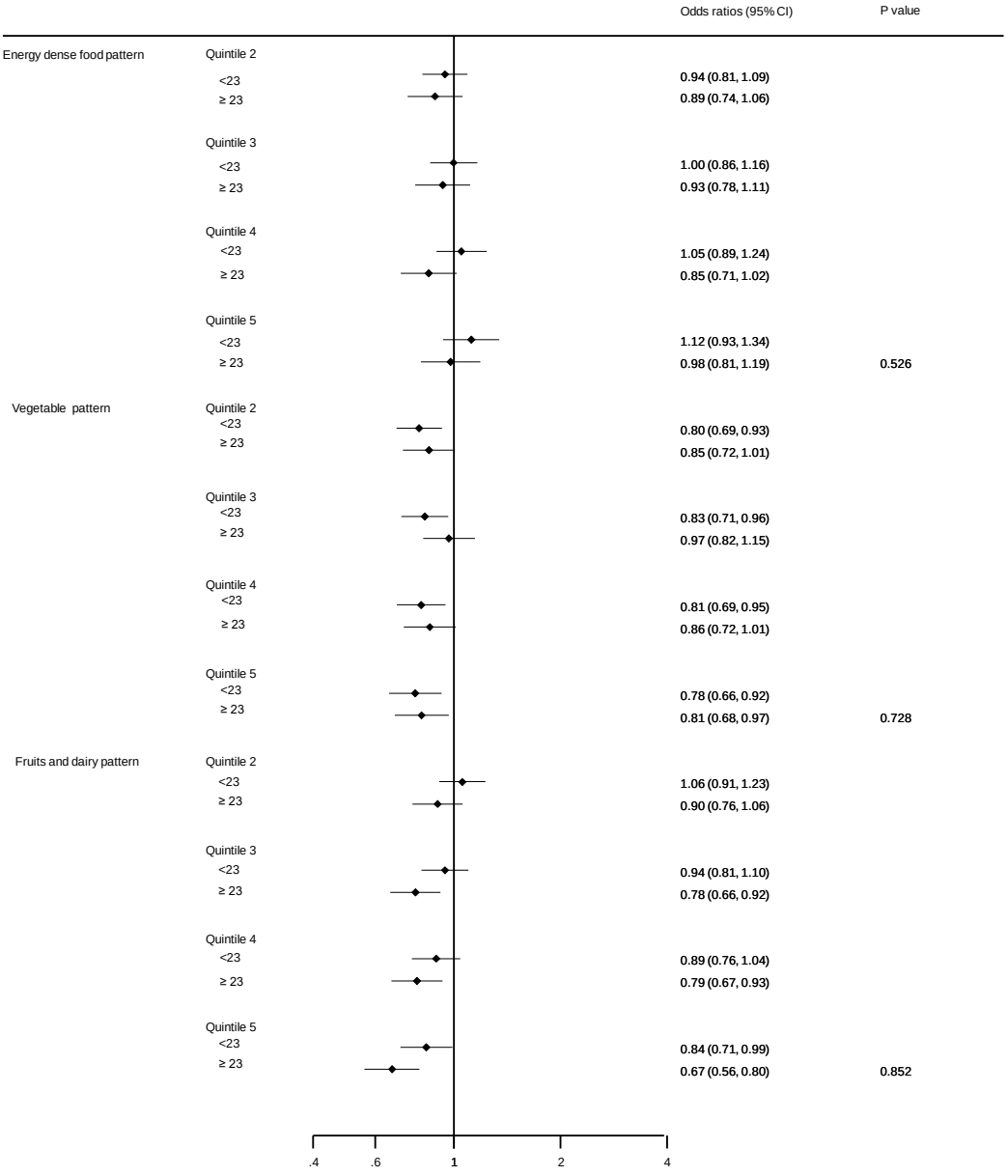

Adjusted for Model 4

**Figure 5.9:** Association of dietary patterns by location



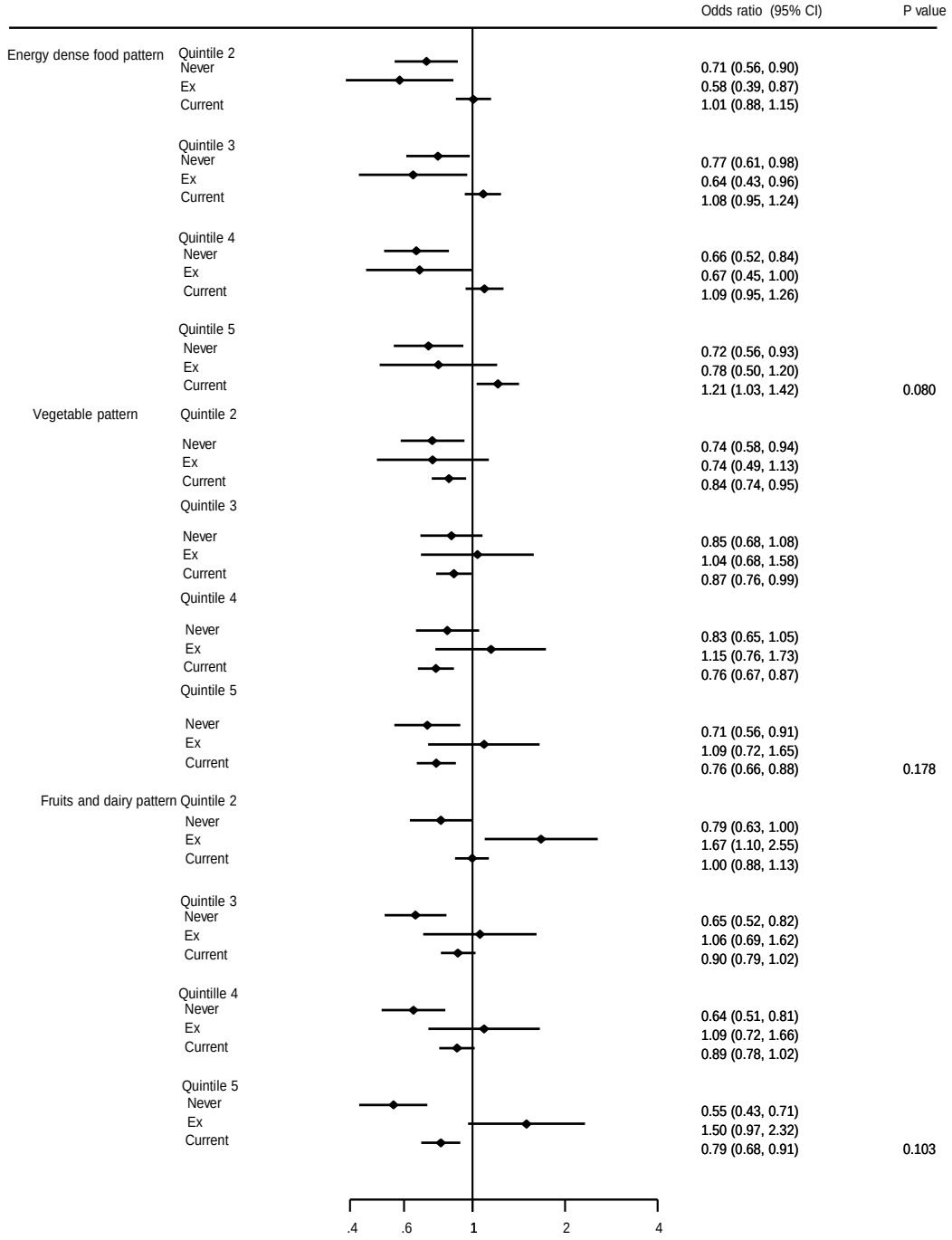

Adjusted for Model 4

**Figure 5.10**: Associations of dietary patterns by BMI



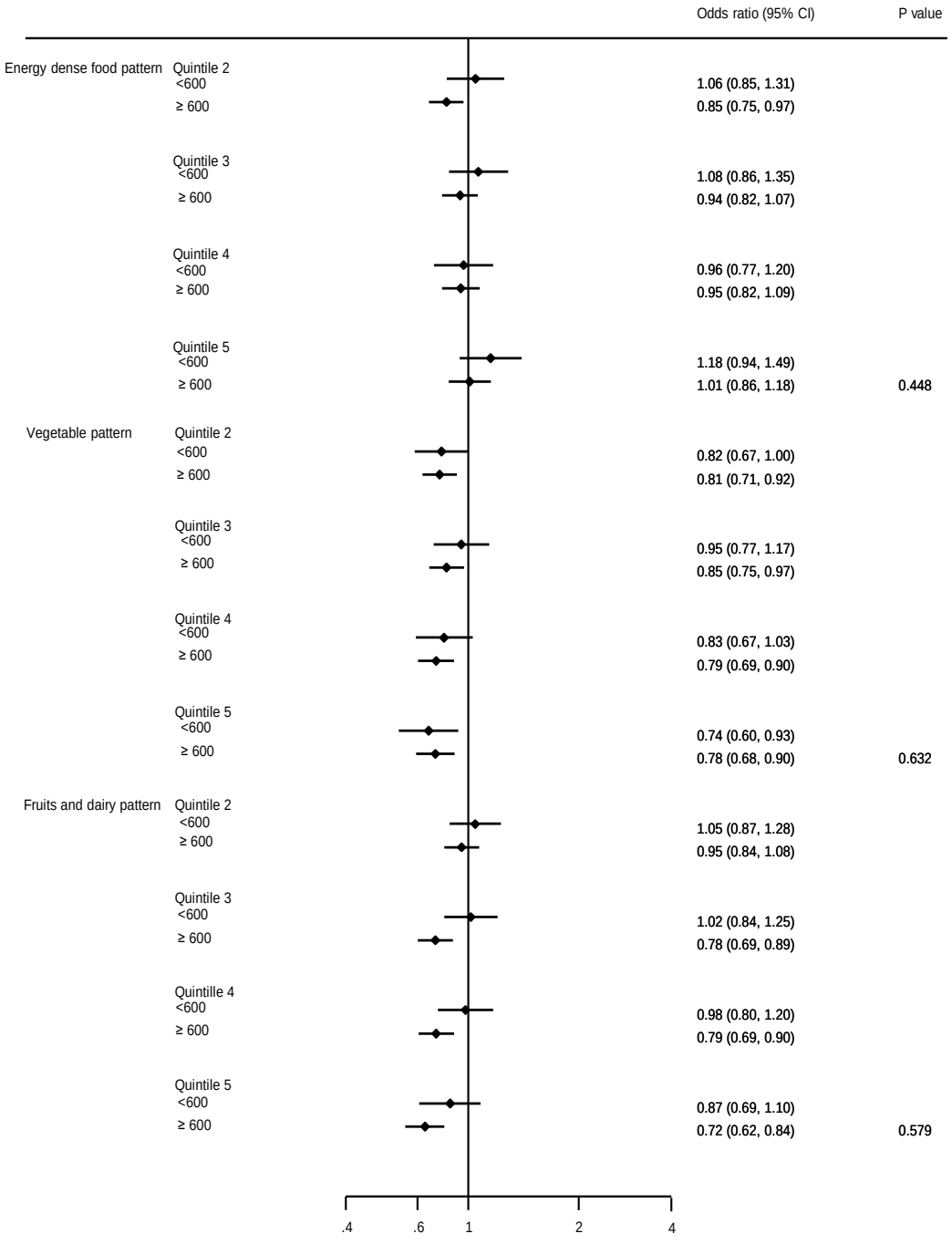

Adjusted for Model 4

**Figure 5.11:** Association of dietary patterns by smoking status



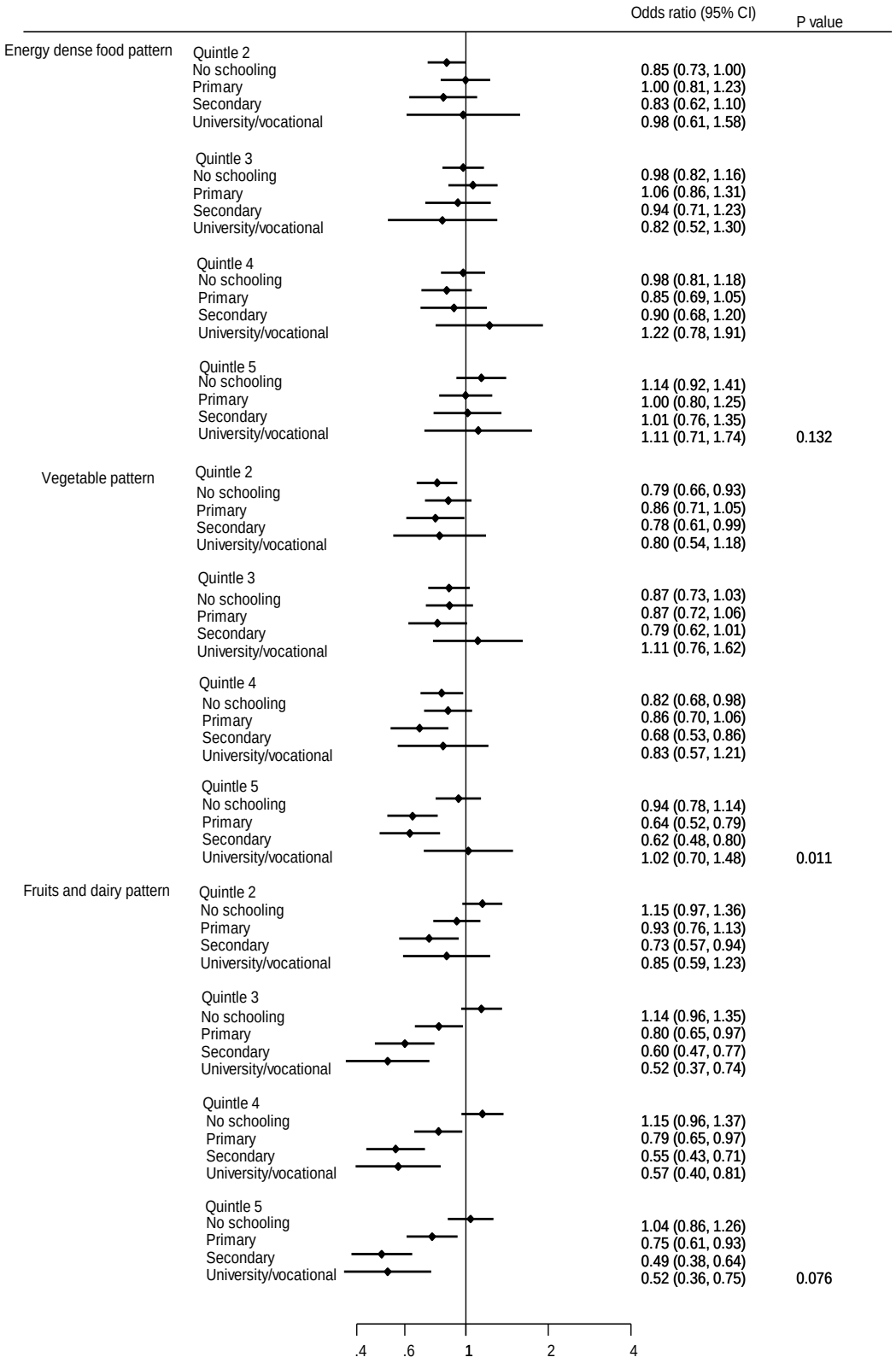

Adjusted for Model 4

**Figure 5.12:** Association of dietary patterns by physical activity



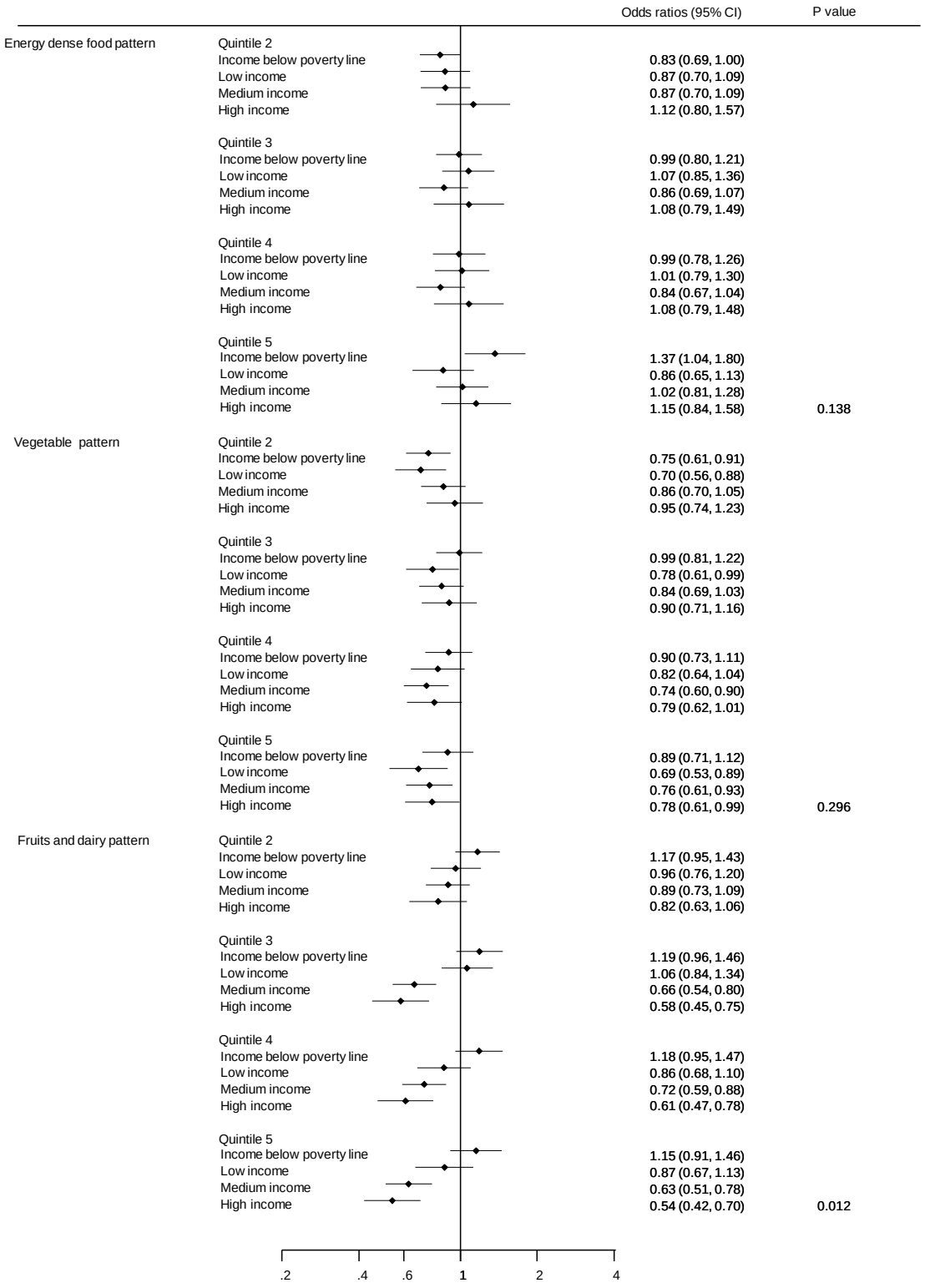

Adjusted for Model 4

**Figure 5.13:** Association of dietary patterns by education level




Adjusted for Model 4.

**Figure 5.14:** Association of dietary patterns by income levels




Adjusted for Model 4.

**Table 5.1:** Factor loading matrix of dietary patterns

| Food items | Mean (g /day) | Energy dense food pattern | Vegetable pattern | Fruits and dairy pattern |
|---|---|---|---|---|
| Green leafy and stem vegetables | 124.6 | -0.04 | **0.60** | -0.02 |
| Potatoes | 64.4 | 0.11 | 0.01 | 0.05 |
| Other vegetables | 456.1 | -0.02 | **0.60** | 0.02 |
| Vitamin C rich fruits | 102.6 | -0.00 | 0.00 | **0.46** |
| Other fruits | 443.6 | 0.02 | 0.14 | **0.45** |
| Pulses | 136.5 | 0.19 | **0.29** | -0.16 |
| Boiled white rice | 970.1 | 0.10 | 0.04 | -0.08 |
| Biryani | 29.5 | **0.39** | -0.0 | -0.04 |
| Bread | 90.0 | 0.13 | 0.05 | -0.08 |
| Deep fried snacks | 32.7 | **0.43** | 0.01 | -0.19 |
| Savoury snacks | 31.2 | **0.39** | -0.03 | 0.08 |
| Sweets | 20.9 | 0.25 | -0.06 | 0.20 |
| Sugar sweetened beverages | 29.5 | **0.36** | -0.10 | 0.08 |
| White meat | 36.6 | 0.23 | 0.17 | 0.03 |
| Red meat | 10.2 | **0.38** | -0.06 | 0.02 |
| Sweet water fish | 258.4 | 0.01 | **0.28** | **0.26** |
| Sea water fish | 17.6 | 0.05 | 0.18 | 0.12 |
| Egg | 21.8 | 0.19 | -0.02 | 0.11 |
| Milk | 69.3 | -0.11 | -0.06 | **0.46** |
| Yoghurt | 8.0 | 0.07 | -0.13 | **0.39** |
| **Variance explained** | | 12.4 | 10.8 | 9.5 |

**Footnotes Table 5.1:**

Factor loadings represent the correlation between factor scores and intakes of food items. Factors obtained by principal component analysis in g/day from a FFQ. Component loadings >0.25 are presented in bold. 20 food groups were entered in the analysis. The variance explained by each factor after they were orthogonally rotated by varimax method was about 33% collectively.

Energy dense food pattern was characterised by deep fried snacks, savoury snacks, biryani, red meat, SSB and sweets. Vegetable pattern was characterised mainly by green leafy and other vegetables, pulses and sweet water fish. Fruits and dairy pattern was characterised by higher loadings of vitamin C rich fruits, other fruits, milk, yoghurt and sweet water fish.

**Table 5.2:** Contribution of food groups to dietary patterns in median intakes (interquartile range)

| | Energy dense food pattern | | Vegetable pattern | | Fruits and dairy pattern | |
|---|---|---|---|---|---|---|
| **Food groups** | **Q1** | **Q5** | **Q1** | **Q5** | **Q1** | **Q5** |
| **Green leafy and stem vegetables** | 89.5 (54.0-171.5) | 111.1 (60.1-190.8) | 43.7 (31.3-57.9) | 257.0 (193.7-330.1) | 71.2 (48.1-131.0) | 132.8 (70.5-221.7) |
| **Potatoes** | 50.0 (21.45-50.0) | 50 (43.6-125) | 50.0 (39.3-125.0) | 50 (39.3-125.0) | 50 (39.3-125.0) | 50 (39.3-125.0) |
| **Other vegetables** | 344.7 (226.7-570.5) | 467.4 (313.1-687.4) | 200.8 (143.7-254.2) | 856.1 (682.1-1090.1) | 275.4 (192.6-423.8) | 554.8 (363.7-782.8) |
| **Vitamin C rich fruits** | 35.6 (18.4-119.7) | 127.6 (47.8-208.4) | 35.6 (18.4-119.7) | 127.6 (43.5-188.3) | 18.4 (0.0-39.9) | 171.2 (127.6-282.7) |
| **Other fruits** | 224.8 (117.0-422.2) | 553.4 (295.9-978.2) | 204.2 (112.3-317.7) | 633.7 (321.8-1079.8) | 144.2 (81.4-211.9) | 924.8 (551.3-1286.5) |
| **Pulses** | 53.4 (29.1-68) | 174.5(68.0-306.0) | 53.4 (29.2-68.0) | 179.0 (68.0-310.5) | 72.5 (53.4-179.7) | 106.9 (53.4-1991.2) |
| **Boiled white rice** | 1161 (645-1161) | 1161 (645-1161) | 1161 (645-1161) | 1161 (645-1161) | 1161 (645-1161) | 1161 (645-1161) |
| **Biryani** | 0.0 | 27.7 (0.0-27.7) | 27.7 (0.0-27.7) | 27.7(0.0-27.7) | 0.0 (0.0-27.7 | 27.7(0.0-27.7) |
| **Bread** | 19.7 (2.7-49.0) | 113.7(44.0-162.2) | 37.9 (11.4-115.0) | 71.6 (19.7-129.5) | 37.3 (6.6-115) | 75.9 (22.4-134.7) |
| **Deep fried snacks** | 0.0 (0.0-7.2) | 51.8 (12.4-93.6) | 9.1 (0.0-31.4) | 10.7 (0.0-48.0) | 10.7 (0.0-48.0) | 10.7 (0.0-48.0) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Savoury snacks** | 5.3 (0.0-9.0) | 60.0 (28.4-103.7) | 11.3 (2.6-33.0) | 18.0 (5.7-51.4) | 6.4 (0.0-14.3) | 40.7 (11.3-74.7) |
| **Sweets** | 6.4 (2.9-11.8) | 30.7 (15.6-49.5) | 12.2 (6.4-28.2) | 15.6 (7.3-33.6) | 9.3 (3.9-14.7) | 28.2 (12.2-44.2) |
| **Sugar sweetened beverages** | 0.0 | 35.8 (16.5-107.3) | 0.0 (0.0-16.5) | 0.0 (0.0-16.5) | 0.0 (0.0-16.5) | 0.0 (0.0-16.5) |
| **White meat** | 7.4 (6.2-16.0) | 48.0 (40.3-88.0) | 13.6 (7.4-40.0) | 48.0 (16.0-73.9) | 7.4 (7.4-29.5) | 48.0 (16.0-61.5) |
| **Red meat** | 0.0 (0.0-6.0) | 13.0 (6.0-39.0) | 6.0 (0.0-13.0) | 6.0 (0.0-13.0) | 6.0 (0.0-6.0) | 6.0 (6.0-16.0) |
| **Sweet water fish** | 165.3 (95.4-253.2) | 259.6 (156.1-413.8) | 130.2 (82.2-199.2) | 336.3 (208.1-545.6) | 129.0 (74.0-201.2) | 326.2 (207.6-518.2) |
| **Sea water fish** | 1.3 (0.0-7.5) | 9.1 (1.3-28.1) | 1.36 (0.0-6.9) | 12.2 (1.3-40.0) | 1.3 (0.0-2.4) | 12.2 (1.3-33.9) |
| **Egg** | 3.9 (3.9-8.4) | 25.3 (25.3-46.4) | 8.4 (3.9-25.3) | 25.3 (3.9-25.3) | 3.9 (3.9-25.3) | 25.3 (8.4-46.4) |
| **Milk** | 16.5 (0.0-107.3) | 16.5 (0.0-107.3) | 16.5 (0.0-107.3) | 35.8 (0.0-107.3) | 0.0 (0.0-16.5) | 107.3 (35.8-250.0) |
| **Yoghurt** | 0.0 | 16.5 (0.0-16.5) | 0.0 (0.0-16.5) | 0.0 (0.0-16.5) | 0.0 | 16.5 (0.0-16.5) |

**Footnotes table 5.2:**

Energy dense food pattern was characterised by deep fried snacks, savoury snacks, biryani, red meat, SSB and sweets. Vegetable pattern was characterised mainly by green leafy and other vegetables, pulses and sweet water fish. Fruits and dairy pattern was characterised by higher loadings of vitamin C rich fruits, other fruits, milk, yoghurt and sweet water fish.

**Table 5.3:** Characteristics of dietary patterns (Quintile 1 vs Quintile 5) in control

| | Energy dense food pattern | | Vegetable pattern | | Fruits and dairy pattern | |
|---|---|---|---|---|---|---|
| **Characteristics** | **Q1** | **Q5** | **Q1** | **Q5** | **Q1** | **Q5** |
| | | | | | | |
| **Age (years)** | 55.4 (9.8) | 46.9 (10.1) | 52.8 (10.7) | 50.9 (9.8) | 51.9 (10.3) | 50.8 (10.5) |
| **Sex (%)** | | | | | | |
| Males | 1046 (69.0) | 1594 (96.2) | 1158 (81.5) | 1585 (90.5) | 1149 (82.2) | 1657 (90.4) |
| Females | 471 (31.0) | 63 (3.8) | 263 (18.5) | 167 (9.5) | 249 (17.8) | 176 (9.6) |
| **BMI ($kg/m^2$)** | 22.0 (3.9) | 24.3 (5.7) | 22.8 (5.9) | 23.9 (6.7) | 22.6 (3.9) | 23.5 (5.4) |
| **Waist-to-hip ratio** | 0.93 (0.08) | 0.97(0.08) | 0.94 (0.08) | 0.96 (0.07) | 0.95 (0.07) | 0.96 (0.08) |
| **History of diabetes (%)** | | | | | | |
| Yes | 123 (8.1) | 154 (9.3) | 135 (9.5) | 210 (12.0) | 142 (10.2) | 186 (10.1) |
| No | 1394 (91.9) | 1503 (90.7) | 1286 (90.5) | 1542 (88.0) | 1256 (89.8) | 1647 (89.9) |
| **History of hypertension (%)** | | | | | | |
| Yes | 191 (12.6) | 209 (12.6) | 169 (11.9) | 276 (15.7) | 173 (12.4) | 243 (13.3) |
| No | 1326 (87.4) | 1448 (87.4) | 1252 (88.1) | 1476 (84.3) | 1225 (87.6) | 1590 (86.7) |
| **Family history of MI (%)** | | | | | | |
| Yes | 70 (4.6) | 164 (9.9) | 82 (5.8) | 142 (8.1) | 76 (5.4) | 165 (9.0) |
| No | 1447 (95.4) | 1493 (90.1) | 1339 (94.2) | 1610 (91.9) | 1322 (94.6) | 1668 (91.0) |
| **LDL-C, (mmol/L)** | 2.79 (0.87) | 2.80 (0.89) | 2.82 (0.89) | 2.81 (0.87) | 2.77 (0.88) | 2.77 (0.88) |
| **HDL- C, (mmol/L)** | 0.90 (0.25) | 0.83 (0.22) | 0.87 (0.24) | 0.82 (0.22) | 0.86 (0.23) | 0.82 (0.85) |
| **Education level (%)** | | | | | | |
| No schooling | 878 (57.9) | 352 (21.3) | 633 (44.6) | 495 (28.3) | 697 (49.9) | 510 (27.9) |
| Primary | 391 (25.8) | 527 (31.9) | 419 (29.5) | 546 (31.2) | 388 (27.8) | 544 (29.7) |
| Secondary | 190 (12.5) | 503 (30.4) | 268 (18.9) | 458 (26.1) | 214 (15.3) | 529 (28.9) |
| Vocational/university | 58 (3.8) | 272 (16.4) | 100 (7.0) | 253 (14.4) | 99 (7.0) | 246 (13.5) |
| **Annual income (Taka) (%)** | | | | | | |
| Income below the poverty line <=56000 | 728 (48.4) | 157 (9.7) | 470 (33.6) | 310 (18.0) | 451 (32.4) | 328 (18.3) |
| Low income | 376 (25.0) | 262 (16.1) | 327 (23.4) | 301 (17.4) | 395 (28.4) | 301 (16.8) |
| Medium income | 284 (18.9) | 549 (33.9) | 384 (27.4) | 558 (32.4) | 377 (27.0) | 573 (31.9) |
| High income | 116 (7.7) | 654 (40.3) | 219 (15.6) | 556 (32.2) | 170 (12.2) | 592 (33.0) |
| **Occupation (%)** | | | | | | |
| Business professional | 292 (19.3) | 1019 (61.6) | 516 (36.3) | 839 (47.9) | 486 (34.8) | 913 (49.9) |
| Manual labour | 463 (30.5) | 196 (11.8) | 330 (23.2) | 348 (19.9) | 333 (23.8) | 345 (18.9) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Non- manual labour | 49 (3.2) | 104 (6.3) | 87 (6.1) | 89 (5.0) | 109 (7.8) | 68 (3.7) |
| Unemployed/retired/students | 712 (47.0) | 336 (20.3) | 488 (34.3) | 476 (27.2) | 470 (33.6) | 504 (27.5) |
| **Smoking status (%)** | | | | | | |
| Never | 405 (26.7) | 537 (32.4) | 431 (30.3) | 579 (33.0) | 369 (26.4) | 612 (33.4) |
| Ex | 107 (7.0) | 106 (6.4) | 110 (7.8) | 131 (7.5) | 106 (7.6) | 118 (6.4) |
| Current | 1006 (66.3) | 1014 (61.2) | 880 (61.9) | 1042 (59.5) | 923 (66.0) | 1103 (60.2) |
| **Total MET-minutes of exercise/week (%)** | | | | | | |
| < 600 | 323 (21.3) | 395 (23.8) | 345 (24.3) | 366 (20.9) | 404 (28.9) | 306 (16.7) |
| ≥ 600 | 1194 (78.7) | 1262 (76.2) | 1076 (75.7) | 1386 (79.1) | 994 (71.1) | 1527 (83.3) |
| **Total Energy intake (kcal/day)** | 2297.2 (659.1) | 4002.2 (991.2) | 2369.1 (720.6) | 3889.8 (1037.2) | 2475.3 (706.8) | 3967.5 (1013.5) |
| **Location (%)** | | | | | | |
| Urban | 409 (27.0) | 904 (55.0) | 767 (49.0) | 559 (45.1) | 621 (44.6) | 1168 (64.1) |
| Rural | 1103 (73.0) | 740 (45.0) | 798 (51.0) | 679 (54.9) | 770 (55.4) | 654 (35.9) |

**Footnotes Table 5.3:**
Energy dense food pattern was characterised by deep fried snacks, savoury snacks, biryani, red meat, SSB and sweets. Vegetable pattern was characterised mainly by green leafy and other vegetables, pulses and sweet water fish. Fruits and dairy pattern was characterised by higher loadings of vitamin C rich fruits, other fruits, milk, yoghurt and sweet water fish.

**Table 5.4:** Association of dietary patterns with myocardial infraction after multivariate adjustments.

| | Q2 | Q3 | Q4 | Q5 | P trend |
|---|---|---|---|---|---|
| **Energy dense food pattern** | | | | | |
| Model 1 | 0.90 (0.84-0.97) | 0.94 (0.87-1.01) | 0.85 (0.79-0.92) | 0.86 (0.80-0.93) | 0.03 |
| Model 2 | 0.87 (0.81-0.94) | 0.89 (0.82-0.95) | 0.78 (0.73-0.85) | 0.72 (0.66-0.79) | <0.001 |
| Model 3 | 0.82 (0.76-0.88) | 0.84 (0.77-0.90) | 0.75 (0.69-0.81) | 0.69 (0.63-0.75) | <0.001 |
| Model 4 | 0.90 (0.83-0.97) | 0.97 (0.90-1.05) | 0.95 (0.88-1.03) | 1.05 (0.95-1.16) | 0.10 |
| Model 5 | 0.90 (0.80-1.01) | 0.97 (0.87-1.10) | 0.95 (0.84-1.07) | 1.05 (0.91-1.21) | 0.11 |
| Model 6 | 0.87 (0.80-0.95) | 0.90 (0.83-0.97) | 0.89 (0.82-0.87) | 0.97 (0.87-1.07) | 0.09 |
| **Vegetable pattern** | | | | | |
| Model 1 | 0.81 (0.75-0.87) | 0.82 (0.76-0.88) | 0.72 (0.67-0.78) | 0.63 (0.59-0.68) | <0.001 |
| Model 2 | 0.80 (0.74-0.87) | 0.84 (0.78-0.89) | 0.74 (0.68-0.79) | 0.64 (0.60-0.70) | <0.001 |
| Model 3 | 0.76 (0.71-0.83) | 0.79 (0.73-0.85) | 0.68 (0.63-0.74) | 0.58 (0.54-0.63) | <0.001 |
| Model 4 | 0.81 (0.73-0.90) | 0.88 (0.78-0.98) | 0.80 (0.72-0.90) | 0.77 (0.68-0.88) | <0.001 |
| Model 5 | 0.81 (0.73-0.91) | 0.88 (0.79-0.98) | 0.80 (0.71-1.90) | 0.77 (0.68-0.88) | <0.001 |
| Model 6 | 0.83 (0.76-0.89) | 0.88 (0.81-0.95) | 0.80 (0.74-0.87) | 0.77 (0.70-0.84) | <0.001 |
| **Fruits and dairy pattern** | | | | | |
| Model 1 | 0.92 (0.85-0.98) | 0.74 (0.69-0.80) | 0.68 (0.63-0.73) | 0.55 (0.51-0.59) | <0.001 |
| Model 2 | 0.94 (0.87-1.01) | 0.78 (0.73-0.84) | 0.72 (0.67-0.78) | 0.58 (0.53-0.62) | <0.001 |
| Model 3 | 0.97 (0.90-1.05) | 0.80 (0.74-0.87) | 0.75 (0.69-0.81) | 0.59 (0.55-0.64) | <0.001 |
| Model 4 | 0.99 (0.91-1.06) | 0.85 (0.79-0.91) | 0.84 (0.78-0.91) | 0.76 (0.69-0.84) | <0.001 |
| Model 5 | 0.98 (0.88-1.10) | 0.85 (0.76-0.95) | 0.84 (0.75-0.94) | 0.76 (0.68-0.87) | <0.001 |
| Model 6 | 0.97 (0.89-1.05) | 0.86 (0.79-0.93) | 0.82 (0.76-0.89) | 0.76 (0.68-0.84) | <0.001 |

After Bonferroni corrections (p value = 0.05/18= 0.0028). The associations remained significant for vegetable pattern and fruits and dairy pattern.

**Footnotes Table 5.4:**

Model 1: adjusted for age and sex

Model 2: Model 1+ smoking status, physical activity, occupation, education and income

Model 3: Model 2 + history of diabetes, history of hypertension, family history of MI

Model 4: Model 3 plus total energy intake.

Model 5: Model 4 + antihypertensives and anti-diabetics use

Model 6: Model 4 + waist-to-hip ratio, LDL-C and HDL-C

# Chapter 6: Correlates of dietary nutrients and their association with acute myocardial infarction in BRAVE

## 6.1 Chapter summary

The main objective of this chapter is to investigate, reliably and in more detail than previously possible, the associations of different dietary nutrients and risk of acute myocardial infarction (AMI) from a large hospital-based case-control study (BRAVE) in Bangladesh. The chapter also describes the cross-sectional association of dietary nutrients with various characteristics to identify potential sources of confounding. Dietary nutrients are postulated to act in many different pathways to affect coronary heart disease (CHD) risk, however, the evidence on the association of how different types of nutrients affect the risk of CHD is debated. In addition, there is scarce evidence on how nutrients affect risk of AMI in a South Asian population. Logistic regression was used to investigate the association of dietary nutrients with the risk of AMI adjusting for potential confounders and mediators.

The findings from this chapter indicate that 1) refined carbohydrates have a no significant association with the risk of AMI, whereas carbohydrates from non-refined sources have an inverse association with the risk of AMI; 2) highest intake of animal protein has significant higher association with the risk of AMI, in contrast plant protein has a weak inverse with the risk of AMI; 3) moderate intake of saturated fatty acids (SFA) from dairy and polyunsaturated fatty acids (PUFA) show an inverse association with AMI; 4) highest quintile of dietary monounsaturated fatty acids (MUFA) has an increased association with MI, but it is non-significant across other quintiles; 5) highest intake of SFA from non-dairy/other sources has no significant association with the risk of AMI; (6) when stratified by age, an increased association is observed for refined carbohydrates and SFA dairy in younger and older age groups respectively. As for substitution analyses, 7) replacing 1% energy from total carbohydrates with SFA and PUFA is associated with lower risk of MI. By contrast, 8) replacement with MUFA and total protein is associated with higher risk. 9) Replacing 1% energy from SFA with total carbohydrate, MUFA and total protein is associated with higher risk; on the contrary, 10) replacement of SFA with PUFA is associated with lower risk of AMI. These analyses highlight that single nutrient approach may not be the most reliable method to assess diet-disease associations. Therefore, the next-generation dietary guidelines should primarily be based on food groups and dietary patterns, considering the complex interactions between different nutrients.

## 6.2 BACKGROUND

The diet-heart hypothesis in 1956 postulated that diets rich in SFA and cholesterol were a major cause of cardiovascular disease (CVD) by increasing the levels of low density lipoprotein (LDL-C) in blood which accelerates the process of atherosclerosis.[1] A number of animal and metabolic studies beginning in the 1950's and 60's identified SFA and PUFA as major dietary influences on serum cholesterol levels, a major risk factor for CVD.[2–5] In addition, the pioneering Seven countries study initiated in the 1940's in United States, also suggested a strong correlation between diets rich in SFAs and high risk of CHD.[6] Since then many studies have been published investigating the role of nutrients, mainly focusing on SFA with the risk of CVD.[7,8] As a consequence of such wealth of evidence, various dietary guidelines have broadly recommended low consumption of total fat (30-35% of total energy intake) and SFA (<10% of energy intake) and also recommended to replace SFA with PUFA and avoid trans-fat.[9]

Nonetheless, evidence from recent meta-analyses of epidemiological studies have challenged the higher association of SFA with CVD.[10,11] The lack of association of SFA with CVD in these reviews may be explained by: (1) earlier evidence was based on observational studies that were prone to measurement error; (2) differences in the effects of highly controlled dietary changes in trials compared to actual dietary patterns in general population; (3) different effects of specific fatty acid isomers of SFA, MUFA and PUFA and, (4) differences in associations of SFA depending on the replacement nutrient and potential over-adjusting in some studies.[11] As for trials investigating substitution of nutrients with other nutrients, the results are not consistent. The dietary recommendations to lower fats mean that the same amount of energy has to be replaced from other nutrients and in most cases the replacement nutrient is carbohydrate. The evidence from replacement of SFA with carbohydrates is conflicting.[12–16] In addition, although most trials support the recommendation of replacing SFA with PUFA to lower the risk of cardiovascular outcomes,[10,17] there are some trials, however, that show no effect.[18]

Due to inconsistent evidence on the association of different nutrients with CVD and CHD often leading to erroneous conclusions, the reductionist approach of investigating single nutrients with disease has been criticized.[19] The contemporary evidence on diet-disease associations now focuses on dietary pattern and food group analysis.[20] The totality of diet including the combinations of different food groups and nutrients is more important in determining risk of disease than single nutrients[21] as discussed in **Chapters 4 and 5**. Nevertheless, it is still important to investigate the association of different nutrients with CHD to better understand the potential complex mechanisms of diet-disease associations.[22]

Dietary nutrients affect blood cholesterol levels, lipids, blood pressure, inflammation, glucose-insulin homeostasis and other biomarkers to affect the risk of CHD, however actual mechanistic pathways are not completely understood.[23] It is also important to consider that it is impossible to separate nutrients from food groups and food groups from dietary patterns; there is an important link between these different approaches.[22] Therefore, it is important to also investigate how nutrients affect the risk of disease which forms a basis to understand the food groups and dietary pattern analyses. The relevance of investigating nutrients also comes partly from the fact that some guidelines still provide recommendations on nutrients[9] and it may have potential for food reformulations (e.g. low fat products).

As discussed, to date the association of different nutrients with CHD is debated, with most of the evidence of diet-CHD associations coming from European and North American origin, with scarce data available from low-and middle-income countries (LMIC) like Bangladesh. Therefore, the primary aim of this chapter is to report the first detailed large-scale study solely focusing Bangladesh, specifically addressing: (1) correlates of nutrients with various characteristics; (2) the association of different food sources of nutrients with the risk of AMI, adjusting for potential confounders; (3) the potential effect of mediators; (3) the potential effect modification by age, sex, geographical location, smoking status, body mass index (BMI) and physical activity and (4) the effect of replacement of nutrients with risk of AMI.

The secondary aim of this chapter is to provide more insight into the association of food group and dietary pattern analyses reported in **Chapters 4 and 5**.

The present analyses differ from previous studies on nutrients and CHD solely based on a population from South Asia in several important ways. First, it is the largest and most detailed investigation of nutrients and CHD filling research gaps about different food sources of nutrients (such as carbohydrates from refined and non-refined sources) and risk of AMI. Second, this study adjusted for several potential confounders and adjusted for total energy intake based on food composition tables from South Asia. Third, it investigates the effect of replacement nutrients with the risk of AMI. Finally, it characterises the shapes of associations with CHD in an understudied population.

## 6.3 METHODS

### 6.3.1 Participants

Details of data collection, inclusion criteria and data management for the analyses in BRAVE are in **Chapter 2**. The present analyses were confined to 7066 cases and 8079 controls that had complete information on age, sex, smoking status, history of diabetes and history of hypertension.

### 6.3.2 Assessment of nutrients in the BRAVE study

Bangladeshi food composition did not have information on different fatty acids therefore the Indian food composition was chosen as being the closest nutritional match thus the contributions of all foods were based on the Indian food composition table.[24] Correlation of energy intake by using these two composition tables was similar (0.98). Daily intake of nutrients was estimated by multiplying the consumption of each food item (g/d) by its nutrient content composition per 100 grams (divided by 100) and summing the nutrient contributions of all foods. Recipe calculation was done similar to what has been reported in **Chapter 2**. It is important to note that there was no information on glycemic index in the food composition table.

Nutrients were collated into groups based on different food sources as shown in **Table 6.1** which are the main exposure variables in the analyses in this chapter. The intake of refined carbohydrates, non-refined carbohydrates, SFA from dairy and other sources, MUFA, PUFA, animal protein and plant protein were expressed in relative contribution of nutrients to total energy intake, in percentage (EN-%) provided by the nutrients out of total daily energy intake. Evaluating nutrients as a percentage of energy is extensively used in clinical settings and dietary guidelines.

To achieve this, first the total energy intake was calculated from nutrient consumption in each participant according to equation based on the Atwater general factor system, which assumes that fat yields 9.0 kcal of energy per gram, and carbohydrate and protein each yield 4.0 kcal/g.[25] Alcohol was not included in the energy intake calculation because it is generally not consumed in the Bangladeshi population due to religious reasons.

*Total Energy (kcal/d) = 9.0 * fat (g/d) + 4.0 * carbohydrate (g/d) + 4.0 * protein (g/d)*

To compute the energy percentages of all nutrients, the energy content of the nutrient was divided by the total daily energy intake then multiplied by 100. For example, the following formula was used to obtain energy percentages from protein:

*EN-% = ((protein (g/d) * 4(kcal)) / total energy intake (kcal/d)) *100%.*

For the analyses, participants were categorised into quintiles of EN-%, which has been done by studies before.[15,26]

### 6.3.3 Description of dietary nutrients and their correlates

Nutrient intake in EN-% by cases and controls were presented in **Table 6.2**. Characteristics of controls were presented comparing Quintile 1 vs Quintile 5 of the distribution of the dietary nutrients with continuous variables reported as means and standard deviations, and categorical variables as percentages in **Table 6.3**. Linear mixed models adjusting for age were used to assess the shape of the association of different nutrients with lipids and waist-to-hip ratio.

### 6.3.4 Contribution of food groups to dietary nutrients in BRAVE controls

To get understanding of which food groups contribute to the different dietary nutrients in BRAVE study, a combined result of food composition and consumption was assessed in controls. First the total nutrient (g/day) from each food group was calculated. For example, for SFA from dairy, total SFA intake of milk and yogurt was calculated.

SFA in milk = (consumption in g/day* nutrition composition of milk)/100

Then the SFA from milk was divided by the total sum of saturated fats from dairy (milk and yoghurt) and multiplied by 100 to obtain the percentage contribution of each food group to the SFA dairy in across controls in BRAVE:

% contribution of milk = (SFA in milk/ SFA in dairy) *100

This was repeated for all other nutrients in the study. Only main food groups contributing at least 1% to total nutrient consumption in the controls were presented.

### 6.3.5 Correlation of nutrients with dietary patterns

To gain insight into which dietary patterns may be correlated with specific nutrients in BRAVE, spearman correlation coefficients of dietary patterns with dietary nutrients were estimated.

### 6.3.6 Association of nutrients with acute myocardial infarction

Multivariate logistic regression models were used to assess the association between different nutrients and risk of AMI. The first quintile was used as reference group, and estimates were presented in odds ratios (ORs) and 95%-confidence intervals, which were obtained using the floating absolute risk (FAR) method. Progressive adjustments of the exposure-outcome associations were conducted to gain insight into the confounding effects of different factors in the BRAVE study.

Five models were applied:

1) Model 1- The minimally adjusted model was adjusted for age and sex.

2) Model 2- additionally adjusted for smoking status (never, ex and current), physical activity (using cut offs of <600 and ≥ 600 Metabolic Equivalent of Task (METs)), annual income (income below the poverty line, low income, medium income and high income), education level (no schooling, primary, secondary, university/vocational) and occupation (business/professionals, manual labour, non-manual labour and unemployed/student/retired).

3) Model 3- additionally adjusted for history of disease related variables: history of diabetes (yes or no), history of hypertension (yes or no) and family history of MI (yes or no)

4) Model 4 -additionally adjusted for total energy intake (kcal/day).

5) Model 5- additionally adjusted for anti-hypertensives and anti-diabetics

Waist-to-hip ratio, blood LDL-C and HDL-C were not included in the models as these are considered potential mediators rather than confounders. Additional sensitivity analyses were also conducted to adjust for these potential mediators in Model 4:

*Mediators*

6) Model 6- Model 4 + waist-to-hip ratio, LDL-C and HDL-C

The "main" associations compared the age and sex model (Model 1) with the primary model (Model 4).

Wald tests were used to get p value of linear trend that models the median values of categories of exposure variable as continuous variables.

The associations of refined carbohydrates, non-refined carbohydrates, SFA from dairy, SFA from other sources, MUFA, PUFA, animal protein and plant protein with AMI risk were each analysed separately according to the methods described above. As all nutrients were expressed as their percentage contribution to total energy consumption, higher levels of

one dietary factor should be interpreted as, at the expense of the same amount of energy from any other macronutrients not included in the model.

### 6.3.7 Subgroup and sensitivity analyses

To assess potential effect modification between exposures and baseline characteristics a covariate was included for the potential effect modifier and for the interaction term between exposure and effect modifier in the primary model (Model 4).

Subgroup analyses were performed for age (<50 years or ≥50 years), sex (males and females), geographical location (urban/rural), smoking status (ex, never, current), BMI (<23 kg/$m^2$ or ≥23 kg/$m^2$) and physical activity. Wald test was performed to get the p value for the interaction term across the quintiles of nutrient consumption and these variables.

To evaluate robustness of the findings, sensitivity analysis was done by excluding people with high and low energy intakes using cut offs from the PURE study (<400 and >5000 kcal/day).[15] In order to correct error for multiple testing, Bonferroni correction was used as sensitivity analysis and results are presented in the footnote of **tables 6.2 and 6.6**.

### 6.3.8 Substitution analysis

The effect of isocaloric replacement of 1% of energy from total carbohydrates and SFA with other nutrients was estimated using multivariable nutrient density models. The coefficients in this model indicated change in outcome by replacement of total carbohydrates and SFA by other nutrients.

The following model represents the standard multivariable model that includes EN-% of all nutrients; total protein, SFA, MUFA, PUFA, remaining (non-SFA) fat (e.g. glycerol, sterols and phospholipids) and total carbohydrates:

Disease risk= Y0 = aProtein + bSFA + cMUFA + dPUFA+ eRemaining fat + fCarbohydrate + gConfounders

a-g are regression coefficients

To investigate the effect of replacement of for example, SFA with total carbohydrate, carbohydrate was considered as an exposure variable in logistic model and all nutrients were adjusted for, except the nutrient to be replaced (SFA), which was excluded from the equation. As the total contribution to total energy intake from all nutrients combined add up to 100 EN-%, and because SFA (EN-%) is the only source of energy excluded in the substitution model, the model can be interpreted as the association of 1 unit energy from total carbohydrate at the expense of the same amount of energy derived from SFA while keeping other nutrients constant.[27]

Disease risk= Ynew = aProtein+ cMUFA + dPUFA+ eRemaining fat+ fCarbohydrate + gConfounders

All these nutrients were analysed as continuous variables in the logistic regression models with adjustment for potential confounders in the Primary model/ model 4 (age, sex, smoking status, physical activity, annual income, education level, occupation, history of diabetes, history of hypertension, family history of MI and total energy intake).

Statistical analyses were performed using STATA v.14 (Statacorp).

## 6.4 RESULTS

### 6.4.1 Nutrient consumption in BRAVE

**Table 6.2** shows the nutrient consumption of cases and controls in BRAVE. In controls the relative contribution of total carbohydrate, total proteins and total fats to total energy intake was 66.6%, 19.7% and 13.7% respectively. As for specific types of carbohydrates, 44.6% was from refined and 22.0% from non-refined sources in controls. Animal protein contributed 7.8% of total energy and plant protein contributed 10.8%. For different types of fatty acids, SFA from dairy contributed 1.1%, SFA from other sources 3.1%, MUFA 3.5% and PUFA 2.7% to total energy intake. Overall, the significant differences can be attributed to the difference in sample size in cases and controls.

### 6.4.2 Association of nutrients with baseline characteristics in controls

The baseline characteristics of the controls in quintile 1 versus quintile 5 of energy percentages from different nutrients are presented in **Table 6.3**. Compared with the lowest intake of refined carbohydrates, controls with the highest intake were older (57.7 (9.8) vs 51.8 (10.2)), less educated, had lower history of diabetes, hypertension and family history of MI, were less physically active (75.6% vs 81.3%) and mostly resided in rural areas. Compared with the lowest intake of carbohydrates from non-refined sources, controls with the highest intake had higher percentage of history of diabetes, hypertension and family history of MI, were more physically active (80.2% vs 74.5%), more educated and had less income below the poverty line.

Compared with the lowest intake of animal protein, controls with the highest intake were slightly older (52.7 (10.3) vs 50.7 (10.1)), more educated, having more percentage of history of hypertension, diabetes and family history of MI. Compared with the lowest intake of plant protein, controls with the highest intake were slightly older (52.2 (10.0) vs 50.5 (10.7)), had more percentage of history of diabetes and hypertension and were mostly residing in urban areas.

For highest intake of SFA from dairy, controls were older (53.5 (10.5) vs 50.8 (10.1)), more educated, had higher percentage of history of diabetes and family history of MI, less likely to be current smokers (61.0% vs 64.5%), more physically active (80.0% vs 73.1%) and mostly resided in urban areas. Compared with the lowest intake of SFA from other sources, controls with the highest intake were more likely to be younger (50.4 (10.7) vs 52.9 (9.7)), more educated, less likely to current smokers (58.1% vs 64.4%), having more percentage of history of hypertension, diabetes and family history of MI.

Highest intake of MUFA in controls was associated with being younger (49.3 (10.7) vs 53.0 (9.7), more educated, having higher history of diabetes and family history of MI and less likely to be currently smoking (59.9% vs 65.9%). Compared with the lowest intake of

PUFA, controls with the highest intake were more likely to be younger (48.1 (10.1) vs 53.6 (10.0)), slightly less physically active, less likely to current smokers (59.9% vs 64.4%), having higher percentage of history of diabetes and family history of MI.

### 6.4.3 Association of nutrients with lipids and waist-to-hip ratio

**Figure 6.1** demonstrates a weak increased and approximately linear association of non-refined carbohydrates, animal protein, SFA from other sources and MUFA with total cholesterol. Whereas carbohydrates from refined sources showed a negative association with total cholesterol. Plant protein showed a u-shaped association with total cholesterol. The shapes of associations of SFA from other sources and MUFA showed a weak association with LDL-C, whereas carbohydrates from refined sources showed an inverse association **(Figure 6.2)**. For HDL-C there were no apparent association with nutrients in men, expect for plant protein which showed a weak inverse association. However, in women, fatty acids showed higher association with HDL-C **(Figure 6.3)**. **Figure 6.4** demonstrates that SFA from other sources, MUFA and PUFA showed higher and approximately linearly associated with waist-to-hip ratio in men, however the association was weak. Whereas refined carbohydrates had weak inverse association with waist-to-hip ratio in men. In females, SFA from dairy had a weak inverse association with waist-to-hip ratio, whereas SFA from other sources had a weak increased association.

### 6.4.4 Association of nutrients with food groups and dietary patterns

*Contribution of food groups to dietary nutrients in BRAVE controls*

**Table 6.4** shows the relative contributions of food groups to different dietary nutrients in the BRAVE controls in EN-%. Most of the carbohydrates from refined sources was derived from boiled white rice (74.2%), followed by bread (12.7%). Carbohydrates from non-refined sources were mainly derived from pulses (39.2%), followed by other fruits (34.0%). Milk was the largest contributor to SFA dairy (84.9%), followed by yoghurt (15.1%). One fourth of SFA from other sources was derived from sweet water fish, followed by 18.2% of boiled white rice and 10.0% from other fruits. Sweet water fish was the largest contributor to MUFA (19.9%) and animal protein (68.6%). PUFA was mainly derived from boiled white rice (30.1%) followed by sweet water fish (13.6%). Plant protein was mainly derived from rice (36.3%) and pulses (33.0%).

*Correlation of nutrients with dietary patterns*

**Table 6.5** shows the correlation of nutrients with dietary patterns. The “energy dense food pattern” was positively correlated with carbohydrate from refined sources (0.30), SFA from other sources (0.44), MUFA (0.50), PUFA (0.47) and plant protein (0.26). The “vegetable pattern” was strongly associated with carbohydrate from non-refined sources (0.50), SFA dairy (0.48), SFA other (0.50), MUFA (0.44) and animal protein (0.51). In contrast the vegetable pattern had a negative association with refined carbohydrates (-0.30). The “fruits and dairy pattern” was associated with plant protein (0.37) and carbohydrates from refined sources (0.26).

### 6.4.5 Refined and non-refined carbohydrates with risk of acute myocardial infarction

In comparison between the highest (quintile 5) versus lowest quintile (quintile 1), in the age and sex adjusted Model 1 refined carbohydrate was associated with higher risk of AMI (OR 1.24 (95% CI 1.16-1.34); p trend 0.01) **(Figure 6.5a)**. There was a non-significant association when adjusted for Model 4 (age, sex, history of diabetes, history of hypertension, family history of MI, physical activity, smoking status, income, education, occupation and total energy intake (OR 1.09 (95% CI 0.99-1.17)), (p trend >0.05) suggesting possible confounding **(Figure 6.5a)**. With additional adjustments for potential mediators in Model 6, only in the highest quintile of refined carbohydrate intake a higher risk of AMI was observed (OR 1.16 (95% CI 1.07-1.28)), however the test for trend across quintiles was non-significant (p trend 0.13) **(Table 6.6)**.

On the contrary, higher intakes of non-refined carbohydrates in Model 1 was associated with lower risk of AMI (highest vs lowest, OR 0.62 (95% CI 0.58-0.67); p trend <0.001) **(Figure 6.5a)**. This association was slightly attenuated in the Model 4, however remained significant (OR 0.70 (95% CI 0.64-0.76); p trend <0.001) **(Figure 6.5a)**. Additional adjustments for potential mediators in Model 6, did not change the results appreciably as compared to Model 4 **(Table 6.6)**.

### 6.4.6 Animal and plant protein with risk of acute myocardial infarction

In comparison between the highest versus lowest quintile, animal protein was associated with higher risk of AMI in Model 1 (OR 1.27 (95% CI 1.19-1.37)) and Model 4 (OR 1.32 (95% CI 1.23-1.44)) (p trend <0.001 for both) **(Figure 6.5a)**. There was no difference in the overall direction of the associations observed with different levels of adjustment for potential confounders and mediators **(Table 6.6)**.

On the contrary, in comparison to the lowest quintile, highest intake of plant-based protein showed a weak inverse association with risk of AMI, however the test for trend was non-significant in the Model 1 (OR 0.93 (95% CI 0.87-1.00); p trend 0.05) and Model 4 (OR 0.87 (95% CI 0.80-0.94); p trend 0.09) **(Figure 6.5a)**. After additional adjustments of potential mediators in Model 6, in the highest quintile plant protein showed a slightly stronger inverse association with the risk of AMI, however the test for trend was non-significant **(Table 6.6)**.

### 6.4.7 Fatty acids with risk of acute myocardial infarction

In the analyses to investigate the association of different types of fatty acids with the risk of AMI, highest quintile (vs lowest quintile) of SFA from dairy in the age and sex adjusted Model 1 was inversely associated with the risk of AMI (OR 0.88 95% CI (0.82-0.95): p trend <0.001) **(Figure 6.5b)**. After adjustment for additional covariates in the primary Model 4, the highest quintile of SFA from dairy was not associated with the risk of AMI. However, only in the quintile 2 of SFA from dairy an inverse association was observed (OR 0.87 (95% CI 0.81-0.94); p trend 0.02) **(Figure 6.5b)**. Associations between the highest quintile of SFA from other sources and the risk of AMI were not significant in Model 1, 2, 5 and 6 models. After adjustments for potential mediators the associations for both types of SFA remained in line with the main results. **(Table 6.6)**.

In comparisons between highest vs lowest quintiles, only the highest intake of MUFA in Model 1 was associated with higher risk of AMI (OR 1.17 95% CI (1.09-1.26); p trend 0.04). After adjustment for additional covariates in Model 4 the highest quintile of MUFA had a stronger association with the risk of AMI (OR 1.23 (95% CI 1.14-1.33)), however the p value of trend became non-significant (p trend = 0.05) **(Figure 6.5b)**. After additional adjustments for potential mediators in Model 6, p value for trend still remained non-significant **(Table 6.6)**.

Higher intakes of PUFA were not associated with the risk of AMI in Model 1 (OR 1.07 95% CI 1.00-1.15). However, a weak inverse association was observed in quintiles 2-4 in Model 4 (p trend 0.05) **(Figure 6.5b)**. After additional adjustments of potential mediators in Model 6, the inverse association observed became stronger (p trend 0.02) **(Table 6.6)**.

### 6.4.8 *Subgroup and sensitivity analyses*

### 6.4.9 Substitution analysis

As illustrated in **Figure 6.13**, replacing 1% energy from total carbohydrates with SFA or PUFA was associated with 10% and 26% lower risk of AMI respectively. On the contrary, replacement of total carbohydrates with MUFA or total protein was associated with 30% and 5% higher risk of AMI respectively.

Replacing 1% energy from SFA with total carbohydrate, MUFA and total protein was associated with a 12%, 45%, 17% higher risk of AMI respectively. On the contrary, replacement with PUFA was associated with 17% lower risk.

## 6.5 DISCUSSION

### 6.5.1 Summary of main findings

In this large case-control study from Bangladesh, the majority of the energy intake of participants came from carbohydrates followed by proteins and fats. A higher intake of refined carbohydrates had no significant association with the risk of AMI, whereas a higher intake of non-refined carbohydrates had an inverse association with the risk of AMI. Highest intake of animal protein had a higher associated with the risk of AMI. On the contrary plant protein showed a weak inverse association with the risk of AMI which was non-significant. As for different types of fatty acids, modest intakes of SFA from dairy and PUFA showed a weak inverse association with AMI. MUFA showed higher association only in the highest quintile but test for trend was non-significant. In contrast, SFA from other sources had no significant association with the risk of AMI. There was no evidence for effect modification of the results by age, sex, location, smoking status, BMI and physical activity for the majority of the nutrients. However, when stratified by age, a strong increased association between highest intake of refined carbohydrates and AMI was only observed in younger age groups compared to older age groups. In addition, for the highest intake of SFA from dairy, an inverse association was observed in younger age groups and an increased association for older age groups. In substitution analyses, replacing 1% energy from total carbohydrates with SFA and PUFA was associated with lower risk of AMI. On the contrary, replacement with MUFA and total protein were associated with higher risk. Replacing 1% energy from SFA with total carbohydrate, MUFA and total protein was associated with higher risk. On the contrary, replacement of SFA with PUFA was associated with lower risk.

### 6.5.2 Correlates of nutrients

The findings of the current study reported that 66.6 % of the total energy was contributed by carbohydrates (about 45% of which came from refined sources), 19.7% (about 11% of which was from plant sources) by proteins and 13.7% by fats. The national nutrition surveys conducted in Bangladesh have also reported that 60% of total calorie intake from carbohydrate based foods, whereas there was a lower percentage of energy consumed from proteins and animal based fats.[28] Consistent with this study, one previous epidemiological study from South Asia, the PURE study also reported that about 65% of the total energy came from carbohydrates from refined and non-refined sources.[15] Contrary to studies from South Asia, evidence from studies conducted in the west have reported a relatively lower percentage of energy from carbohydrates consumed.[15] Whereas higher intakes of protein from animal sources and totals fats (especially SFA) have been reported.[15,26] Overall, higher intakes of refined carbohydrates, non-refined carbohydrates

and animal protein were reported in rural areas. Whereas in urban areas higher consumption of SFA from dairy and plant protein was reported.

In terms of sociodemographic correlates, those who were more educated and had higher income levels had a higher consumption of most of the nutrients. The present study reported that fatty acids tended to be associated with less likely to be current smokers. Whereas people who had higher consumption of carbohydrates from non-refined sources and SFA from dairy were more physically active. As for history of disease overall there were slight differences in those with the highest and lowest intake of nutrients consumption. Majority of the people with higher nutrient consumption had slightly higher reported history of diabetes, hypertension and family history of MI.

The findings of the current study of different types of nutrients indicated that dietary nutrients may associate differently with various characteristics. Overall the nutrients showed weak associations with lipids and waist-to-hip ratio. The data reported that higher intakes of refined carbohydrates were associated with lower waist-to-hip ratio, total cholesterol and LDL-C. In contrast, carbohydrates from non-refined sources had no association with waist-to-hip ratio but had increased association with total cholesterol and LDL-C. Reducing LDL cholesterol in blood is one of the key targets for the prevention and treatment of CVD.[29] There were gender differences in the association of refined carbohydrates with HDL-C, with males having increasing HDL-C with higher intake and females having no association. Higher intake of SFA from dairy sources had no effect on cholesterol levels. Although there is scarce evidence on how dairy fats per se have an effect on LDL-C and HDL-C concentrations,[30] a crossover randomised controlled trial (RCT) also reported that consumption of a high-fat dairy and Dietary Approaches to Stop Hypertension (DASH)–type diet did not increase LDL-C and HDL-C significantly compared with a low-fat dairy and DASH diet.[31] As for other fatty acids, higher intakes of SFA from other sources and MUFA were associated with higher waist-to-hip ratio, total cholesterol and LDL-C. Although a diet low in SFA has been recommended to reduce LDL-C in the blood and resultantly reduce the risk of CHD. However, this approach does not consider that single biomarker (LDL-C) may not be the best predictor of the overall effect of the nutrient on CHD. Other biomarkers such as triglycerides, HDL-C, ratio of total cholesterol to HDL-C, ratio of apolipoprotein B (ApoB) to apolipoprotein A1 (ApoA1) studied altogether may be better to explain the risk of CHD.[32] Higher intakes of SFA from dairy and other sources, MUFA and PUFA showed an increased association with HDL-C in females only. In males there was no association. Such observed gender differences in various associations could be due to less number of female participants in the BRAVE study.

In contrast PUFA was only associated with higher waist-to-hip ratio. Higher intakes of animal protein were weakly associated with higher waist-to-hip ratio, total cholesterol and

LDL-C. As for plant protein a u-shaped association was seen with total cholesterol and LDL-C and a lower association with HDL-C. A systematic review study reported that consumption of soy protein with isoflavones resulted in lower total and LDL-C concentrations as compared to the consumption of animal protein.[33] This diversity in the direction and magnitude of the associations potentially highlight distinct nutrient pathways and how these affect the risk of CHD. It also highlights the heterogeneity that occurs between different dietary nutrients that should be considered when investigating disease risk. However, it is important to consider that the whole food matrix consists of many different nutrients and this makes it challenging to interpret these associations.[34] Additionally the studies only adjusted for age and sex so there is possibility of confounding by other variables.

The food sources of nutrients have been shown to be an important factor to better understand the association of nutrients with CHD risk. Populations from different countries may have different food sources of nutrients. A large proportion of carbohydrates from refined sources was derived from boiled white rice. The largest contributors to carbohydrates from other sources were pulses and other fruits. Milk was the largest source of SFA from dairy, whereas sweet water fish, boiled white rice and other fruits were the highest contributors to other sources of SFA. Sweet water fish was the largest contributor to MUFA and animal protein. Plant protein was mainly derived from boiled white rice and pulses. It is important to note that the SFA, MUFA and PUFA content will also be dependent on the cooking oils of the various dishes (e.g. the added fat for cooking of fish, vegetables, rice etc). These oils are 'assumed' and have an assumed quantity in the recipe and will therefore determine the quantities and proportions of SFA, MUFA and PUFA.

The "energy dense food pattern" was positively correlated with carbohydrate from refined sources and different fatty acids. The "vegetable pattern" was strongly associated with carbohydrate from non-refined sources, fatty acids and animal protein. The "fruits and dairy pattern" was associated with SFA refined carbohydrates and plant protein. Although this means that the possibility of confounding by other foods and nutrients in the results cannot be excluded, this may highlight that association of nutrients should not be considered independently of the dietary patterns within which they exist.

### 6.5.3 Refined and non-refined carbohydrates and risk of acute myocardial infarction

In the current analyses refined carbohydrates initially demonstrated an increased association in the age and sex adjusted model, which became weak and statistically insignificant after further adjustments in Model 4 suggesting the role of confounding. In Model 4, in addition to other confounders, energy intake was also adjusted for which may explain the null association. This means that amount of calories from carbohydrates are more important than the exposure of the carbohydrates itself; etiological association of carbohydrates itself is less important. Although evidence has suggested that higher intake of refined carbohydrates in food raises LDL-C, in addition to raising the blood glucose and insulin levels and thereby is expected to increase the risk of CVD.[35] However, there is inconsistent evidence from observational studies owing to different populations and heterogeneous sources of carbohydrates consumed.[15,36,37] In the BRAVE study 44.6% of the contribution of energy in controls was from refined carbohydrates. The major source of refined carbohydrates in this population was boiled white rice (74.2%) and thus the overall non-significant association reported, is congruous to the null association observed between rice with AMI reported in **Chapter 4**. This result is also consistent with the PURE study which assessed the dietary intake of 135,335 subjects from 18 countries (including South Asia) reported that higher intakes (up to 77% of energy) of total carbohydrates (mostly contributed by refined sources) had no significant association with the risk of AMI after multivariate adjustments.[15] However when stratified by age groups, the associations varied in the current study; the younger group showed an increased association with the risk of AMI, in contrast no association was seen in the older groups. This may be explained by the different types of carbohydrates consumed by the age groups. The younger group consumed more fried snacks than the older group in this study as reported in **Chapter 3**.

Associations differed for non-refined carbohydrate, with a significant inverse association with the risk of AMI in this study. The major contributors of non-refined sources of carbohydrates in this study are from pulses, fruits and vegetables. These food items had an inverse association with the risk of AMI as reported in **Chapter 4** and this may explain the strong inverse association. In addition, carbohydrates from non-refined sources have low glycemic index, which may not have profound effect on raising blood sugar levels.[38]

A number of potential mechanisms explain how carbohydrate quality affects the risk of CHD. Glycaemic index (GI) is a measure that ranks food groups based on their incremental glucose response of a test food relative to a reference food (e.g. white bread).[36] Dietary carbohydrates that produce low glucose response (such as those from plant sources) have been postulated to improve glucose levels and insulin control and thus reduce the risk of CHD. In contrast, evidence from observational studies suggest that carbohydrates with

high GI (such as refined carbohydrates and added sugars) increases insulin resistance and triacylglycerol concentrations and lowers HDL-C. [36]

### 6.5.4 Animal and plant protein and risk of acute myocardial infarction

The findings from the current analysis suggested that animal protein was associated with higher risk of AMI, whereas plant protein had a weak inverse association with the risk of AMI. There is inconsistent evidence on how different sources of proteins may affect the risk of CHD and how cultural variations in diet, concomitant compounds consumed with the protein, and preparation of food, all which may play a key role in determining CHD risk.[34] In addition, differences in the sources of protein may have different effect on the risk of CHD. In this study 36.3% of the plant-based protein was derived from boiled white rice, 33.0% from pulses and only 9% from other vegetables. This may explain the inverse association of plant-based protein. There is a general perception that plant based foods and resultantly plant based proteins are beneficial for cardiovascular health.[34]

For animal protein the main food source was sweet water fish (68.6%), and the association observed is congruous to the strong increased association of sweet water fish with AMI in **Chapter 4**. As aforementioned, the evidence on the role of plant based and animal protein with CVD is mixed, depending on the population studied, how protein groups are categorised and what sources of protein are consumed.[34] The Nurses' Health study reported that both animal (from beef, pork, lamb, chicken or fish) and vegetable proteins were associated with lower risk of MI.[39] Similarly, analyses of different food sources of protein (e.g., red meat, white meat, fish, dairy, eggs, legumes, nuts, beans, etc.) have demonstrated that each protein has a different association with the risk of CHD.[40]

Dietary protein sources comprise a heterogenous category of foods that contain a variety of non-protein compounds that may have different cardiometabolic effects and thus the associations may be explained by the non-protein component.[16] Generally, plant-based protein sources are low in SFA, and high in fibre, contain micronutrients (such magnesium, potassium, carotenoids, vitamin C, B). In addition, the different amino acid profiles of plant and animal proteins may also contribute to the differing vascular effects. Plant proteins tend to be lower in the sulphur-containing amino acids like methionine, as well as tryptophan, threonine, lysine and leucine.[16] Whereas in animal proteins there are high levels of lysine and methionine, which have shown to induce hypercholesterolemia in rabbits.[16]

#### *6.5.5 Fatty acids and risk of acute myocardial infarction*

For individual fatty acids, the current analyses reported an inverse association only with modest intakes of SFA from dairy, however the there was no association in the highest

quintile. Earlier it was postulated that dairy products increase the risk of CVD due to high SFA content that may increase LDL-C levels and lead to atherosclerosis.[41] Based on this evidence various dietary guidelines have recommended consumption of low fat dairy products rather than full fat dairy products to reduce the risk of CVD.[42,43] However, evidence from a recent meta-analysis has indicated that dairy products have no association with the risk of CHD,[44] challenging the guidelines to reduce SFA intake. In addition, recent research investigating dairy fats and CVD have challenged the common belief that SFA in dairy is detrimental for heart health.[45] In particular, a meta-analysis reported that odd chained SFA from dairy are cardio-protective[10] and another study reported that SFA in dairy reduced the risk of CVD.[46] On the other hand, a study investigating dairy fats in 3 cohorts from United States reported no association with CVD.[47] Consistent with the current evidence, this study reported that SFA from dairy sources had no significant association on lipid markers. This emphasises the importance of investigating individual SFA to investigate the risk with CHD. However, when stratified by age groups, a higher association was observed between SFA from dairy and AMI in older age groups and an inverse association in younger age groups. This may be explained by the different sources and amount of dairy products consumed by the two age groups; the older group consumed more milk and less yoghurt as compared to the younger group as reported in **Chapter 3**. There is evidence that the probiotics in yoghurt decreases inflammation and may lower the risk of AMI.[48]

SFA have short chains (1-6 saturated carbons), medium chains (7-12) and long chains (13 or more). These structural differences have differential effects on the risk of CHD.[49] Dairy fats include medium chained fatty acids including butyric acid, phytanic acid, cis-palmitoleic acid and trans-palmitoleic acid. Studies have shown that some of these fatty acids have antidiabetic properties.[16] Phytanic acid which is a product of ruminal degradation of chlorophyll, induces brown adipocyte differentiation and the uncoupling protein in brown adipocytes, leading to increased thermogenesis. This may decrease body fat and risk of CVD.[16] In addition, both butyric acid and phytanic acid may act synergistically as peroxisome proliferator-activated receptors agonists, to lower insulin sensitivity.[16] Furthermore, it is important to note that in whole or reduced fat milk and yogurt products, fats are consumed along with minerals such as calcium, phosphorous, magnesium and potassium, elements that have blood pressure lowering effects.[16] There is some evidence that the calcium present in dairy products may attenuate the effect of dairy fats on blood lipid levels.[50]

The current analyses reported that SFA from other sources had no significant association with the risk of AMI. A traditional view of dietary SFAs indicated that dietary SFAs were associated with higher risk of CHD by increasing the levels of LDL-C.[6] However, several

recent meta-analyses have reported no association between SFA and CHD risk.[10,11,51] Specifically, a meta-analysis of 37 observational studies showed no association between SFA intake and risk of CHD (risk ratios were 1.03 95% CI 0.98-1.07).[10] In another meta-analysis of prospective studies, de Souza et al also did not find associations between SFA and CHD.[51] It is important to note however, that these meta-analyses suffered from heterogeneity due to a lack of consistency in methods used by different studies, resulting in large heterogeneity in the estimates. In addition, the heterogeneity of effect across circulating composition of specific SFAs could partly also be explained by biology, as circulating SFAs reflect both consumption and metabolism and synthesis.[10] "For example, the influence of metabolism seems particularly relevant for the de novo synthesis of even-numbered SFAs, compositions of which are largely determined by dietary factors, including carbohydrate and alcohol consumption, and other metabolic pathways rather than direct dietary intake, which may explain the non-significant associations." [10]

Consistent with the current evidence, the traditional view of detrimental effect of SFA is also challenged by the current study. Earlier evidence was mostly based on ecological and less robust observational studies,[52] did not take into account the multifactorial aetiology of disease and had inadequate reliance on single biomarkers to assess CVD risk rather than investigating disease end points.

In addition, earlier hypothesis was based on the evidence that SFA increases the levels of LDL-C, leading to atherosclerosis. In this study higher intake of SFA from other sources was also found to be weakly associated with higher total cholesterol and LDL-C, however a causal inference cannot be made as these estimates are cross-sectional and do not consider the effect of SFA on other biomarkers in the blood. It is important to consider that SFA also influence other lipid makers (HDL-C, apolipoproteins and triglycerides) and therefore the ratio of HDL-C to total cholesterol may be a more relevant marker to assess the risk of CHD than LDL-C alone. Nonetheless, the current study reported low consumption of SFA from other sources (3.1%) as compared to what has been reported in western populations, and this may also explain the null association observed. The PURE study also reported that low levels of SFA (< 7%) had no association with the risk of AMI.[15] In the current study, the major sources of SFA from other sources were sweet water fish (25%), boiled white rice (18%), snacks (13%) and other fruits (10%). However, there could have been under reporting of snacks / fast foods in this study, that are high is SFA which could which could have affected the observed associations.

The lack of overall associations of MUFA and PUFA with CHD are also in line with a previous meta-analysis that reported no association between MUFA and n6-PUFA (the main contributor to total PUFAs in the diet) with CHD.[10] In the current study, PUFA was inversely associated with the risk of AMI only in the middle quintiles, but the trend was non-

significant. PUFAs include mainly omega-3 and omega-6 fatty acids. The predominant sources of long-chain n-3 PUFAs in the diet are oily fish and other types of seafood and of n-6 PUFAs are vegetable oils. There is evidence to suggest that n-3 PUFAs reduces inflammatory responses, modulate enzymes or molecules associated with various signally pathways involving cell function and have effect on gene expression in lipid metabolism, reduces inflammation and smooth muscle cell proliferation.[53] In addition, a meta-analysis reported that n−3 fatty acids supplementation lowered systolic and diastolic blood pressure by 4- and 3-mm Hg, respectively, in hypertensive patients.[54] There is evidence to suggest that PUFA are associated with lower risk of CVD by having beneficial effects on blood lipids, insulin resistance and inhibition of thrombosis.[55] However, in this study PUFA was not associated with LDL-C and HDL-C.

The lack of significant association of MUFA and CHD in this study is consistent with available evidence, which remains conflicting about whether MUFA promote or protect against atherosclerosis.[56–58] Although the PREDIMED trial conducted in Spain, reported that diets higher in MUFA (mainly from olive oil and nuts) compared with a low fat diet reduced AMI in high-risk individuals.[59] In the current study however, the major source of MUFA was sweet water fish, (which may also explain the weak increased association in the highest quintile) therefore it is difficult to compare across different studies due to different food sources of nutrients consumed. Consistent to the findings of the BRAVE study, the Nurses' Health study (largest study that disaggregated by food sources) reported MUFA from animal food sources (beef and pork) had a significant association with CHD. Whereas MUFA from plant sources had an inverse association.[60] This suggests that the food source of MUFA affects its association with CHD.

Overall, the evidence of the current study on different fatty acids is consistent with the previous study (PURE study) having participants from Bangladesh, which reported that SFA, MUFA and PUFA had no significant association with AMI.[15] PURE study is a large non-western cohort study of 18 countries that also has participants from South Asia.

### 6.5.6 Substitution analysis

As aforementioned, various dietary guidelines have focused on reducing SFA intake.[9] However, it is also important to note that reduction in SFA according to current dietary guidelines means that this has to be replaced by same amount of energy from other nutrients, which in most cases is carbohydrates. The evidence from meta-analyses that reported no association of SFA with CVD did not take into account the differences in the effects of the replacement nutrients, specifically carbohydrates and PUFA, which may be the main cause of heterogeneity among studies.[61] Therefore, investigating the association

of isolated SFA or any other nutrient is misleading as it implies that replacement of it by any macro-nutrient will result in similar results.[17] LMICs like Bangladesh have diets high in refined carbohydrates (white rice and bread) and low in fats, therefore it is important to investigate how replacement of one nutrient with another effects the risk of AMI. The focus of recent research and dietary guidelines has therefore shifted to an increased emphasis on the importance of the replacement nutrients specifically SFA rather than investigating single nutrients only.[51,62]

In the current study it was reported that replacement of SFA with PUFA lowered the risk of AMI by 17% (OR 0.83, 95% CI 0.74-0.94), which agrees with the majority of the previously published reviews and clinical trials. Specifically a meta-analysis of 11 observational studies having 5429 incident CHD cases in the US and Europe reported that replacing 5% energy from SFA with same amount of energy from PUFA was associated with a 13% lower risk of CHD (HR 0.87, 95% CI 0.77-0.97).[12] Similarly, a Cochrane review of randomized controlled trials showed that substituting SFA with PUFA reduced the risk of CVD events by 27% (HR 0.73, 95% CI 0.56-0.96).[14] In addition, there is evidence from meta-analysis of 60 clinical trials that PUFA replacing SFA lowers the levels of LDL-C, with no effects on HDL-C.[63] It is also suggested that the effects of SFAs on lipoproteins may be modulated by the availability of PUFA, such that LDL-C is only increased by SFAs if PUFA intake is below ~5% of total energy.[64]

In the BRAVE study replacement of SFA with MUFA was associated with higher risk of AMI. There is mixed evidence of effects on CHD with MUFA as a replacement nutrient for SFA. A small Finish study, having 183 fatal and 382 non-fatal CHD events, reported that substitution of SFA with MUFA led to a higher risk of CHD, whereas substitution with PUFA resulted in a lower risk of CHD,[65] which is congruous with the findings of the BRAVE study. In contrast, a meta-analysis of 11 prospective cohort studies found no effect of replacing SFA with MUFA on coronary events.[12] Moreover, the PREDIMED cohort study reported that replacing SFA with MUFA is inversely associated with the risk of AMI.[66] These inconsistent results may be explained by the different sources of MUFA consumed from different populations.

The data reported that replacement of SFA with carbohydrates had a higher risk of AMI. Mixed results have also been reported when SFA is replaced by total carbohydrates. A meta-analysis reported that replacing SFA with carbohydrates had higher risk with CHD, which is in agreement with the results of this study.[12] Similarly, the EPIC-Netherlands study reported that substitution of 5% of energy from SFA with carbohydrate was associated with higher risk of ischemic heart disease (IHD).[13] In contrast, a Cochrane review of RCTs showed no association with CVD when SFA was replaced with

carbohydrates.[14] The PREDIMED trial also reported no effect of replacing 5% of energy from SFA with carbohydrates on CVD.[66]

In the current study a 1% replacement of energy from SFA with total protein had 30% higher risk of AMI, this could be explained because of the high portion of animal protein came from fish, which was seen to be detrimental for the risk of AMI in **Chapter 4**. This is also in agreement to the EPIC-Netherlands study which reported that replacing 5% of energy from SFA with protein (mostly animal protein) increased the risk of IHD by 29% (HR 1.29, 95% CI 1.08-1.54).[13] However, when protein types were examined in this trial, only animal protein was associated with increased risk of CHD, whereas plant protein showed no association. In contrast, in the current study replacement of SFA with plant protein was associated with a weak increased association.

Carbohydrates replacement analyses in the current study reported that replacement with SFA and PUFA lowered the risk of AMI. Whereas, replacement of carbohydrate with MUFA and total protein had higher risk of AMI. This is in contrast to evidence from the PURE study that reported no association with AMI when carbohydrate was replaced by the above mentioned nutrients.[15]

### 6.5.7 Strengths and limitations

Several strengths and limitations merit careful consideration. This is the largest observational study from solely based on a population from South Asia that investigates the association of different food sources of dietary nutrients with the risk of AMI. In addition, this analysis adjusted for multitude of potential confounders and mediators. Nonetheless, owing to the observational nature of the study, potential unmeasured and residual confounding cannot be excluded. In addition, in this study trans-fat intake was not measured separately which may affect the results, especially in the substitution analyses. Trans-fats were not measured separately as the food composition were not comprehensive enough to distinguish trans-fat consumption. As trans-fats were not additionally adjusted for this may contribute to residual confounding. The way to measure trans fats is in blood, which was not available for the BRAVE study.

The study used FFQ to measure dietary intake, which is prone to measurement error leading to under or over estimation of the observed associations. Measurement error may occur due to misreporting of food intake and uncertainty in the estimating of portion sizes. Systematic error can occur if reporting is associated with participant's characteristics such as there is evidence that under-reporting of energy intake is associated with BMI.[67] Such type of measurement error can bias the associations in either direction and is more of a concern. When the error originates from variations in individual food choices, which may simply differ from one day to another, the error is characterised as random and is common to all individuals in a population. As this error occurs at random or is unpredictable, this would lead to attenuation of the observed associations.

In addition, the dietary nutrient intake was estimated from food composition table, which also has many limitations. The composition table reports a single composition of a specific food, however the components of can food vary considerably in composition; depending on the environment, location, animal breed, cultivation method, storage, different manufacturers, processing methods and cooking method.[15,68] For this chapter the Indian Food composition table[24] was used as the Bangladesh Food composition table[69] lacked information on individual type of fatty acids. As the diets of the countries are broadly similar, this may be considered as a reliable database. In addition, the results observed for the nutrients and risk of AMI in this chapter were consistent with the food groups/sources of these nutrients as reported in **Chapter 4**, highlighting the reliability of the results. However, the possibility of confounding by other nutrients cannot be ignored. In addition, there may be a possibility that the observed associations are due to multiple testing or Type I error. To correct this, Bonferroni adjustment was used as a sensitivity analysis, after which only the associations of other carbohydrates and animal protein remain with AMI remained significant.

Nevertheless, it is important to note that it is the interactions between different nutrients that constitute food groups determines the effect on CHD risk.[20] In addition, because of the collinearity between different nutrients in the complex food matrix, it is difficult to exclude the effects of other nutrients while observing the association of single nutrients with CHD risk.[15] However, the analysis on substitution analysis allowed to investigate the effect of several nutrients in this study.

It is also important to consider that it is difficult to translate single nutrient based dietary guidelines to general public and therefore the research on nutritional epidemiology in the last decade has focused more on food groups and dietary patterns than specific nutrients.[70] However, investigating the association of nutrients with risk of AMI is important to enhance our understanding of the food group and dietary pattern analyses.

### 6.5.8 Implications

As described earlier in this chapter, the findings should be interpreted with caution since measurement errors, inherent in any self-reported dietary assessments, may affect the observed associations. Alternative explanations for these findings have also been discussed, as the potential importance of food sources of fatty acids, and the range of nutrients that foods contain. Future research needs to evaluate and address measurement error carefully, while also investigating these potential alternative explanations of the observed associations. If the observed associations are further supported by future methodologically-robust studies (e.g., prospective epidemiological studies), these findings may have important implications for public health policies and nutrition research priorities in Bangladesh and elsewhere, such as limiting animal protein and increasing intake of carbohydrates from non-refined sources in this population. In addition, the findings support shift from a conventional nutrient-based to a more food- or dietary patterns-based dietary advice approach. This is because nutrients are highly intercorrelated, and therefore distinguishing the risk association for one nutrient from another is often somewhat complicated.

In addition, public health guidelines, national governments and industries which promote reducing the intake of SFA, should also take the replacement nutrient into consideration. According to the findings of this study if SFA is replaced with carbohydrates it increases the risk of CHD, in contrast when SFA is replaced by PUFA it reduces the risk of CHD, although causality cannot be inferred from observational studies. Finally, studies involving circulating nutrients as objective biomarkers of diet should be considered rather than self-reported measures of diet.[10]

### 6.5.9 Conclusion

In conclusion, findings from this large case-control study from Bangladesh reported that refined carbohydrates had no significant association with the risk of AMI, whereas carbohydrates from non-refined sources had an inverse association with the risk of AMI. Highest intake of animal protein had significant higher association with the risk of AMI; in contrast plant protein had a weak inverse with the risk of AMI. Modest intakes of SFA from dairy and PUFA showed an inverse association with AMI. For MUFA there was a higher association in the highest quintile, but the overall association was non-significant across quintiles. In contrast highest intake of SFA from other sources had no significant association with the risk of AMI. These analyses highlight the heterogeneity in CHD risk depending on the different sources of nutrients consumed. As for substitution analyses, replacing 1% energy from total carbohydrates with SFA and PUFA was associated with lower risk of AMI. By contrast, replacement with MUFA and total protein were associated with higher risk. Replacing 1% energy from SFA with total carbohydrate, MUFA and total protein was associated with higher risk. On the contrary, replacement of SFA with PUFA was associated with lower risk. As the food we consume consists of a myriad of nutrients that may act synergistically or antagonistically to affect the risk of CHD. Therefore, the next-generation dietary guidelines should primarily be based on food groups and dietary patterns, considering the complex interactions between different nutrients.

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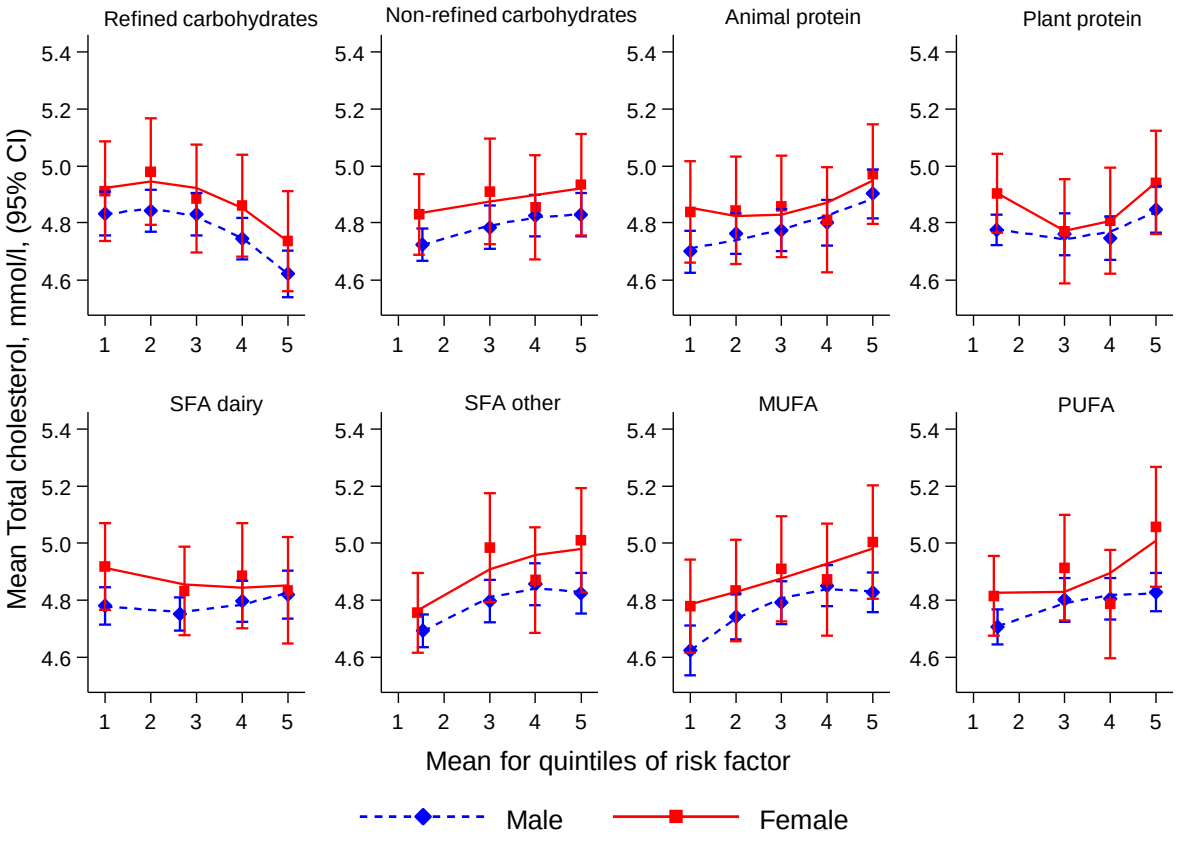
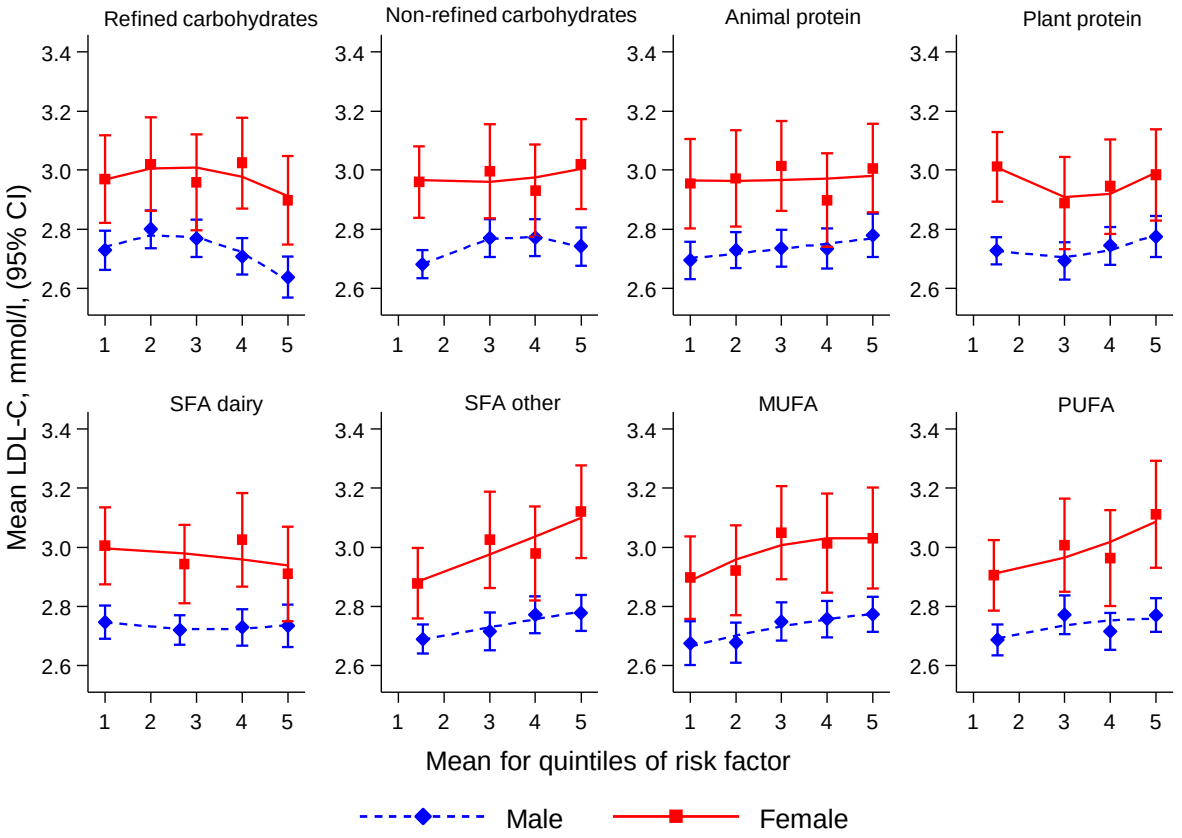
**Figure 6.1:** Cross-sectional association of nutrients with total cholesterol in BRAVE controls.




Response means are adjusted to age 40

Mean (95% CI) of total cholesterol (y-axes) by sex-specific quintiles of dietary nutrients (En-%), adjusted to age=40 years. Estimates were obtained with linear mixed models

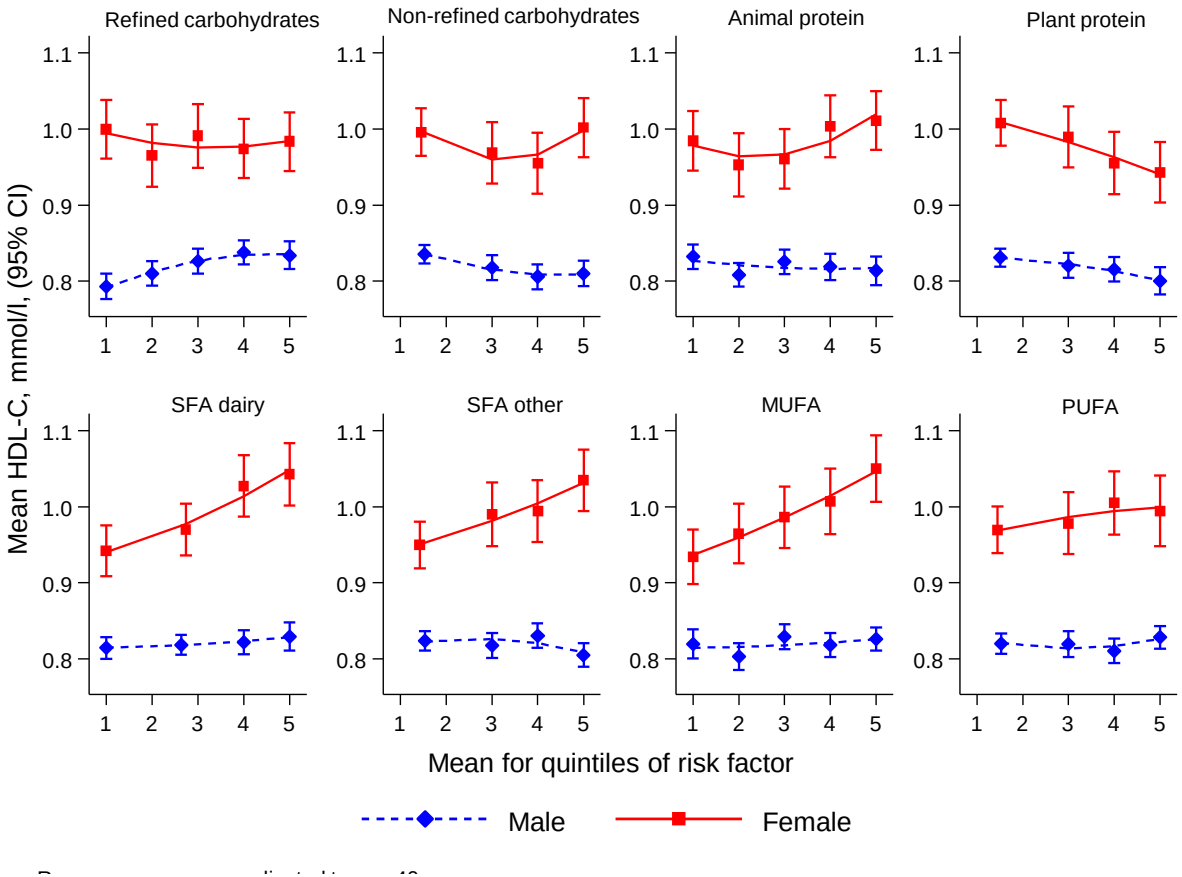
**Figure 6.2:** Cross-sectional association of nutrients with LDL-C in BRAVE controls.




Response means are adjusted to age 40

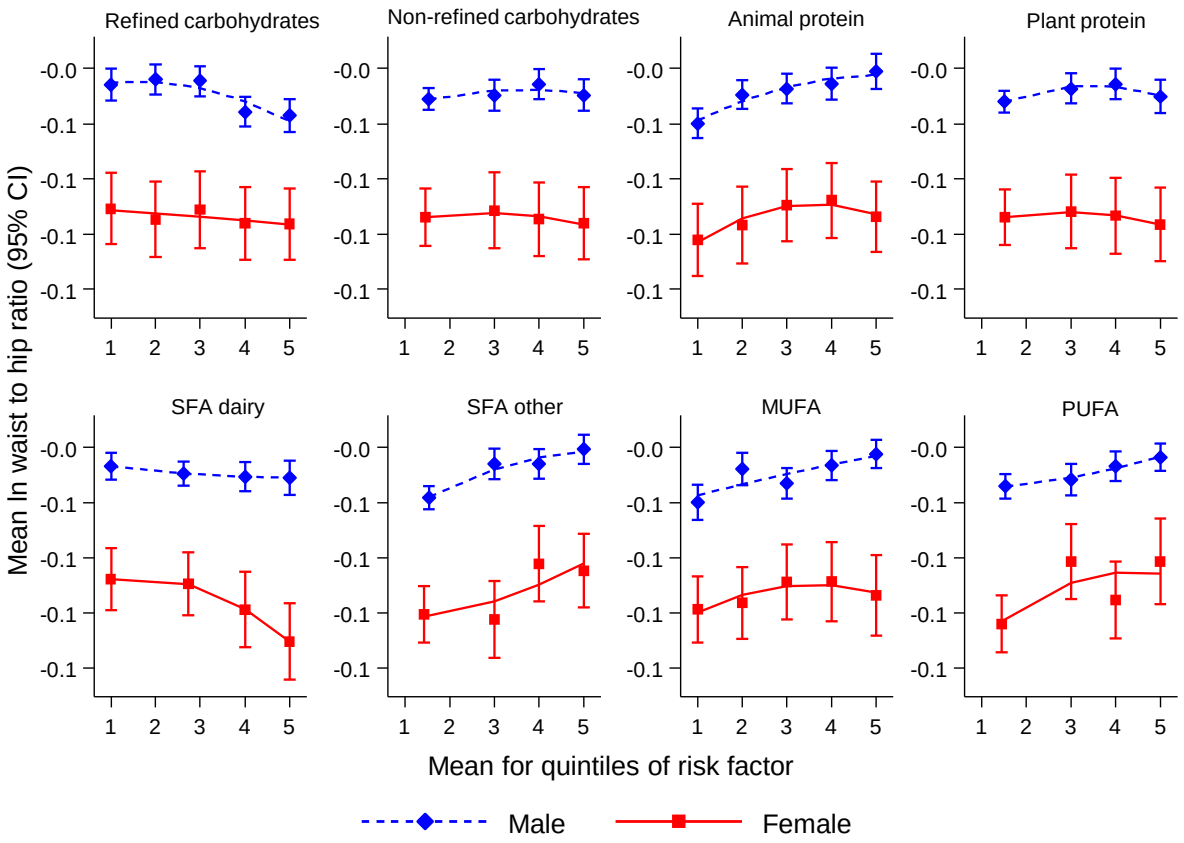
Mean (95% CI) of LDL-C (y-axes) by sex-specific quintiles of dietary nutrients (En-%), adjusted to age=40 years. Estimates were obtained with linear mixed models

**Figure 6.3:** Cross-sectional association of nutrients with HDL-C in BRAVE controls.




Mean (95% CI) of HDL-C (y-axes) by sex-specific quintiles of dietary nutrients (En-%), adjusted to age=40 years. Estimates were obtained with linear mixed models

**Figure 6.4:** Cross-sectional association of nutrients with waist-to-hip ratio in BRAVE controls.




Response means are adjusted to age 40

Mean (95% CI) of log of wait to hip ratio (y-axes) by sex-specific quintiles of dietary nutrients (En-%), adjusted to age=40 years. Estimates were obtained with linear mixed models

**Figure 6.5a:** Association of nutrients with risk of myocardial infraction

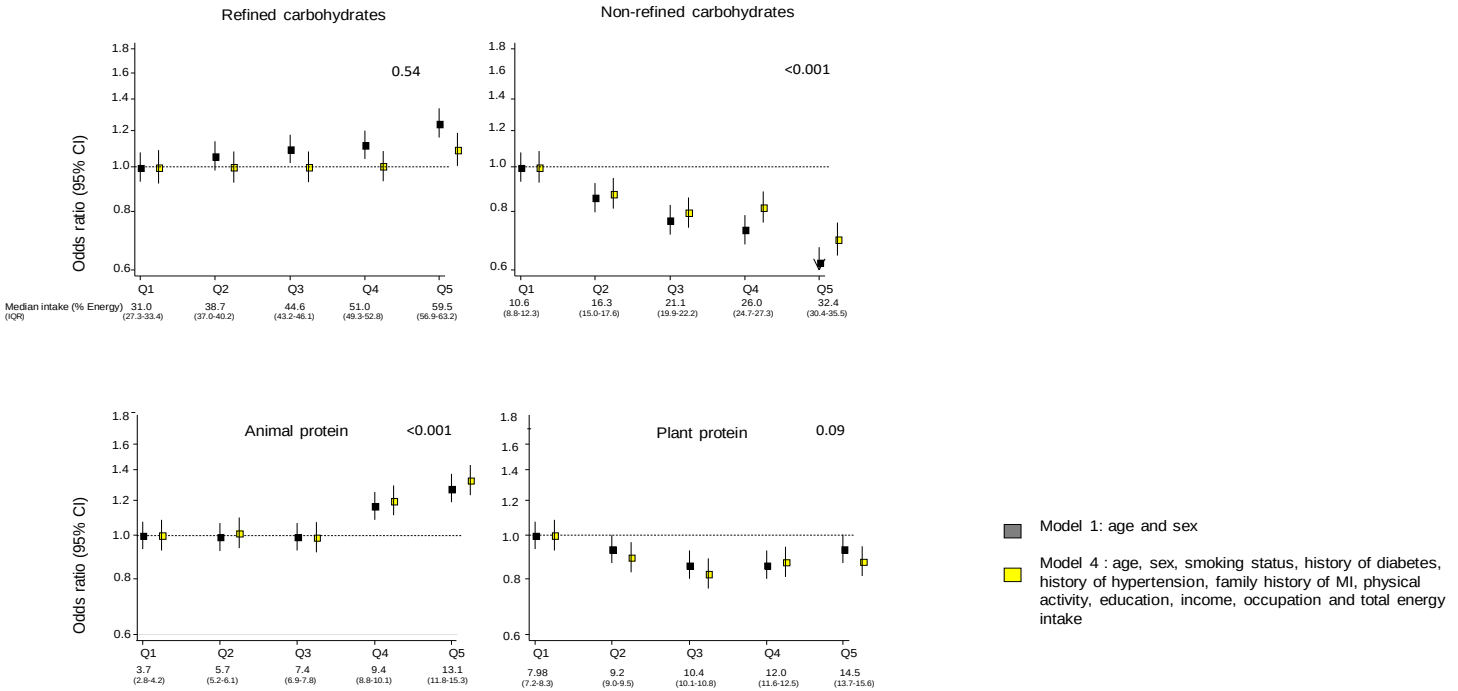
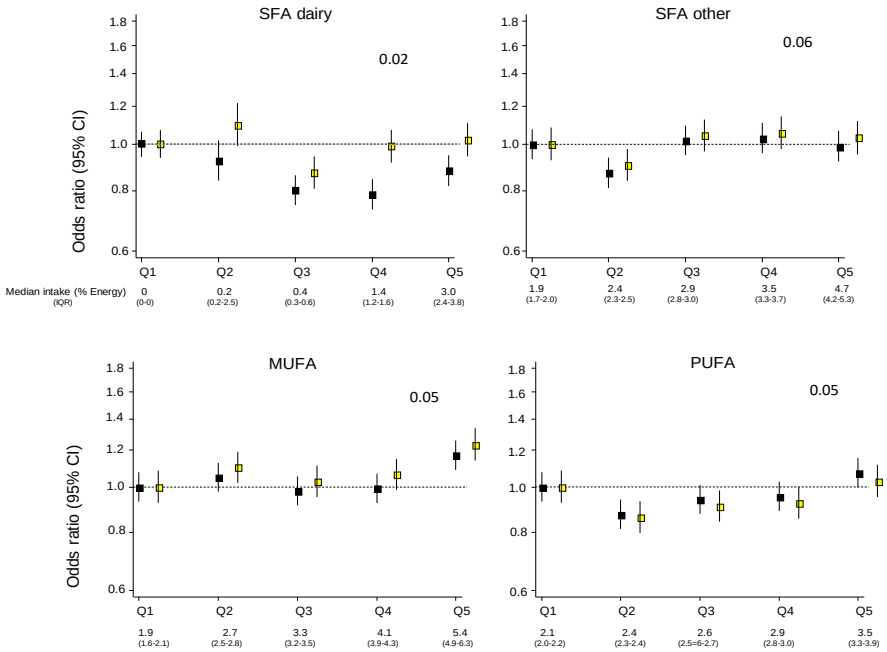


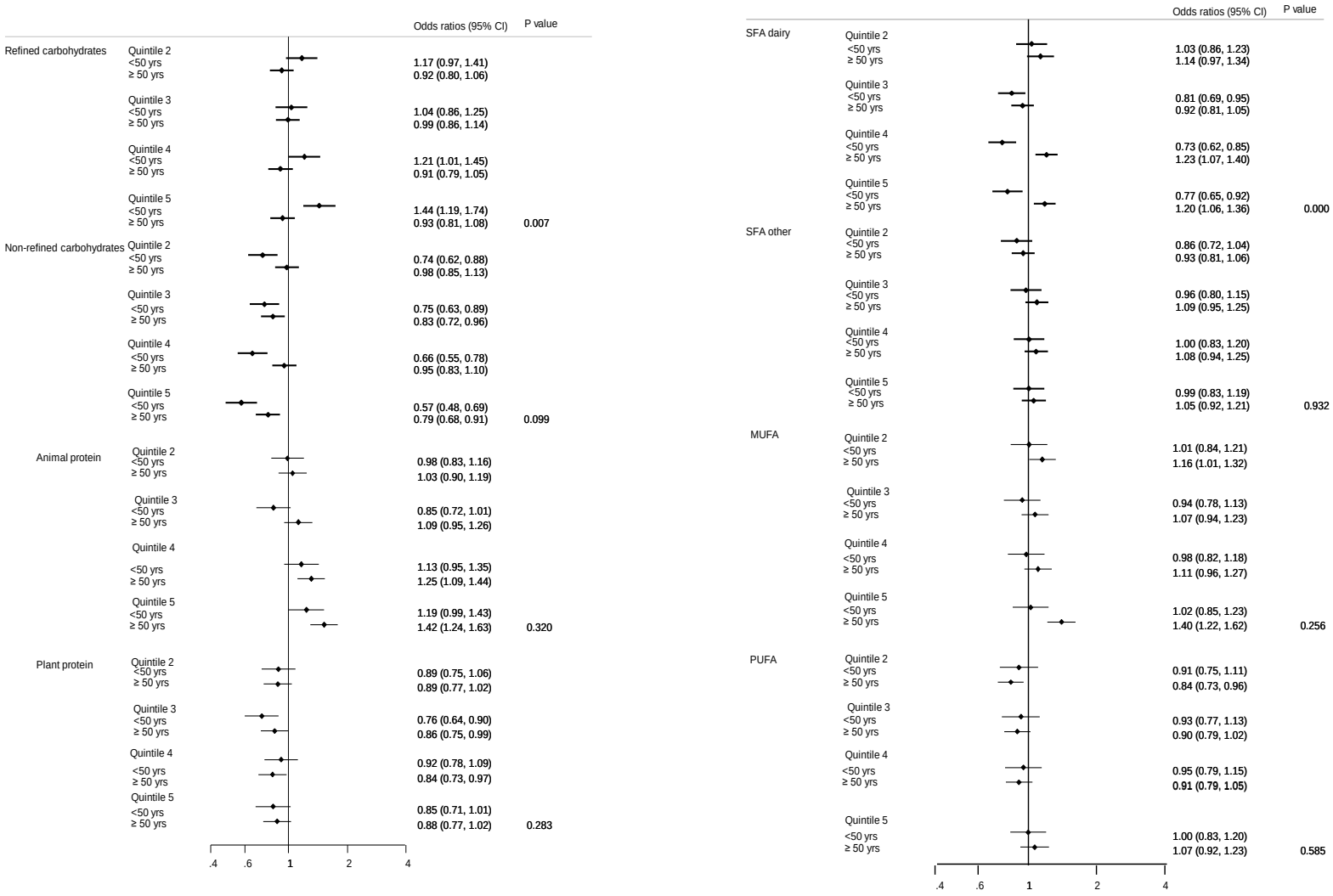
**Figure 6.5b:** Association of nutrients with risk of myocardial infraction




Model 1: age and sex

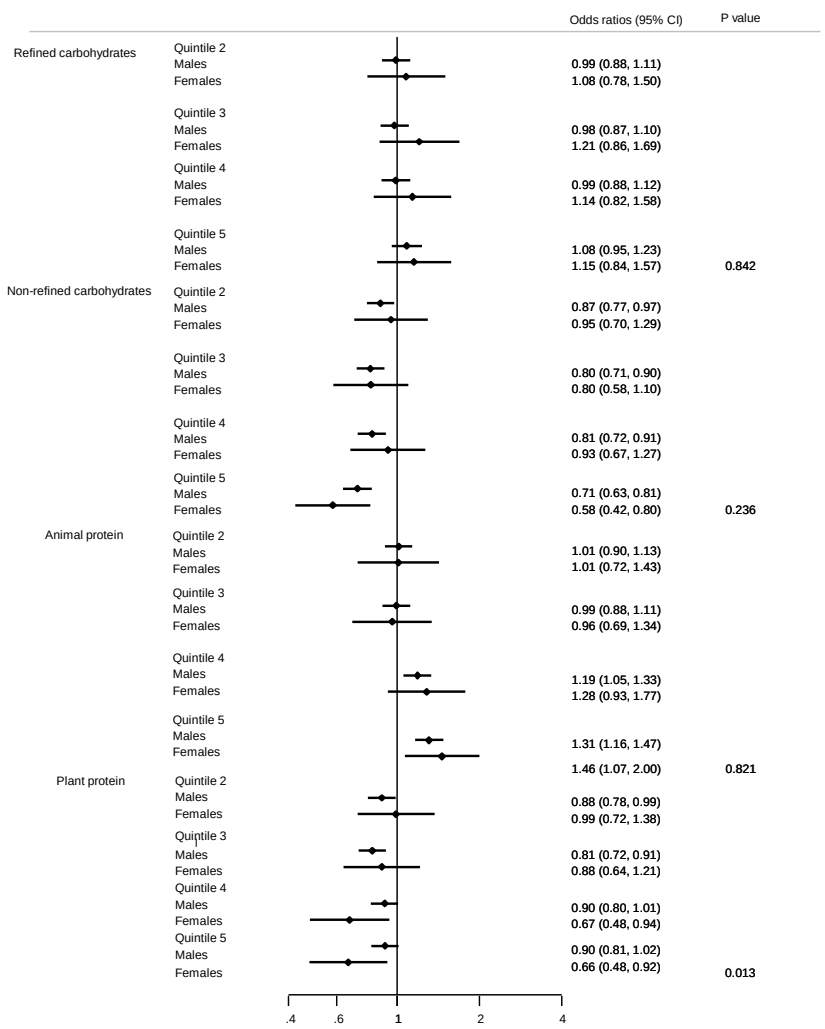
Model 4 : age, sex, smoking status, history of diabetes, history of hypertension, family history of MI, physical activity, education, income, occupation and total energy intake

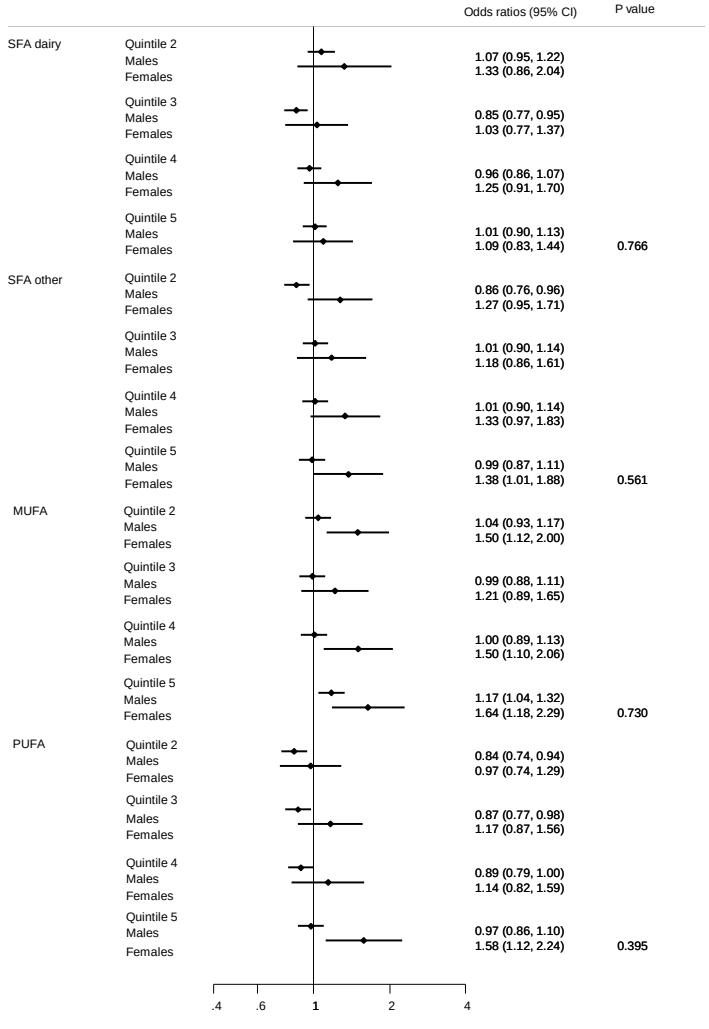
**Figure 6.6:** Association of nutrients by age




The associations were adjusted for Model 4

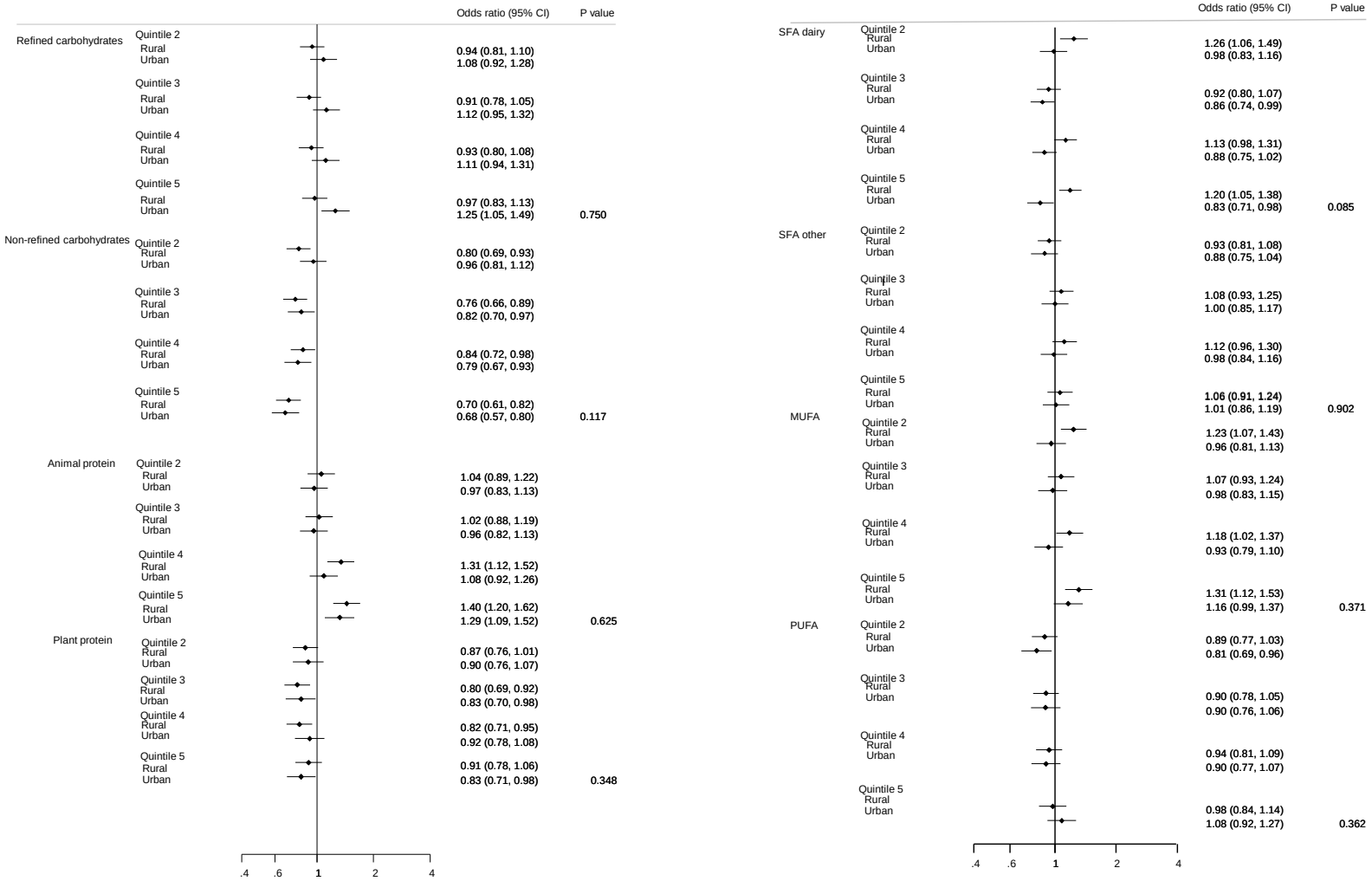
**Figure 6.7:** Association of nutrients by sex



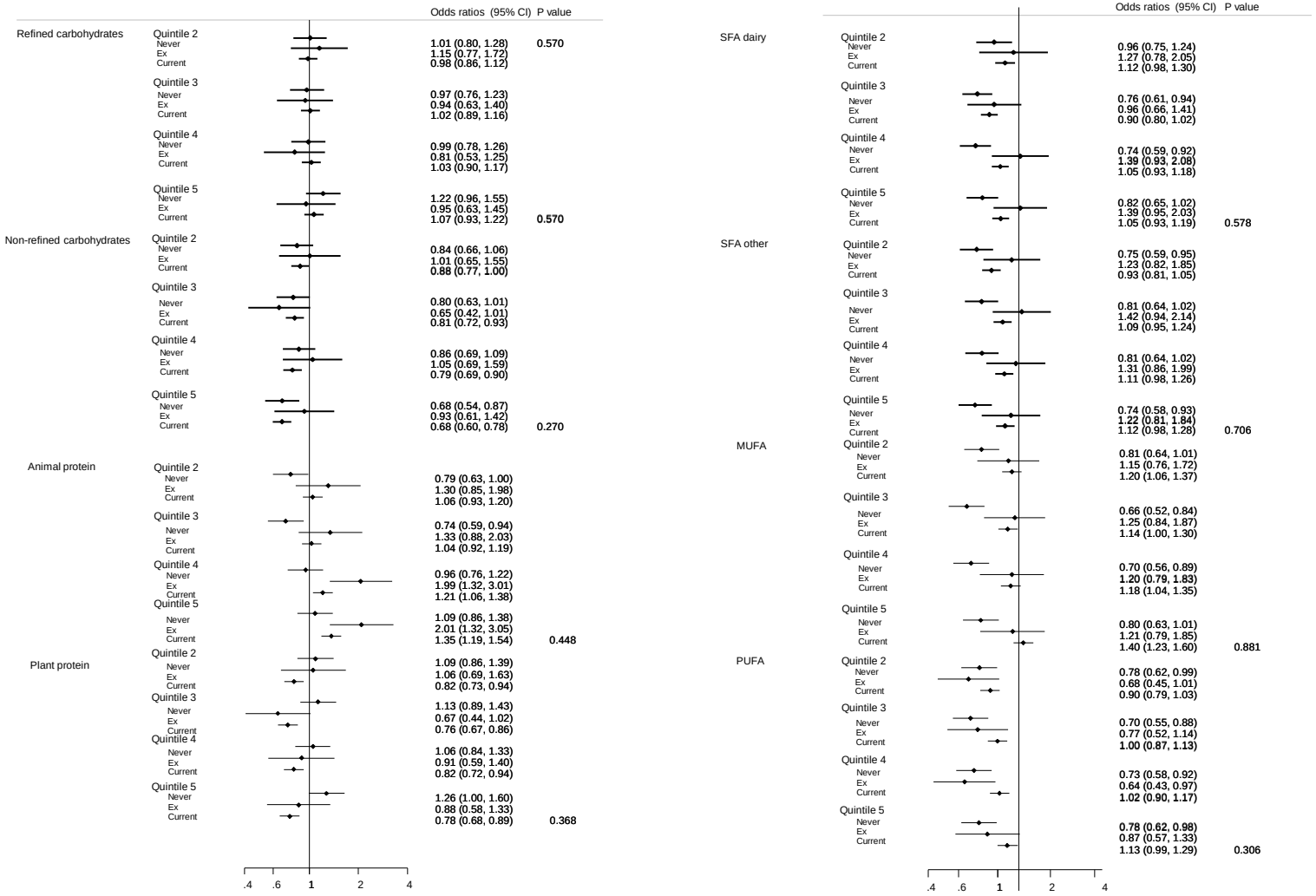

The associations were adjusted for Model 4

**Figure 6.8:** Association of nutrients by location



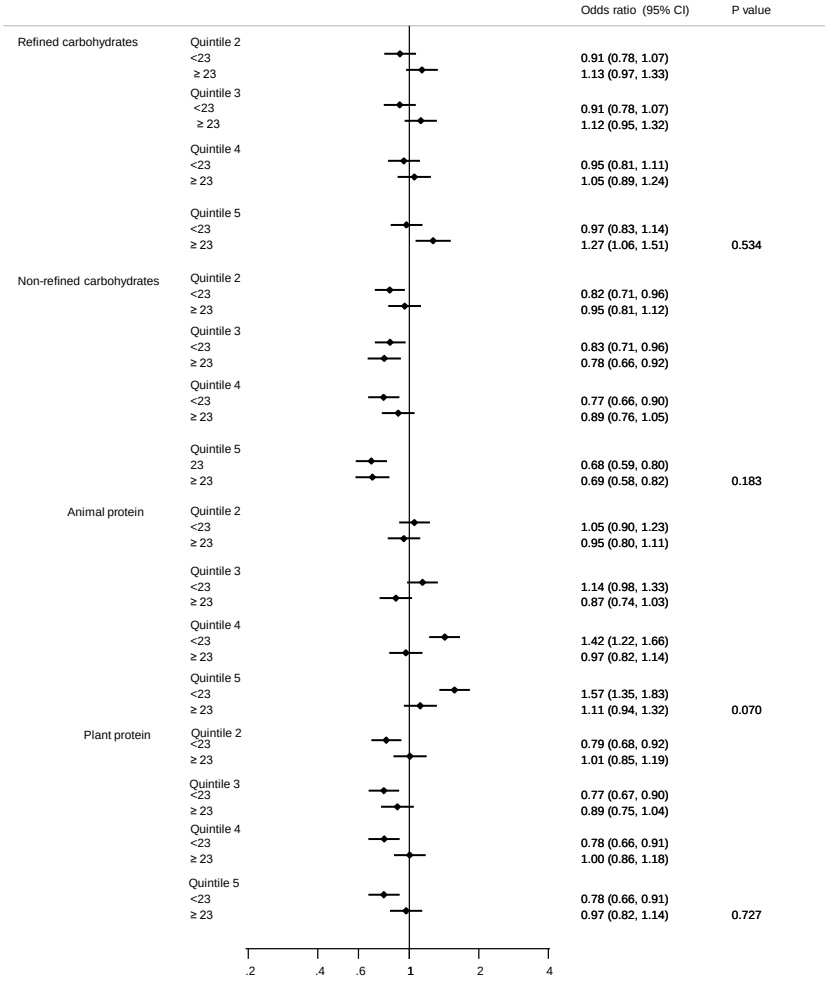

The associations were adjusted for Model 4

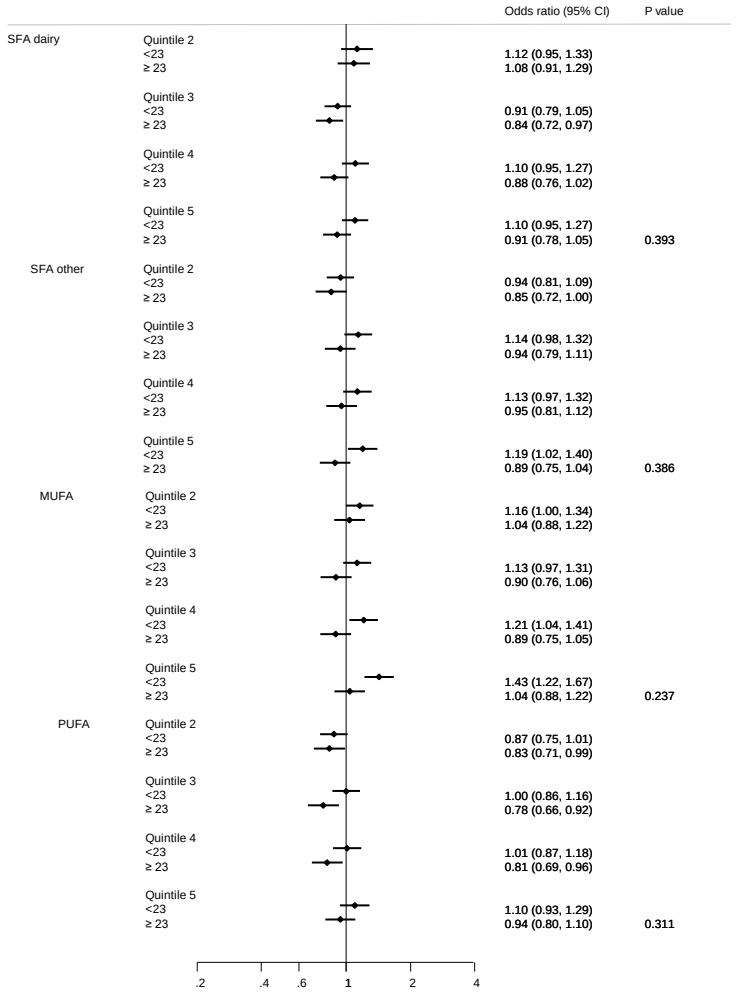
**Figure 6.9 :** Association of nutrients by smoking




The associations were adjusted for Model 4

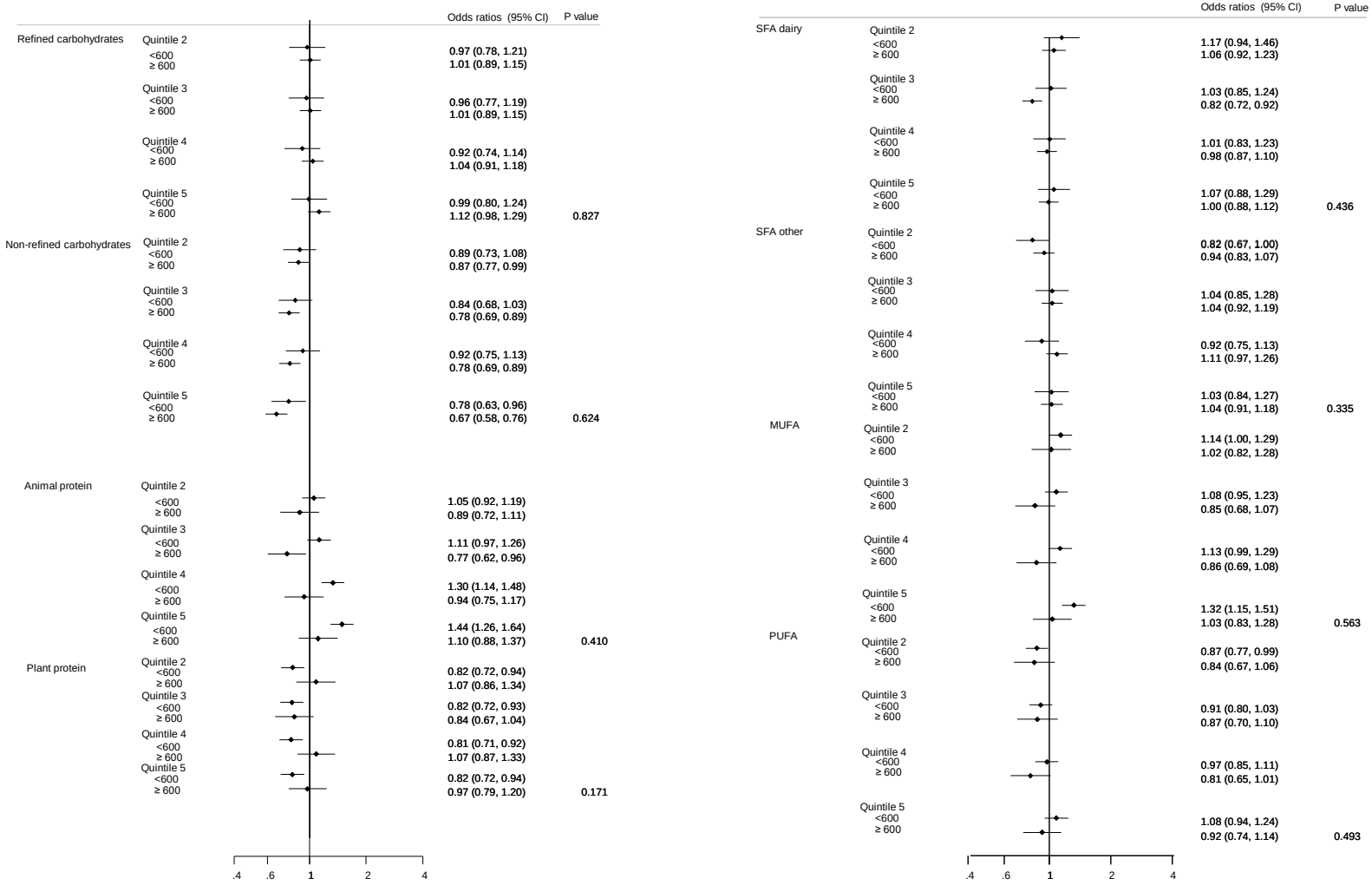
**Figure 6.10:** Associations of nutrients by BMI




The associations were adjusted for Model 4

**Figure 6.11:** Association of nutrients by physical activity




The associations were adjusted for Model 4

**Figure 6.12:** Association of nutrients and AMI by excluding participants with high and low energy intakes using cut offs from PURE study

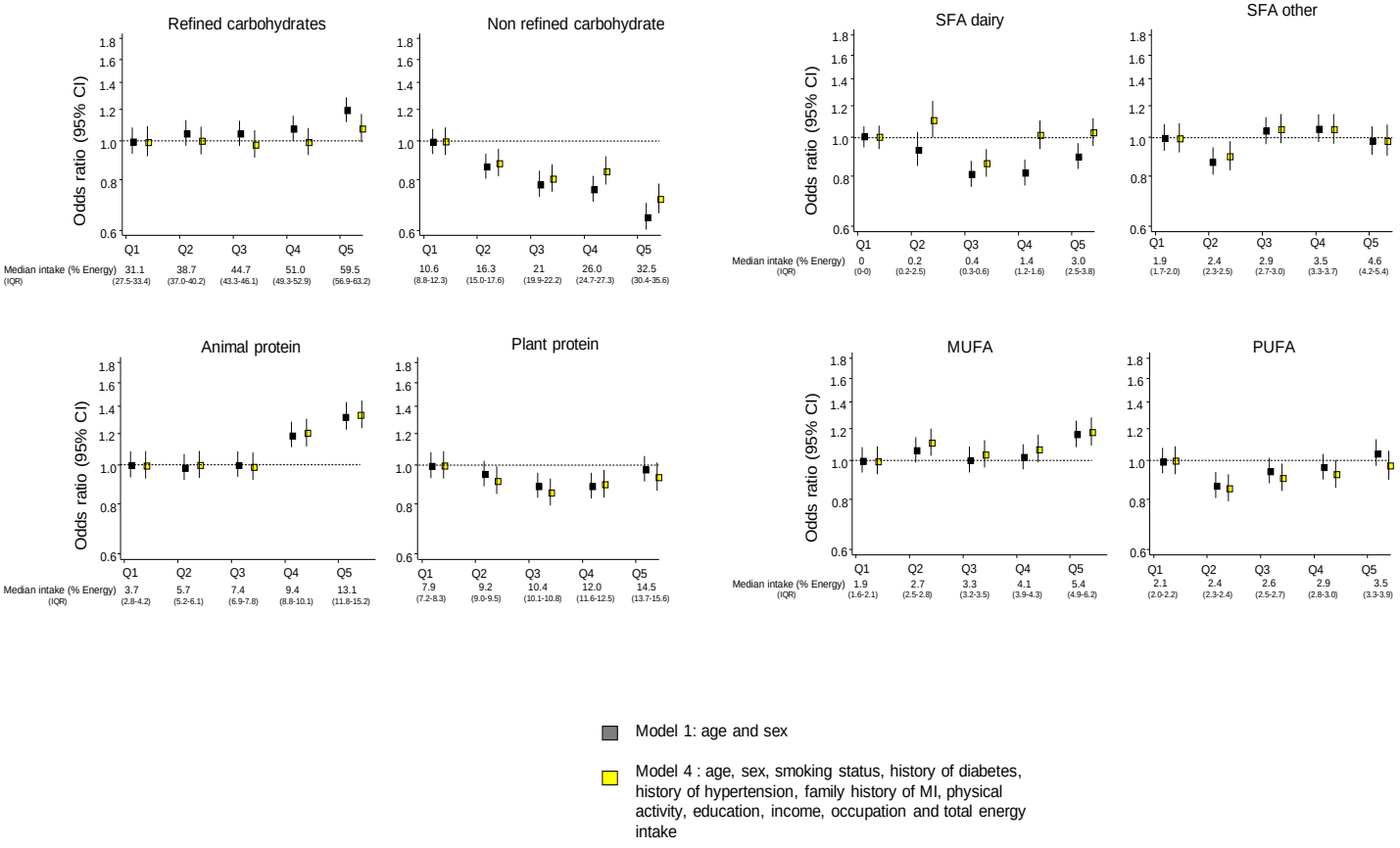
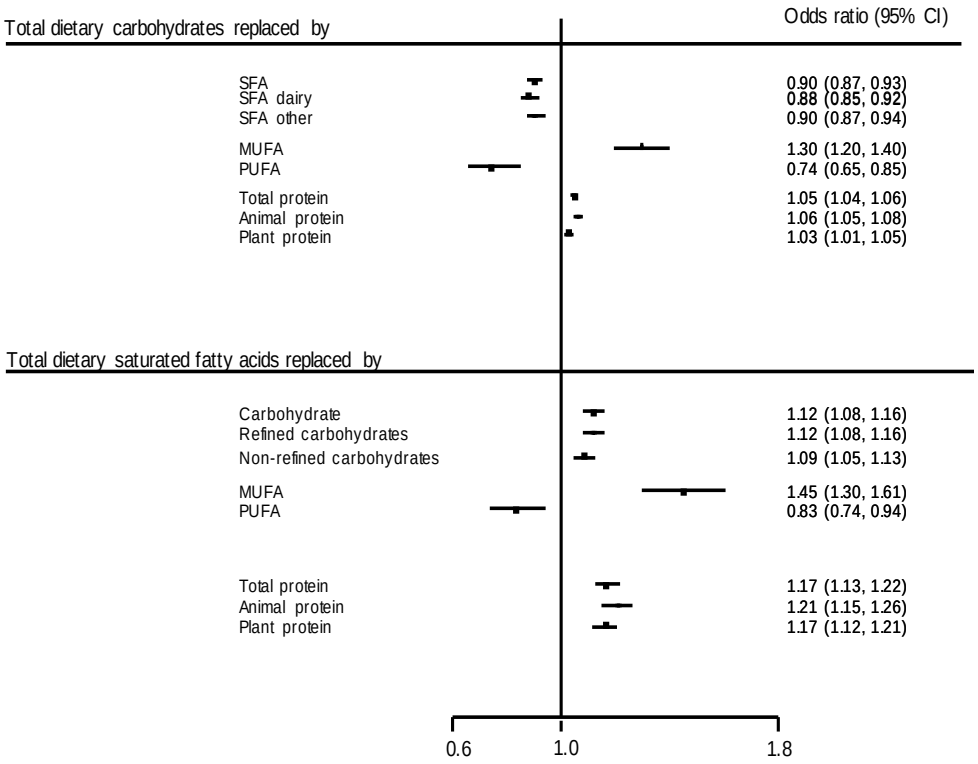


**Figure 6.13:** Association with AMI for of replacement of total carbohydrates and total saturated fatty acids with other nutrients




The associations were adjusted for Model 4

**Table 6.1**: Nutrients by food items.

| Nutrients | Food items |
|---|---|
| **Refined carbohydrates** | Boiled white rice, biryani, ruti, chapatti, paratha, bread, soft drinks, juices, lassi<br>Deep fried snacks: Puri, singarao aloo, singara koliza, samosa, chop roll and pakora<br>Savoury snacks: Jhal pattie, muglai paratha, chanachur, haleem, chotpoti, jhal muri, desi pitha, chana boot, crisps and noodles<br>Sweets: Cakes/pastries, laddu and sweet biscuits |
| **Non-refined carbohydrates** | Vegetables: Amaranth stem, drumstick leaves, colocasia leaves, spinach, cabbage ash gourd, brinjal, okara, bitter gourd, bottle gourd, ridge gourd, snake gourd, unripe papaya, pointed gourd, water lilly, tomato, cucumber, green long beans, banana blossom, sponse gourd, carrots runner beans, potatoes, sweet potatoes<br>Fruits: Guava, orange, grapefruit, banana, custard apple, hog plum, burmese grapes, papaya, emblic (amla), monkey jack, wood apple, jambolan, pineapple, coconut, mango, jackfruit, sapota, apple, grapes, asian pears<br>Pulses: Green gram (muger daal), lentil (musrur daal) and bengal gram (buter daal).<br>Dairy: Milk and yoghurt |
| **Animal Protein** | White meat: Chicken and duck meat<br>Red meat: Beef and goat meat<br>Fish: Rohu, catla, clown knife, hilsa, pangas, catfish, walking catfish, climbing perch, tilapua, tengra, barb, mola carplet, striped snake-head, spotted snake-head, byne, nola, silve carp, mirgel carp, koral, air, rissha, lobster, shrimps, pomfret, bombay duck, croaker, rita, churi, hangori, parsey, giant sea perch and goby<br>Eggs<br>Dairy: Milk and yoghurt |
| **Plant Protein** | Boiled white rice, ruti, chapatti, paratha, bread<br>Vegetables: Amaranth stem, drumstick leaves, colocasia leaves, spinach, cabbage ash gourd, brinjal, okara, bitter gourd, bottle gourd, ridge gourd, snake gourd, unripe papaya, pointed gourd, water lilly, tomato, cucumber, green long beans, banana blossom, sponse gourd, carrots runner beans, potatoes, sweet potatoes<br>Fruits: Guava, orange, grapefruit, banana, custard apple, hog plum, burmese grapes, papaya, emblic (amla), monkey jack, wood apple, jambolan, pineapple, coconut, mango, jackfruit, sapota, apple, grapes, asian pears |
| **SFA dairy** | Milk and yoghurt |
| **SFA other** | All other food items except dairy |
| **MUFA** | All food items |
| **PUFA** | All food items |

**Table 6.2:** Baseline nutrient intake of participants in BRAVE

| | **Cases** | | | **Control** | | | **P value** |
|---|---|---|---|---|---|---|---|
| **Nutrients (% Energy)** | N | Mean | SD | N | Mean | SD | |
| Total carbohydrate | 6995 | 66.0 | 6.7 | 8020 | 66.6 | 6.5 | <0.001 |
| Carbohydrate refined | 6995 | 45.3 | 10.9 | 8020 | 44.6 | 11.0 | <0.001 |
| Carbohydrate non-refined | 6993 | 20.7 | 8.1 | 8020 | 22.0 | 8.3 | <0.001 |
| Total protein | 6995 | 20.1 | 3.6 | 8020 | 19.7 | 3.5 | <0.01 |
| Animal protein | 6994 | 8.2 | 4.1 | 8010 | 7.8 | 3.8 | <0.001 |
| Plant protein | 6995 | 10.8 | 2.7 | 8020 | 10.8 | 2.5 | NS |
| Total fat | 6995 | 13.9 | 4.3 | 8020 | 13.7 | 4.2 | NS |
| SFA dairy | 6990 | 1.1 | 1.4 | 7984 | 1.1 | 1.4 | NS |
| SFA other sources | 6995 | 3.1 | 1.2 | 8020 | 3.1 | 1.3 | NS |
| MUFA | 6995 | 3.6 | 1.5 | 8020 | 3.5 | 1.4 | <0.001 |
| PUFA | 6995 | 2.8 | 0.7 | 8020 | 2.7 | 0.6 | 0.001 |

P value was obtained using two samples t test. According to Bonferroni correction p values <0.005 were considered significant.

Total protein includes plant protein, animal protein and includes a proportion of protein from other sources as well, so therefore the total of animal and plant protein does not add up to total protein.

**Table 6.3:** Characteristics of controls in quintile 1 versus quintile 5 of different nutrients

| | **Carbohydrate refined** | | **Carbohydrate non-refined** | | **Animal protein** | | **Plant protein** | |
|---|---|---|---|---|---|---|---|---|
| **Characteristics** | **Q1** | **Q5** | **Q1** | **Q5** | **Q1** | **Q5** | **Q1** | **Q5** |
| | | | | | | | | |
| **Age (years)** | 51.8 (10.2) | 57.7(9.8) | 51.4 (10.2) | 52.0 (10.2) | 50.7 (10.1) | 52.7 (10.3) | 50.5 (10.7) | 52.2 (10.0) |
| **Sex (%)** | | | | | | | | |
| Males | 1441 (86.5) | 1285 (84.8) | 1213 (84.9) | 1525 (86.5) | 1468 (88.2) | 1244 (84.7) | 1367 (88.5) | 1374 (86.8) |
| Females | 225 (13.5) | 230 (15.2) | 216 (15.1) | 238 (13.5) | 197 (11.8) | 225 (15.3) | 177 (11.5) | 209 (13.2) |
| **BMI ($kg/m^2$)** | 23.7 (6.9) | 22.5 (3.7) | 22.9 (4.0) | 23.4 (5.5) | 22.6 (3.8) | 23.5 (4.1) | 23.5 (4.2) | 23.4 (3.9) |
| **History of diabetes (%)** | | | | | | | | |
| Yes | 214 (12.9) | 115 (7.6) | 122 (8.5) | 187 (10.6) | 110 (6.6) | 209 (14.2) | 121 (7.8) | 208 (13.1) |
| No | 1400 (92.4) | 1452 (87.1) | 1307 (91.5) | 1576 (89.4) | 1555 (93.4) | 1260 (85.8) | 1432 (92.2) | 1375 (86.9) |
| **History of hypertension (%)** | | | | | | | | |
| Yes | 267 (16.0) | 143 (9.4) | 138 (9.7) | 274 (15.5) | 192 (11.5) | 230 (15.7) | 179 (11.6) | 261 (16.5) |
| No | 1399 (84.0) | 1372 (90.6) | 1291 (90.3) | 1489 (84.5) | 1473 (88.5) | A239 (84.3) | 1365 (88.4) | 1322 (83.5) |
| **Family history of MI (%)** | | | | | | | | |
| Yes | 165 (9.9) | 77 (5.1) | 89 (6.2) | 148 (8.4) | 87 (5.2) | 129 (8.8) | 125 (8.1) | 110 (7.0) |
| No | 1501 (90.1) | 1438 (94.9) | 1340 (93.8) | 1615 (91.6) | 1578 (94.8) | 1340 (91.2) | 1419 (91.9) | 1473 (93.0) |
| **Education level (%)** | | | | | | | | |
| No schooling | 496 (37.1) | 635 (42.7) | 625 (43.7) | 510 (28.9) | 727 (43.7) | 485 (33.0) | 481 (31.2) | 549 (34.7) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Primary | 380 (28.4) | 466 (31.3) | 433 (30.3) | 503 (28.6) | 483 (29.0) | 451 (30.7) | 488 (31.7) | 448 (28.3) |
| Secondary | 290 (21.7) | 265 (17.8) | 253 (17.7) | 515 (29.2) | 320 (19.2) | 341 (23.2) | 379 (24.6) | 381 (24.0) |
| Vocational/university | 170 (12.7) | 122 (8.2) | 118 (8.3) | 234 (13.3) | 135 (8.1) | 191 (13.1) | 193 (12.5) | 205 (13.0) |
| **Annual income (Taka) (%)** | | | | | | | | |
| Income below the poverty line <=56000 | 232 (20.3) | 478 (31.9) | 435 (30.7) | 398 (22.9) | 424 (25.6) | 363 (25.3) | 339 (22.3) | 379 (24.2) |
| Low income | 243 (14.9) | 447 (29.8) | 363 (25.6) | 310 (17.8) | 482 (29.1) | 231 (16.1) | 301 (19.8) | 296 (18.9) |
| Medium income | 494 (30.3) | 418 (27.8) | 411 (29.0) | 526 (30.2) | 514 (31.0) | 418 (29.1) | 489 (32.2) | 484 (30.9) |
| High income | 564 (34.5) | 158 (10.5) | 208 (14.7) | 506 (29.1) | 237 (14.3) | 424 (29.5) | 390 (25.7) | 408 (26.0) |
| **Occupation (%)** | | | | | | | | |
| Business professional | 816 (49.0) | 504 (33.3) | 518 (36.3) | 841 (47.7) | 598 (35.9) | 648 (44.1) | 701 (45.5) | 721 (45.6) |
| Manual labour | 237 (14.2) | 461 (30.5) | 403 (28.2) | 257 (15.6) | 450 (27.0) | 266 (18.1) | 331 (21.5) | 238 (15.0) |
| Non-manual labour | 79 (4.8) | 94 (6.2) | 88 (6.1) | 91 (5.1) | 123 (7.4) | 57 (3.9) | 82 (5.2) | 103 (6.5) |
| Unemployed/retired/students | 532 (32.0) | 455 (30.0) | 419 (29.3) | 573 (32.5) | 494 (29.7) | 498 (33.9) | 428 (27.8) | 521 (32.9) |
| **Smoking status (%)** | | | | | | | | |
| Never | 525 (31.5) | 455 (30.0) | 436 (30.5) | 566 (32.1) | 484 (29.1) | 471 (32.1) | 531 (34.4) | 470 (29.7) |
| Ex | 131 (7.9) | 103 (6.8) | 93 (6.5) | 136 (7.7) | 134 (8.0) | 104 (7.0) | 100 (6.5) | 131 (8.3) |
| Current | 1010 (60.6) | 957 (63.2) | 900 (63.0) | 1061 (60.2) | 1047 (62.9) | 894 (60.9) | 913 (59.1) | 982 (62.0) |
| **Total MET-minutes of exercise/week (%)** | | | | | | | | |
| < 600 | 312 (18.7) | 369 (24.4) | 365 (25.5) | 350 (19.8) | 360 (21.6) | 308 (21.0) | 361 (23.4) | 391 (24.7) |
| ≥ 600 | 1354 (81.3) | 1146 (75.6) | 1064 (74.5) | 1413 (80.2) | 1305 (78.4) | 1161 (79.0) | 1183 (76.6) | 1192 (75.3) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Total Energy intake (kcal/day)** | 3633.9 (1109.0) | 2445.3 (580.0) | 2646.4 (739.9) | 3381.8 (984.4) | 2760.0 (818.9) | 3394.6 (1153.9) | 3199.4 (1062.5) | 3046.3 (845.1) |
| **Location (%)** | | | | | | | | |
| Urban | 743 (44.8) | 649 (43.1) | 615 (43.5) | 821 (46.7) | 832 (50.2) | 540 (37.0) | 606 (39.9) | 876 (55.4) |
| Rural | 917 (55.2) | 858 (56.9) | 798 (56.5) | 937 (53.3) | 826 (49.8) | 918 (63.0) | 914 (60.1) | 705 (44.6) |

**Table 6.3 (cont):** Characteristics of controls in quintile 1 versus quintile 5 of different nutrients

| | SFA dairy | | SFA other sources | | MUFA | | PUFA | |
|---|---|---|---|---|---|---|---|---|
| **Characteristics** | **Q1** | **Q5** | **Q1** | **Q5** | **Q1** | **Q5** | **Q1** | **Q5** |
| | | | | | | | | |
| **Age (years)** | 50.8 (10.1) | 53.5 (10.5) | 52.9 (9.7) | 50.4 (10.7) | 53.0 (9.7) | 49.3 (10.7) | 53.6 (10.0) | 48.1 (10.1) |
| **Sex (%)** | | | | | | | | |
| Males | 1816 (84.9) | 1351 (85.8) | 1303 (82.4) | 1429 (89.2) | 1307(80.4) | 1395 (91.5) | 1260 (80.6) | 1443 (93.2) |
| Females | 322 (15.1) | 224 (14.2) | 279 (17.6) | 173 (10.8) | 318 (19.6) | 129 (8.5) | 303 (19.4) | 105 (6.8) |
| **BMI ($kg/m^2$)** | 23.1 (3.9) | 23.1 (5.5) | 22.5 (3.8) | 24.1 (7.1) | 22.5 (3.8) | 23.9 (4.0) | 22.7 (5.5) | 23.9 (3.9) |
| **History of diabetes (%)** | | | | | | | | |
| Yes | 182 (8.5) | 212 (13.5) | 135 (8.5) | 195 (12.2) | 120 (7.4) | 187 (12.3) | 119 (7.6) | 178 (11.5) |
| No | 1956 (91.5) | 1363 (86.5) | 1447 (91.5) | 1407 (87.8) | 1505(92.6) | 1337 (87.7) | 1444(92.4) | 1370 (88.6) |
| **History of hypertension (%)** | | | | | | | | |
| Yes | 288 (13.5) | 217 (13.8) | 203 (12.8) | 248 (15.5) | 218 (13.4) | 207 (13.6) | 216 (13.8) | 204 (13.2) |
| No | 1850 (86.5) | 1358 (86.2) | 1379 (87.2) | 1354 (84.5) | 1407(86.6) | 1317 (86.4) | 1347(86.2) | 1344 (86.8) |
| **Family history of MI (%)** | | | | | | | | |
| Yes | 133 (6.2) | 132 (8.4) | 66 (4.2) | 160 (10.0) | 74 (4.5) | 134 (8.8) | 81 (5.2) | 133 (8.6) |
| No | 2005 (93.8) | 1443 (91.6) | 1516 (95.8) | 1442 (90.0) | 1551 (95.5) | 1390 (91.2) | 1482 (94.8) | 1415 (91.4) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Education level (%)** | | | | | | | | |
| No schooling | 866 (40.5) | 547 (34.8) | 803 (50.8) | 392 (24.5) | 825 (50.8) | 348 (22.8) | 736 (47.1) | 362 (23.4) |
| Primary | 608 (28.4) | 477 (30.3) | 425 (26.9) | 527 (32.9) | 417 (25.7) | 501 (32.9) | 424 (27.1) | 499 (32.3) |
| Secondary | 451 (21.1) | 354 (22.5) | 259 (16.4) | 433 (27.1) | 284 (17.5) | 423 (27.8) | 301 (19.3) | 441 (28.5) |
| Vocational/university | 213 (10.0) | 194 (12.3) | 95 (6.0) | 248 (15.5) | 99 (6.0) | 251 (16.5) | 102 (6.5) | 245 (15.8) |
| **Annual income (Taka) (%)** | | | | | | | | |
| Income below the poverty line <=56000 | 598 (28.3) | 412 (26.6) | 541 (34.3) | 297 (19.0) | 572 (35.3) | 257 (17.2) | 532 (34.3) | 214 (14.0) |
| Low income | 501 (23.7) | 284 (18.3) | 432 (27.4) | 238 (15.3) | 432 (26.7) | 252 (16.9) | 370 (23.9) | 295 (19.3) |
| Medium income | 624 (29.6) | 439 (28.4) | 417 (26.4) | 473 (30.3) | 420 (26.0) | 472 (31.6) | 430 (27.7) | 531 (34.8) |
| High income | 389 (18.4) | 413 (26.7) | 187 (11.9) | 552 (35.4) | 194 (12.0) | 513 (34.3) | 219 (14.1) | 486 (31.9) |
| **Occupation (%)** | | | | | | | | |
| Business professional | 872 (40.8) | 641 (40.8) | 471 (29.8) | 853 (53.3) | 482 (29.7) | 804 (52.8) | 494 (31.6) | 857 (55.4) |
| Manual labour | 438 (20.5) | 354 (22.5) | 453 (28.6) | 209 (13.0) | 443 (27.3) | 229 (15.0) | 394 (25.2) | 244 (15.8) |
| Non-manual labour | 145 (6.8) | 50 (3.1) | 101 (6.4) | 80 (5.0) | 102 (6.2) | 86 (5.6) | 84 (5.4) | 98 (6.2) |
| Unemployed/retired/students | 682 (31.9) | 528 (33.6) | 557 (35.2) | 459 (28.7) | 598 (36.8) | 405 (26.6) | 591 (37.8) | 349 (22.6) |
| **Smoking status (%)** | | | | | | | | |
| Never | 609 (28.5) | 488 (31.0) | 437 (27.6) | 550 (34.3) | 435 (26.8) | 503 (33.0) | 441 (28.2) | 521 (33.7) |
| Ex | 148 (6.9) | 127 (8.0) | 127 (8.0) | 121 (7.6) | 119 (7.3) | 108 (7.1) | 115 (7.4) | 100 (6.4) |
| Current | 1381 (64.5) | 960 (61.0) | 1018 (64.4) | 931 (58.1) | 1071 (65.9) | 913 (59.9) | 1007 (64.4) | 927 (59.9) |
| **Total MET-minutes of exercise/week (%)** | | | | | | | | |
| < 600 | 576 (26.9) | 320 (20.3) | 373 (23.6) | 342 (21.3) | 388 (23.9) | 378 (24.8) | 349 (22.3) | 386 (24.9) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ≥ 600 | 1562 (73.1) | 1255 (80.0) | 1209 (76.4) | 1260 (78.7) | 1237 (76.1) | 1146 (75.2) | 1214 (77.7) | 1162 (75.1) |
| **Total Energy intake (kcal/day)** | 2702.1 (806.7) | 3173.2 (960.2) | 2658.7 (728.7) | 3450.0 (1130.7) | 2582.9 (757.3) | 3482.8 (1119.3) | 2811.6 (865.2) | 3421.6 (1045.6) |
| **Location (%)** | | | | | | | | |
| Urban | 1118 (52.4) | 1025 (65.5) | 680 (43.1) | 780 (49.2) | 689 (42.5) | 746 (49.6) | 656 (42.1) | 794 (51.9) |
| Rural | 1016 (47.6) | 540 (34.5) | 899 (56.9) | 805 (50.8) | 933 (57.5) | 759 (50.4) | 903 (57.9) | 737 (48.1) |

**Table 6.4:** Percentage contribution of food groups

| | Refined carbs | Non-refined carbs | Animal protein | Plant protein | SFA dairy | SFA other sources | MUFA | PUFA |
|---|---|---|---|---|---|---|---|---|
| **Green leafy stem** | | 2.1 | | 4.1 | | 2.6 | | 4.3 |
| **Potatoes** | | 7.7 | | 1.3 | | 0.3 | | 1.0 |
| **Other vegetables** | | 9.4 | | 8.6 | | 3.9 | | 2.0 |
| **Vitamin C rich fruits** | | 3.1 | | 1.4 | | 0.4 | | 1.4 |
| **Other fruits** | | 34.0 | | 7.1 | | 10.0 | 4.3 | 4.3 |
| **Pulses** | | 39.2 | | 33.0 | | 1.7 | 1.9 | 6.3 |
| **Boiled white rice** | 74.2 | | | 36.3 | | 18.2 | 14.6 | 30.1 |
| **Biryani** | 1.4 | | | | | 5.1 | 6.6 | 5.6 |
| **Bread** | 12.7 | | | 7.3 | | 2.0 | 4.2 | 5.3 |
| **Deep fried snacks** | 2.5 | | | | | 4.2 | 12.8 | 8.7 |
| **Savoury snacks** | 1.6 | | | | | 8.8 | 4.0 | 4.5 |
| **Sweets** | 3.5 | | | | | 6.8 | 7.6 | 3.5 |
| **Sugar-sweetened drinks*** | 1.0 | | | | | | | |
| **White meat** | | | 3.8 | | | 2.7 | 3.1 | 1.3 |
| **Red meat** | | | 3.5 | | | 2.6 | 2.0 | |
| **Sweet water fish** | | | 68.6 | | | 25.1 | 19.9 | 13.6 |
| **Sea water fish** | | | 12 | | | | | |
| **Egg** | | | 5.9 | | | 7.5 | 7.7 | 3.1 |
| **Milk** | | 4 | 4.5 | | 84.9 | | 10.3 | 1.6 |
| **Yoghurt** | | | | | 15.1 | | 1.0 | |

**Table 6.5:** Spearman correlation of nutrients with dietary patterns.

| Nutrients | Energy dense food pattern | Vegetable pattern | Fruits and dairy pattern |
|---|---|---|---|
| **Refined carbohydrates** | 0.01 | **-0.30** | **0.26** |
| **Non-refined carbohydrates** | **0.30** | **0.50** | -0.13 |
| **Animal Protein** | 0.11 | **0.51** | 0.14 |
| **Plant Protein** | **0.26** | 0.11 | **0.37** |
| **SFA dairy** | 0.02 | **0.48** | -0.07 |
| **SFA other** | **0.44** | **0.50** | **-0.21** |
| **MUFA** | **0.50** | **0.44** | -0.06 |
| **PUFA** | **0.47** | **0.28** | 0.05 |

**Table 6.6**: Association of nutrients with risk of AMI after multivariate adjustments

| | Q2 | Q3 | Q4 | Q5 | P trend |
|---|---|---|---|---|---|
| **Refined carbohydrates** | | | | | |
| Model 1 | 1.06 (0.98-1.14) | 1.09 (1.02-1.18) | 1.12 (1.04-1.20) | 1.24 (1.16-1.34) | 0.01 |
| Model 2 | 1.07 (1.00-1.16) | 1.09 (1.02-1.18) | 1.13 (1.04-1.21) | 1.29 (1.19-1.39) | 0.004 |
| Model 3 | 1.08 (1.00-1.16) | 1.12 (1.03-1.21) | 1.17 (1.09-1.27) | 1.36 (1.26-1.47) | <0.001 |
| Model 4 | 1.00 (0.92-1.09) | 1.00 (0.93-1.08) | 1.00 (0.93-1.08) | 1.09 (0.99-1.17) | 0.54 |
| Model 5 | 1.00 (0.90-1.12) | 1.00 (0.90-1.12) | 1.00 (0.90-1.12) | 1.09 (0.97-1.23) | 0.50 |
| Model 6 | 1.00 (0.93-1.09) | 1.20 (0.94-1.11) | 1.03 (0.95-1.11) | 1.16 (1.07-1.28) | 0.13 |
| | | | | | |
| **Non-refined carbohydrates** | | | | | |
| Model 1 | 0.86 (0.80-0.92) | 0.77 (0.72-0.83) | 0.73 (0.68-0.79) | 0.62 (0.58-0.67) | <0.001 |
| Model 2 | 0.86 (0.80-0.93) | 0.77 (0.71-0.82) | 0.76 (0.70-0.82) | 0.62 (0.57-0.67) | <0.001 |
| Model 3 | 0.85 (0.79-0.92) | 0.74 (0.69-0.80) | 0.74 (0.68-0.80) | 0.61 (0.56-0.66) | <0.001 |
| Model 4 | 0.88 (0.81-0.95) | 0.80 (0.74-0.86) | 0.82 (0.76-0.89) | 0.70 (0.64-0.76) | <0.001 |
| Model 5 | 0.87 (0.78-0.97) | 0.80 (0.71-0.89) | 0.82 (0.73-0.92) | 0.70 (0.62-0.78) | <0.001 |
| Model 6 | 0.85 (0.80-0.92) | 0.75 (0.70-0.82) | 0.78 (0.72-0.85) | 0.66 (0.60-0.71) | <0.001 |
| | | | | | |
| **Animal protein** | | | | | |
| Model 1 | 1.01 (0.92-1.07) | 1.00 (0.92-1.07) | 1.16 (1.08-1.25) | 1.27 (1.19-1.37) | <0.001 |
| Model 2 | 0.99 (0.92-1.07) | 0.98 (0.91-1.06) | 1.18 (1.09-1.27) | 1.28 (1.19-1.38) | <0.001 |
| Model 3 | 0.98 (0.91-1.06) | 0.97 (0.90-1.05) | 1.16 (1.07-1.24) | 1.23 (1.13-1.32) | <0.001 |
| Model 4 | 1.01 (0.94-1.09) | 0.99 (0.92-1.07) | 1.20 (1.11-1.29) | 1.32 (1.23-1.44) | <0.001 |
| Model 5 | 1.01 (0.91-1.13) | 0.99 (0.88-1.10) | 1.19 (1.07-1.33) | 1.33 (1.19-1.49) | <0.001 |
| Model 6 | 0.96 (0.88-1.04) | 0.96 (0.89-1.04) | 1.16 (1.07-1.26) | 1.28 (1.18-1.40) | <0.001 |
| | | | | | |
| **Plant protein** | | | | | |
| Model 1 | 0.93 (0.87-1.00) | 0.86 (0.80-0.93) | 0.86 (0.80-0.93) | 0.93 (0.87-1.00) | 0.09 |
| Model 2 | 0.95 (0.88-1.03) | 0.88 (0.82-0.95) | 0.88 (0.82-0.95) | 0.90 (0.83-0.97) | 0.34 |
| Model 3 | 0.94 (0.87-1.01) | 0.84 (0.78-0.90) | 0.85 (0.78-0.91) | 0.83 (0.77-0.90) | 0.04 |
| Model 4 | 0.89 (0.83-0.97) | 0.82 (0.76-0.89) | 0.87 (0.80-0.94) | 0.87 (0.80-0.94) | 0.09 |
| Model 5 | 0.89 (0.80-1.00) | 0.83 (0.74-0.92) | 0.87 (0.78-0.97) | 0.88 (0.78-0.98) | 0.09 |
| Model 6 | 0.89 (0.82-0.97) | 0.83 (0.77-0.90) | 0.86 (0.79-0.93) | 0.84 (0.77-0.91) | 0.14 |
| | | | | | |

| | Q2 | Q3 | Q4 | Q5 | P trend |
|---|---|---|---|---|---|
| **SFA dairy** | | | | | |
| Model 1 | 0.92 (0.84-1.02) | 0.80 (0.75-0.86) | 0.77 (0.73-0.85) | 0.88 (0.82-0.95) | <0.001 |
| Model 2 | 0.96 (0.87-1.06) | 0.85 (0.79-0.92) | 0.84 (0.78-0.91) | 0.94 (0.88-1.02) | 0.01 |
| Model 3 | 0.98 (0.88-1.08) | 0.86 (0.80-0.93) | 0.88 (0.82-0.95) | 0.96 (0.89-1.04) | 0.05 |
| Model 4 | 1.10 (0.99-1.21) | 0.87 (0.81-0.94) | 0.99 (0.91-1.07) | 1.02 (0.94-1.10) | 0.002 |
| Model 5 | 1.10 (0.97-1.24) | 0.87 (0.79-0.97) | 0.99 (0.90-1.09) | 1.02 (0.93-1.14) | 0.005 |
| Model 6 | 1.13 (1.02-1.25) | 0.88 (0.80-0.95) | 1.02 (0.94-1.0) | 1.02 (0.94-1.11) | <0.001 |
| | | | | | |
| **SFA other sources** | | | | | |
| Model 1 | 0.87 (0.81-0.94) | 1.02 (0.95-1.09) | 1.03 (0.96-1.10) | 0.99 (0.92-1.07) | 0.008 |
| Model 2 | 0.89 (0.82-0.96) | 1.02 (0.95-1.10) | 1.01 (0.94-1.04) | 0.97 (0.90-1.04) | 0.04 |
| Model 3 | 0.88 (0.82-0.95) | 1.01 (0.94-1.09) | 1.00 (0.93-1.08) | 0.93 (0.86-1.00) | 0.04 |
| Model 4 | 0.90 (0.84-0.98) | 1.04 (0.97-1.13) | 1.05 (0.98-1.14) | 1.03 (0.95-1.12) | 0.06 |
| Model 5 | 0.91 (0.82-1.01) | 1.04 (0.93-1.16) | 1.06 (0.94-1.17) | 1.03 (0.92-1.15) | 0.05 |
| Model 6 | 0.89 (0.82-0.96) | 1.04 (0.96-1.12) | 1.03 (0.94-1.11) | 0.99 (0.91-1.08) | 0.05 |
| | | | | | |
| **MUFA** | | | | | |
| Model 1 | 1.05 (0.98-1.13) | 0.98 (0.91-1.05) | 0.99 (0.93-1.07) | 1.17 (1.09-1.26) | 0.04 |
| Model 2 | 1.07 (1.00-1.16) | 1.00 (0.92-1.07) | 1.00 (0.93-1.08) | 1.11 (1.03-1.20) | 0.28 |
| Model 3 | 1.06 (0.98-1.15) | 0.97 (0.90-1.05) | 0.99 (0.91-1.07) | 1.09 (1.00-1.18) | 0.33 |
| Model 4 | 1.10 (1.02-1.19) | 1.03 (0.95-1.11) | 1.06 (0.98-1.15) | 1.23 (1.14-1.33) | 0.05 |
| Model 5 | 1.10 (0.99-1.23) | 1.03 (0.92-1.18) | 1.06 (0.95-1.18) | 1.23 (1.10-1.38) | 0.03 |
| Model 6 | 1.10 (1.01-1.19) | 1.01 (0.93-1.10) | 1.05 (0.97-1.14) | 1.20 (1.11-1.31) | 0.11 |
| | | | | | |
| **PUFA** | | | | | |
| Model 1 | 0.87 (0.81-0.94) | 0.94 (0.88-1.01) | 0.95 (0.89-1.03) | 1.07 (1.00-1.15) | 0.009 |
| Model 2 | 0.89 (0.82-0.96) | 0.95 (0.88-1.07) | 0.95 (0.88-1.02) | 1.02 (0.94-1.10) | 0.20 |
| Model 3 | 0.87 (0.80-0.94) | 0.91 (0.84-0.98) | 0.91 (0.84-0.98) | 0.96 (0.88-1.03) | 0.14 |
| Model 4 | 0.86 (0.80-0.93) | 0.91 (0.84-0.98) | 0.92 (0.85-1.00) | 1.03 (0.95-1.11) | 0.05 |
| Model 5 | 0.86 (0.78-0.96) | 0.91 (0.81-1.01) | 0.92 (0.83-1.03) | 1.03 (0.92-1.15) | 0.01 |
| Model 6 | 0.83 (0.76-0.90) | 0.88 (0.81-0.96) | 0.90 (0.83-0.98) | 0.94 (0.86-1.03) | 0.02 |
| | | | | | |

After Bonferroni corrections (p value = 0.05/48= 0.001). The associations remained significant only for non-refined carbohydrates and animal protein.

**Footnotes table 6.6:**

Model 1: adjusted for age and sex

Model 2: Model 1+ smoking status, physical activity, occupation, education and income

Model 3: Model 2 + history of diabetes, history of hypertension, family history of MI

Model 4: Model 3 plus total energy intake.

Model 5: Model 4 + anti-hypertensives and diabetics

Model 6: Model 4 + waist-to-hip ratio, LDL-C and HDL-C

# Chapter 7: Discussion

The main aim of this thesis was to investigate the association of diet and risk of acute myocardial infarction (AMI) in a relatively understudied population of Bangladesh, using data from the Bangladesh Risk of Acute Vascular Events (BRAVE) case-control study. This thesis employed epidemiological methods to: (1) describe the baseline diet in this population; (2) assess precisely the association of dietary food groups, dietary patterns and nutrients with various lifestyle, sociodemographic and other factors and (3) investigate, in detail, the associations of food groups, dietary patterns and nutrients with AMI adjusting for potential confounders and mediators. The thesis follows the public health approach of describing diet in terms of food groups first. However, as isolated food groups do not consider how they are consumed together in form of meals, therefore dietary pattern analyses were conducted as a second step to provide a holistic approach to study diet-disease associations. Lastly, as food groups do not relate well to potential biological mechanisms, dietary nutrients were discussed next. **Figure 7.1** shows the schematic diagram summarising the link between the three dietary approaches. In **Chapter 1**, the rationale for conducting this research was discussed. In **Chapter 2** the BRAVE study methods were defined including the dietary instrument. Furthermore, the characteristics of cases and controls were described and the association of conventional and other non-dietary related risk factors of CHD were investigated. In **Chapter 3** the correlates of food groups in BRAVE controls were described. In **Chapter 4** the association of different food groups with risk of AMI was investigated. In **Chapter 5** the dietary patterns were identified, their correlates were assessed and its association with risk of AMI was investigated. In **Chapter 6** the dietary nutrients were described, their correlates were assessed and its association with risk of AMI was investigated.

The aim of this current Chapter is to provide:

1) a summary of the main findings already reported in the thesis;

2) discuss their strengths and limitations;

3) highlight future studies that are needed to help clarify the associations of diet with AMI in this population;

4) discuss key public health implications and recommendations of the findings.

## 7.1 Summary of main findings

### 7.1.1 *Studies on diet and risk of CHD in South Asia (Chapter 1)*

The literature review highlighted that there was scarce evidence on diet and risk of CHD from South Asia. Overall there were seven unique studies; six on food groups, four on dietary patterns and one on nutrients and risk of CHD. Out of these seven studies four studies reported data from Bangladesh. As compared to the BRAVE study these studies had lower number of participants from Bangladesh. In addition, two of the studies (INTERHEART and PURE)[1,2] did not report estimates specific for Bangladesh, emphasising the need to conduct research on diet and CHD in this population. Overall, the evidence from this literature review concluded that although the burden of CHD is rising in this region, there is paucity of evidence about its potential dietary determinants.

### 7.1.2 *Diet in the BRAVE study* ***(Chapters 3, 5-6)***

This analysis described the baseline diet of the population. The BRAVE controls consumed about 67% of their total energy from carbohydrates, predominantly from refined sources. Protein consumption was 19.7% and total fat consumption was 13.7%. These results were echoed in the food group assessment with boiled white rice having the highest consumption, followed by vegetables, fruits, sweet water fish and pulses. Consumption of biryani, local snacks, sweets, meat and dairy products was relatively low in this population.

Principal component analysis (PCA) was used to derive dietary patterns. The study identified three distinct dietary patterns in the BRAVE study; the “energy dense food pattern” was characterised by higher correlation of fried snacks and savoury snacks, biryani (a type of flavoured rice with meat made in oil), red meat, sugar sweetened beverages (SSB) and sweets; (2) the “vegetable pattern” was characterised mainly by leafy vegetables, other vegetables, pulses and sweet water fish and (3) the “fruits and dairy pattern” was characterised by vitamin C rich fruits, milk, other fruits, yoghurt and sweet water fish.

### 7.1.3 *Cross-sectional correlates of diet* ***(Chapters 3, 5-6)***

This analysis examined the cross-sectional associations of dietary food groups, patterns and nutrients with a wide range of lifestyle, sociodemographic and other characteristics recorded in the BRAVE controls. Overall, dietary food groups, patterns and nutrients showed modest associations with the various characteristics showing the role of potential confounding. In particular, higher consumption of fruits and vegetables was associated with healthy lifestyle behaviours such as less likely to be currently smoking and being more physically active. On the contrary, higher consumption of red meat and biryani was

associated with being less physically active. There were few strong correlations between food groups suggesting how foods are eaten together and justifying the rationale for conducting dietary pattern analyses. Furthermore, the current study found that overall higher scores on the three dietary patterns identified in this study were associated with higher socio-economic status and healthy behaviours. Those in the higher scores were younger, more educated, had higher annual income, less likely to be current smokers, and were more physically active. Additionally, the nutrients showed association with food groups and dietary patterns suggesting the inter-connections between these three dietary approaches. Furthermore, nutrients showed weak associations with blood lipids and waist-to-hip ratio.

7.1.4 *Association of food groups and risk of acute myocardial infraction* ***(Chapter 4)***

**Figure 7.2** shows the summary diagram of main association from **Chapter 4**. Findings of the analyses on the association of food groups with AMI reported an inverse association between vegetables (green leafy and stem, potatoes and other vegetables), fruits (vitamin C rich and other fruits), yoghurt, certain spices (cumin, coriander and garlic) and risk of AMI. In contrast, higher consumption of biryani and fish was associated with higher risk of AMI. There was no evidence of association of higher consumption of red meat, white meat, white rice and bread with the risk of AMI. Consumption of 1-2 portions a day of pulses had an inverse association with AMI. On the contrary, more than 1 egg per day was associated with higher risk with AMI. Most of the associations were not modified by age, sex, geographical location, smoking status, BMI and physical activity. However, age group did appear to have an effect on the associations with consumption of other fruits, milk and pulses. Other fruits had a stronger inverse association in the younger group in comparison to the older group. The interaction of milk with age group, demonstrated that higher consumption of milk was associated with increased association only in the older age group. The interaction of pulses with age group observed that a stronger inverse association was observed in older age groups compared to younger age groups.

7.1.5 *Association of dietary patterns and risk of acute myocardial infraction* ***(Chapter 5)***

**Figure 7.2** shows the summary diagram of main association from **Chapter 5**. Results on the analyses of dietary patterns with risk of AMI reported that the “energy dense food pattern” had no significant association with the risk of AMI. On the contrary the “vegetable pattern” and “fruits and dairy” pattern was inversely associated with risk of AMI largely independent of potential confounders and mediators. These associations were not modified by age, sex, geographical location, smoking status, BMI and physical activity, apart from interaction of “vegetable pattern” with age group and education level and “fruits and dairy

pattern" with income levels. The "vegetable pattern" had an inverse association only in the younger age group and in those who had primary and secondary education levels. A stronger association between highest quintile of "fruits and dairy pattern" and AMI was observed in high income level group as compared to income levels below poverty line.

7.1.6 *Association of nutrients and risk of acute myocardial infraction* ***(Chapter 6)***

**Figure 7.2** shows the summary diagram of main association from **Chapter 6**. As for dietary nutrients, higher intake of refined carbohydrates had a weak but non-significant association with the risk of AMI, while non-refined carbohydrates were associated with lower risk of AMI. Plant protein showed a weak inverse association, on the contrary animal protein showed an increased association with the risk of AMI. As for specific fatty acids, moderate intake of SFA from dairy and PUFA showed an inverse association with AMI. In contrast highest quintile of dietary MUFA has an increased association with MI, but it was non-significant across other quintiles. Whereas, SFA from other sources had no significant association with the risk of AMI. These associations were not modified by age, sex, geographical location, smoking status, BMI and physical activity, apart from the interaction of refined carbohydrates and SFA from dairy with age group. This former association showed that a higher intake of refined carbohydrates was associated with higher risk of AMI in the younger age groups. The latter observed that a higher intake of SFA from dairy had an increased association in the older age group and an inverse association in the younger age group. For substitution analyses, replacing 1% energy from total carbohydrates with SFA and PUFA was associated with lower risk of AMI. In contrast, using MUFA and total protein as the replacement were associated with higher risk. Replacing 1% energy from SFA with total carbohydrate, MUFA and total protein was associated with higher risk. On the contrary, replacement of SFA with PUFA was associated with lower risk.

## 7.2 Strengths

This thesis has several important strengths. First, the current diet-CHD study is the most powerful study conducted thus far involving a South Asian population, with almost 5 times more CHD events than all previous South Asian studies combined. Furthermore, while many relevant previous epidemiological studies in this region were based on self-reported outcomes, all CHD outcomes were clinically validated in the current study. Second, this entails a detailed investigation of the dietary basis of CHD among South Asians Bangladeshi population, and assess, in a single comprehensive study, a wide range of food sources of major nutrients (e.g., animal protein, dairy fats, and refined carbohydrates) and local foods (e.g., biryani or mixed rice and spices) in relationship with potential CHD risk. Third, the study employs a validated country-specific food frequency questionnaire (FFQ) to estimate intake of different types and subtypes of food groups and derived total energy intake using food composition table from Bangladesh and similar settings. The FFQ is a widely used method for dietary assessment which has low respondent burden and administration costs.[3] Furthermore, the study uses standardised measures to collect data on wide range of lifestyle, biochemical and other characteristics and therefore is a rigorous case-control study. Fourth, in contrast with previous studies conducted in South Asia, the current analyses use three different nutritional epidemiology approaches (food groups, dietary patterns and nutrients) to quantify the associations with AMI (and hence ensures the “totality of evidence”). This study also explores and characterises the shape of associations between various food groups and risk of AMI in Bangladesh and quantifies any mutual “substitution effect” of individual nutrients. Fifth, it conducts, where possible, additional sensitivity and subgroup analyses to supplement and contextualize the main findings. Sixth, the study involved has approximately equal number of participants from urban and rural areas in Bangladesh, providing good representation of diverse settings. Finally, this study aims to fill a crucial research gap on diet and risk of CHD among the South Asians and serves as a stimulus for further detailed scientific work on this topic.

## 7.3 Limitations

Despite the strengths mentioned above, the limitations of this work, as summarised below, merit careful consideration.

### *7.3.1 Study design-based limitations:*

*Selection bias:*

Selection bias can occur if there is bias introduced in study design if the study population is not representative of the general population.[4] Like any epidemiological study, potentials for selection bias remains a possibility in the BRAVE study, which may under- or over-estimate the true associations. However, the recruiting hospital of the BRAVE study is the largest tertiary-care referral cardiology hospital in Bangladesh. Therefore, given the unavailability of coronary care units in nearby districts and an easy transport access to the recruiting hospital, this referral hospital has a nationwide catchment, receiving AMI cases from all over the country. Consequently, there was broadly an equal urban-rural split, and good representation of age groups and socio-demographic factors among the study participants. Additionally, the study recruited all AMI cases and controls with a standardised selection protocol and by using consistent operating procedures. Nonetheless, the possibility that the controls in this study (who are typically the accompanying healthy visitors of other admitted patients) could differ from the general population, cannot be ruled out entirely.

*Information bias:*

Information bias may occur if there are recording errors in self-administered questionnaires, misreporting due to memory and misinterpretation of information.[4] As a case control study, there is a possibility of recall bias in the BRAVE study since diseased individuals (i.e., AMI cases) may recall the responses differentially than healthy controls. For example, the cases may be more likely to overestimate risk factors of CHD like smoking and physical inactivity inflating the ORs, and controls may be likely to underestimate. In addition, the data on diet was collected by a FFQ, which also has a possibility of recall bias if cases and controls recall their diets differently. To minimise these potential misinterpretations, a team of trained research physicians (rather than participants themselves) administered the study questionnaire. These trained interviewers adequately explained all questions to the participants, and where appropriate, cross-checked the responses for all self-reported measures of risk factors such as smoking, history of disease, physical activity with other family members or attendants of the participants. Similarly,

for the FFQ, to minimize any response bias related to food description, frequency or portion size, the interviewers used illustrative food charts and samples of dishes/cutleries so that the respondents could provide dietary history as accurately as possible.

*Reverse causation:*

Given the observational nature of the study there is also possibility of reverse causation as participants may change their diets and lifestyle due to pre-existing medical history (diabetes, hypertension). However, controls with chronic conditions (such as malignancy, any infection, leprosy, inflammatory disorders, hepatitis or chronic kidney disease, recent history of surgery or were pregnant) and history of CVD, which may have affected the hypotheses studied in BRAVE, were not recruited in the study.

*Residual confounding:*

Residual confounding can be caused by unmeasured confounding factors, additional confounding factors that were not adjusted for (e.g. use of nutritional supplements) and not precisely measured confounding factors (e.g. socioeconomic factors).[5] Although the analyses were adequately adjusted for all conventional CHD risk factors as well as other potential confounders such as medical history; the possibility of residual confounding from cannot be ruled out. There is lack of data on additional factors which could be relevant (such as health systems related factors, contamination of food items, environmental pollution -indoor air pollution while preparing food, water contamination-water used for food preparation) and concomitant nutritional deficiencies (such as iron and vitamin D), which may have impacted the observed associations. In addition, there may be errors in self-reported measures such as medical history, socio-economic status and physical activity that may also affect the observed associations.

### *7.3.2 Methodological limitations:*

*Missing data and multiple testing:*

There was missing data in cases and controls. Methods such as multiple imputation may be used, but they are still in development[6] and are beyond the scope of this thesis. However, sensitively analysis of conventional risk factors with AMI using multiple imputation in **Appendix 4** yielded results similar to the main analysis. Additionally, as multiple analyses were done some of the significant findings may be due to multiple testing. Nevertheless, sensitivity analysis by using Bonferroni correction for the main analyses were in line with the main results.

*PCA to derive dietary patterns:*

The use of PCA in deriving dietary patterns in **Chapter 5** has limitation of being subjective in which way the food groups are categorized before applying PCA. Subjective decisions are also made in choosing the number of factors, type of analytical approach (use of rotation), the value of the factor loading chosen to describe the factors and naming of the dietary patterns which may influence the results.[7,8] Furthermore, although the original aim of PCA is to reduce detailed information into smaller number of interpretable variables that have the characteristic of explaining high variability in the target behaviour. However, variance explained by dietary data available in literature is usually small.[9] Nevertheless, the analyses conducted in thesis detailed the methodology of the deriving the dietary patterns and several sensitivity analyses yielded similar dietary patterns. Additionally, there are many different methods used to derive dietary patterns as discussed in **Chapter 5**. There is a need to standardize the methods for more reliable within and cross country comparisons such as the dietary patterns development project.[10]

*Analyses based on nutrients:*

The dietary nutrient intake in **Chapter 6** was estimated from food composition tables, which also has many limitations. The composition table reports a single composition of a specific food, however the components of can food vary considerably in composition; depending on the environment, location, animal breed, cultivation method, storage, different manufacturers, processing methods and cooking methods.[2] Moreover, the analyses of nutrients with risk of AMI should be interpreted carefully. This is because of the collinearity between different nutrients in the complex food matrix, it is difficult to exclude the effects of other nutrients while observing the association of single nutrients with CHD risk.

*7.3.3: Dietary tool (FFQ)limitations:*

The FFQ measures dietary intake over the last year so it has limitation of recall bias. It also doesn't take into account the within person variation or the changes in diet that occurs over a period of time.[11] The dietary instrument used in this study has inherent limitation of bias that can lead to under or over-reporting. Non-differential misclassification may occur when the degree of misclassification of exposure status among those with and those without the disease is the same; e.g. random error which will attenuate the associations observed. Differential misclassification may cause bias in either direction and is particularly likely, when exposure reporting is associated with a characteristic such as higher BMI may

be associated with underreporting of food items. Therefore, other methods to measure dietary intake may be considered. A combination of 24-hour recalls, food diaries and weighted records may be used.[12] However, it is important to consider that in large epidemiological studies this will raise the cost of the study. A study examined costs of conducting 24-hour recalls in Africa and South Asia reported that the average cost of a 24-hour recall survey was approximately $247 per household.[13] In addition, to measure long term dietary intake it is recommended to take repeated dietary assessments which may not be financially feasible. Advances should be made to develop low cost interactive computer based technologies and mobile phone applications to collect dietary data and link it to food composition tables to ensure quick and accurate data collection and processing.[3]

In future the circulating levels of nutrients in the blood of the cases and controls of BRAVE study may be tested to see if they are associated with the risk of AMI. Previous evidence has shown that these biomarkers of diet (such as circulating fatty acids, essential fatty acids, urine nitrogen- for protein, sucrose and fructose) are highly correlated with the actual dietary intake, these are free of biases due to social desirability to report healthy foods, and are not dependent on memory and knowledge of the participants to describe and report their dietary intake.[14] Furthermore, the dietary nutrient intake was estimated from food composition table, which also has many limitations as components of can food vary considerably in composition.

## 7.4 Scientific research implications of current findings

This section highlights the future research implications of the current study and future studies that I have been involved with to help elucidate the association of diet and risk of CHD in South Asians.

### *7.4.1 The need of future studies in South Asia*

As discussed in this thesis, there are many studies investigating the association of diet with the risk of CHD from high income countries (HICs) like USA and UK, however, there is scarce data available from South Asian populations such as Bangladesh. The present analysis is the largest detailed study on diet and CHD solely based on a Bangladeshi population. It confirms previous observed association of some food groups with CHD in western populations and has yielded some novel insights on the association of diet with CHD specific to Bangladesh. However, due to the limitations of the case-control design (recall and selection biases and residual confounding from unmeasured sources) causality cannot be assumed. Therefore, results of this study should be used for hypothesis generating and should be replicated to similar settings.

Ideally it will be best to carry out an adequately powered randomised controlled trials (RCTs) to overcome the limitations of a case-control design. However, it may not be feasible to carry out a RCT in a LMIC. First, if researchers have to investigate diet and its association with CHD, it will require a huge population to be randomized to different dietary interventions and have to follow participants for years or decades to observe clinical end points.[15] For example in the PREDIMED trial, about 7000 participants were randomized to different dietary interventions and were followed for about 5 years to obtain 277 CVD events.[16] Second, to ask people to adhere to a certain diet for such a long period of time is an insurmountable task. Often people with adverse health are lost to follow up, leading to biases in the study. Third, evidence base is needed to set up such trails, which is limited in South Asian populations. Fourth, RCTs have ethical considerations. It is unethical to ask people to follow a diet for a long period of time without knowing the potential health consequences of the diet under investigation. Fifth, in a resource-constrained LMIC, in the absence of expensive RCTs, well-conducted large observational studies provide useful relevant evidence.

*7.4.2 The Bangladesh Longitudinal Investigation of Emerging Vascular Events (BELIEVE): Ongoing study in Bangladesh*

To more reliably inform public health policy and dietary guidelines it is essential that the findings in the BRAVE study are replicated in other observational studies. In this regard during the time of my PhD I was involved in the design and coordination of the BELIEVE study. I prepared questionnaires, manuals for field workers and coordinated the field work with the team in Bangladesh. This study was initiated in 2016 and has been designed as a blood-based prospective cohort that aims to recruit up to 150,000 community-based participants from Bangladesh. It is a substantially large prospective bio-resource with extensive baseline questionnaire and physical measures, as well as stored blood samples that will allow many different types of assay (e.g. genetic, proteomic, metabonomic, biochemical and haematologic). The study will serve as a unique scientific resource for Bangladesh to study current and future ethically-approved studies relating to lifestyle and other emerging causes of vascular and nonvascular diseases, to help shape local (and global) preventive policies. The follow up in BELIEVE is currently in progress to ascertain clinical end points. It will be important to replicate the current findings in BELIEVE study, when sufficient numbers of incident CHD events have been ascertained. I will be working on this study as part of my post-doctoral research.

*7.4.3 Recommendations for future studies*

BRAVE study highlights the existing gaps in the case-control evidence and encourages future research to elucidate the reasons for the observed association between diet and risk of MI. It is important to carry out studies to (1) investigate the contamination of fish and other food items with toxic metals; (2) examine if associations vary by preparation or cooking method (frying vs steaming vs barbequing); (3) test association of different types of dairy products (fermented vs non fermented, sweetened vs unsweetened and high fat vs low fat); (4) investigate the genetic determinants of diet or the inter-individual variation in relation to diet; (5) investigate if indoor air pollution due to cooking methods has an effect on risk of CHD; (6) conduct studies in other parts of Bangladesh to observe if these associations persist (e.g. rural vs urban settings); (7) test dietary health promotion strategies and interventions in Bangladesh and (8) carry out RCTs with risk factors of CHD as endpoints such as blood lipids and blood pressure.[15]

Combined evidence from different types of studies will be best to establish public health guidelines, encompassing dietary recommendations on food groups and dietary patterns. The future research in LMICs can be carried out in collaboration with HICs so that a comparison of patterns of diet and the association with CHD could be observed for the different regions. Moreover, studies like BRAVE should also be conducted in other South

Asian countries such as India and Pakistan, to see whether dietary patterns and the association with MI vary importantly among different South Asian populations.

## 7.5 Avenues for future nutritional epidemiology

The future of nutritional epidemiology is likely to be based upon many encouraging recent advances in the so-called "omics" approaches. These evolving technologies can help better understand inter-individual variability to diet, propel discoveries of novel objective biomarkers of diet, enhance screening capacities of high risk populations for targeted dietary interventions, and provide further insights into the biological pathways.[17,18] While the promise of such major future progress in nutrition epidemiology remains high, relevant future bio-assessments and research is required to incorporate these new avenues within the current BRAVE study.

### *7.5.1 Personalised nutrition*

While the current dietary guidelines are based on population averages, the combination of omics and bioinformatics has increased interest for personalized nutrition.[17,18] In this regard, once the relevant genetic modifiers of various nutrient-CHD links are identified, it may eventually lead to personalised medicine for CHD prevention by developing tailored and optimal diets for individuals depending on their genetics, medical history and other lifestyle circumstances.[17,19] For example, there are considerable inter-individual differences in the response to the lipid levels to dietary cholesterol and fatty acid consumption reported in trials.[20] There is also evidence now that dietary SFA interacts with APOE genotype.[21] A case-control study investigated 3 variants in the APOE gene (the ε2-, ε 4-, and -419T allele) with SFA. It was observed that carriers of those three alleles combined had a stronger association of SFA with AMI risk as compared to non-carriers of those alleles.[21]

Personalised nutrition can use data from emerging technologies and traditional nutritional assessment methods to understand the mechanisms due which there are different responses to dietary intake in individuals, to identify novel biomarkers to predict disease and provide personalized dietary advise to people for effective prevention and management of disease.[19] The BRAVE case-control study will have genetic information in due course and that can be used to investigate diet-gene interactions. However, it is important to note that personalized medicine is still in its infancy and more research is required to translate this into clinical setting.

*7.5.2 Dietary assessment using metabolomics*

Metabolomics (large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms) analysis offers opportunity to assess habitual diet better than single biomarkers.[19] New metabolomic technologies can profile large number of metabolites from ingestion and absorption of different foods. Studies have identified new biomarkers for food exposures such as urinary proline betaine and 4-hydroxy-proline betaine as a biomarker for citrus foods, plasma ether-linked phospholipids and plasmalogens as biomarkers for dietary fat sources and urinary trimethylamine-N-oxide and carnitines as biomarkers for red meat and fish.[19] In this context, if such objective biomarkers of diet were available in the BRAVE study, these could have been used to further reinforce the associations observed for different food groups.

Additionally, many studies have used metabolomics to study the dietary patterns and understand the role of diet in prevention of CHD. One such study investigated the metabolite profiles associated with high adherence to dietary recommendations - the Alternative Healthy Eating Index (AHEI) - and the extent to which metabolites associated with AHEI to predict incident CVD.[22] It was observed that the metabolic profiles of fatty acids (higher ratio of PUFA, omega-3, omega-6 and lower ratio of saturated, MUFA and conjugated fatty acids relative to total fatty acids) were associated with higher AHEI score (indicating healthier diet).[22] However, it is important to note that the metabolomics approach is still emerging, and is, therefore, yet to identify all potential biomarkers that can reliably predict dietary patterns.

*7.5.3 Causal inference using Mendelian Randomization (MR)*

It is well established that RCTs provide the best level of evidence for causality. However, randomised nutritional trials on CHD in general population are often unfeasible because of the requirements of large sample size, prolonged follow-up to accrue sufficient number of CHD events, and appropriate ethical considerations.[17] By contrast, MR is a novel technique that uses relevant genetic data to establish causal links between diet and risk of disease. This approach aims to mimic a conventional RCT as genotypes are assigned randomly to individuals at birth and can therefore eliminate confounding and reverse causation.[19] MR analyses may also be used to investigate the causal relationship of dietary factors with CHD risk, and an appropriate genetic instrument could be single nucleotide polymorphisms (SNPs) related to intake of food groups or nutrients.[23] A study used MR to obtain association of n-6 PUFAs (linoleic acid) with CHD using SNPs relevant to the genes FADS1, FADS2 and NTAN1. It was observed that linoleic acid was associated with lower risk of

diabetes but had no association with CHD.[24] Another example of using genetic instruments include 18 loci identified in the US Framingham study that showed strong evidence of interaction with five different types of fatty acids on inflammatory biomarkers.[25] Specifically, omega-6 fatty acid interacted with two SNPs on chromosome 7, which are located near the CHCHD3 gene. This gene is known for maintaining crista integrity and mitochondrial function.[26] The disruption of crista structure has been postulated to be associated with CVD, thus the interaction of omega-6 fatty acids with SNPs near this gene may have an impact on CVD risk.[25] Furthermore, a study of 147 individuals assessed the association of SNPs with the reported Prudent and Western dietary pattern scores. The study found that the Western diet was associated with SNP rs113152482 and gene expression of PFKFB3 in blood.[27] PFKFB3 is involved in glycolysis, protein kinase signalling and neuropeptide expression.[27]

However, it is important to note that genome-wide association studies (GWAS) based on MR investigate lifelong risk of disease as compared to observational studies that give a more real-world assessment of disease risk. Furthermore, the instruments used in GWAS studies need to be strong, having high proportion of variance explained to predict life-long risk of disease.

## 7.6 Potential public health implications of the current findings

As discussed above, the BRAVE study yielded novel insights into the association of diet and risk of CHD in a relatively understudied population of Bangladesh. However, as the study is observational, any potential causal role of diet with CHD cannot be assumed. Hence it is important to investigate the dietary determinants of CHD to identify the factors for future research, provide better guidance to change the behaviours of people and to reduce the burden of CHD. Although a case-control study is not the best evidence to inform policy, however if future prospective studies and RCTs confirm the diet-CHD associations observed, this study may have several public health implications. As mentioned previously, the findings of this thesis underscore the need for future studies for detailed assessments of causal relevance to CHD.

### *7.6.1 Dietary guidelines:*
### *Shift from nutrients to food groups and dietary patterns*

This section describes the importance of food groups and dietary patterns in formulating dietary guidelines as compared to single nutrients. Previously, dietary recommendations have focused on single nutrients, mainly on reducing total fat and SFA, and to replace SFA with PUFA to reduce the risk of CHD.[28] The only dietary guidelines available from Bangladesh recommends to limit total fat intake to 15-30% and SFA to <10%.[29] However, recent studies have negated the increased association of total fat with CVD.[30,31] In the present analyses, SFA from dairy sources had an inverse association, whereas SFA from other sources had no significant association, with the risk of CHD. These findings highlight that there is heterogeneity in the associations of SFA with CHD depending on different sources of food. There is also evidence from other studies that dietary dairy fat reduces the risk of cardio-metabolic outcomes.[30,32] The 2015 Dietary Guidelines Advisory Committee suggested that low fat diets have no effect on the association of CVD and therefore, dietary recommendations should be based on food items and dietary patterns.[33] Consistent with the recent evidence, the present analyses also highlights that it is misleading to have dietary guidelines based on single nutrients, such as recommending a lower fat intake. Although the range of total SFA consumed in this study is within the dietary recommendation, it is important to consider that different sources of SFA may have differential effects on the risk of CHD.

It is also important to note that we don't consume isolated nutrients but whole foods. An array of different nutrients make up these food items and nutrition composition of these food groups vary considerably.[34] The current analyses used three different approaches to

investigate the association of diet with CHD in this population. This highlights the importance of food groups and dietary patterns, rather than single nutrients in relation to CHD risk.[35,36] Health policies focusing on isolated nutrients often lead to paradoxical dietary choices. For example, low fat products in the market may have high carbohydrate content or sodium content. If one of the macronutrients is reduced it has to be replaced by equal energy by another macronutrient. Moreover, the substitution analyses have shown that replacing SFA with carbohydrate will have different effects on the risk of CHD than replacing SFA with PUFA, as also observed in this study. Hence, there is no effect of a single nutrient in an absolute sense, because this effect may change based on the substitution nutrient and the different food sources it comes from.[34] Misguidance by the low fat dietary guidelines introduced in the last decades, have led to excess intake of carbohydrates and/or sugar, which in turn have increased the risk of cardio-metabolic diseases.[37,38]

In addition, it is very difficult for people to estimate their daily percentage of calories and specific nutrient intake. Although it is important to investigate the association of nutrients with CHD to understand the biological mechanisms of the disease, it is important to note, that the antagonistic and/or synergy interactions between nutrients that constitute the whole food matrix, determine the associations .[15,36] For example, milk is not just made up of SFAs, but has other essential nutrients as well such as, calcium, vitamin D.[39] The combined effect of these multiple nutrients determines the effect on disease, opposed to just SFAs. Further to the considering whole foods, it is important to consider that the associations, of food groups with CHD, may differ due to preparation methods. For example, the association between potatoes and CHD would be predicted to change depending on whether the potatoes are fried or boiled. In addition, the thesis highlights that as food groups are eaten together as part of a meal not in isolation, it is also important to study dietary patterns. Dietary pattern analysis allows for the interaction between food groups and thus provides a more holistic approach towards investigating diet-disease associations.

Taken together, dietary recommendations based on isolated nutrients are misleading. Therefore, guidelines and health policies should focus on food groups and dietary patterns. It is important for dietary guidelines to take into account the totality of evidence; inter-relation between food groups, dietary patterns and nutrients when formulating these guidelines.[34] A systematic top down approach should be considered in which dietary patterns should be given the most importance followed by food groups and then nutrients.[34] Using the evidence from this study, it may be recommended to adopt the “vegetable pattern” and “fruits and dairy pattern”. These dietary patterns include high

consumption of fruits, vegetables, pulses, dairy products and relatively low levels of sweet water fish. Such dietary guidelines will be better able to inform the general public and will be easier to follow by the people in Bangladesh.[15] One example of dietary pattern-based guideline is by the 2015 US Dietary Guidelines Advisory Committee (DGAC) that identified 3 patterns that were associated with reduced risk of chronic diseases. These patterns included some common food items such as high intakes of fruits, vegetables, whole grains and nuts and legumes.[34] It is important to note that individual food choices depend on may factors such as education, socio-economic status (e.g. income), culture, biology, lifestyle (e.g. exercise, sleep, television watching), availability and affordability of foods.[17,19] These factors may play an important role in affecting the dietary exposure.[19] Therefore, in order to ensure effective implementation of dietary patterns-based guidelines, the guidelines should be informed by not only research-based evidence but also social, cultural, economic and environmental factors that affect dietary habits and food availability.[40]

The recommended foods should be widely available, affordable and accessible to the general population.[40] The guidelines should be easily understood by the people, should be culturally appropriate and tailored to different age groups and people with different medical conditions.[40] Implementing the guidelines is multifaceted in nature and requires support from different stakeholders. Government, food industry and clinicians have an important role to play in supporting dietary pattern-based guidelines.[40] Governments can implement the guidelines by incorporating these into national nutrition guidelines. Food industry can cooperate with the government to meet the needs of the people and on how foods are marketed. Clinicians can play a major role in health promotion campaigns and imparting knowledge. It is very important to educate the people about healthy dietary patterns and health care professionals can play a key role in giving nutrition education.[17] Communication channels should be established or incorporated in already established platforms to inform the general public about healthy dietary.[40]

Additionally, to have long-term sustainable improvements in dietary intake, policy changes will be required including taxes on unhealthy foods, subsidies for production of healthy foods, food labelling, regulation of how food is marketed and creating healthy environments. Multiple approaches are therefore needed to translate existing nutritional knowledge into policies and public health practice, which will be discussed more in detail in the subsequent sections.[17]

*7.6.2 Implications for policy makers- focus on CHD based nutrition policy*

In Bangladesh, the focus of nutrition policies is to combat under-nutrition in children and mothers. The 2015 National Nutrition Policy of Bangladesh[41] is focused primarily on maternal and child nutrition. Although the policy has a clause that mentions "ensure appropriate nutrition for adults and elderly persons suffering from malnutrition-related NCDs", however, the policy lacks discussion on how this can be implemented. The BRAVE study highlights that there is a need to allocate strategic priorities and resources in Bangladesh to promote nutrition research and relevant prevention policy formulations.

In this regard, findings of the BRAVE study may help bring greater focus on formulating nutrition-based policy for CHD prevention in Bangladesh. The findings, for example, highlight the importance of having fruits and vegetables to potentially reduce risk of CHD, and to commission diet quality assessment studies of local fish, which may be contaminated (e.g., with toxic heavy metals) and increased coronary risk among BRAVE participants.

Furthermore, lifestyle practices (including diet patterns) tend to change as result of increased urbanization, economic development and industrialization over the decades, potentially contributing to a rise in non-communicable diseases (NCDs).[42] Bangladesh is also now facing a dual burden of disease due to NCDs and diseases related to under-nutrition. The positive impact of policy measures on NCDs is potentially substantial and the results from the BRAVE study highlight the need to develop and scale-up prevention strategies that promote cardio-beneficial lifestyle behaviours including healthy diet, physical activity and tobacco control.

These nutrition related policies should be supplemented with appropriate population awareness, agriculture, environmental and fiscal policies. This is highly relevant to resource-poor settings since, as reported by the multinational. For example the nutritional policy can be developed along the lines of the WHO Global Action Plan to reduce NCDs,[43] which advocates formulating fiscal policies to improve nutrition. Specifically, the government may provide subsidies to local farmers to increase production, availability, affordability and consumption of fruits and vegetables. This is highly relevant to resource-poor settings since, as reported by the multinational PURE cohort study, the costs of fruits and vegetables relative to household income is significantly high in low-income countries in comparison to high-income counterparts.[44] In addition, tax strategies can be used to increase prices of unhealthy foods and beverages (such as sugar tax). There is evidence that taxation and subsidies related to nutrition influences individual behaviour.[45]

Furthermore, there should be policies to ensure regulation of marketing of potentially unhealthy foods such as processed foods by the private sector. Government stakeholders should also have policies to make sure that the food items are not contaminated (such as testing fish for arsenic, mercury contamination of foods and testing of dairy products). Furthermore, policies should be developed to encourage hygienic food preparation so that nutrition quality of the food is restored. In order to achieve long-term impact, the Government of Bangladesh should encourage dietary pattern-based guidelines and ensure informed food selection by the consumers. There should also be multi-sectoral efforts to involve all relevant stakeholders from food, fishery and livestock, agriculture, education, health and nutrition areas to implement policy on CHD.

Furthermore, the current study may have important policy implications for female CHD cases. Only a small proportion of all AMI cases (~12%) in this study were female. This can be due to a potential lack of access and awareness of women about CHD, leading to less hospitalisation. This has an important policy message of increasing awareness of CHD among women, overcoming the barriers related to access to healthcare and dedicating more funds to address this important public health issue (for instance, through conducting qualitative research).

In Bangladesh there are a few nutritional related programmes present. For example the Health, Nutrition and Population Sector Intervention Program (HNPSIP, 2016–2021) by World Bank highlights aims to combat the burden of NCDs by promoting healthy lifestyles and environments.[46] Despite the presence of these policies and programmes there is lack of implementation and monitoring by the government.[47] Recently there is a programme called the National Plan of Action for Nutrition (NPAN2) 2016-2025 which is based on the national nutrition policy. The plan is focused on delivering nutrition specific (direct interventions) to combat maternal and child malnutrition.

There is a need to have an action plan for NCDs including CHD based on the points discussed above. However, as the findings are based on observational study, the main policy implication of the study is to have increased focus of diet-based policies for CHD. At the same time appropriate guidelines need to be underpinned by future scientific work on diet and CHD in this population.

*7.6.3 Implications for clinical practice and health systems*

For clinicians, BRAVE provides evidence to give health promotional messages to the people on the role of conventional risk factors of CHD such as tobacco smoking, physical activity and healthy diet.[48] Generally, clinicians are the most respected source of lifestyle modification information. However, it is important to consider that the case-control design of BRAVE has limitations and therefore the results need to be replicated in large cohort studies before it can have implications for clinical practice and health systems.

Overall Bangladesh lacks a strong focus on CHD prevention at the primary care level. Clinicians provide advice on broad dietary modification to CHD patients, but this is done mostly at the secondary and tertiary care levels. Already existing platforms such as the NCD corners at the Upazila Health complexes established by the government of Bangladesh can be used to disseminate messages on healthy diet and other healthy lifestyle behaviours. These platforms although developed by the government are largely non-functional or under-staffed. There is, therefore, a need for the primary care physicians to provide nutritional advice (such as on healthy dietary pattern), underpinned by local scientific evidence, to promote CVD prevention. If dietary advice is delivered effectively, there is evidence that it can bring beneficial changes in patient's dietary habits and lower risk factors of CHD such as hypertension and high cholesterol levels.[49]

However, for many clinicians, barriers within the health system can limit their ability to implement the strategies for the promotion of healthy lifestyle. Examples of some of these barriers include inadequate funds or incentives for health promotion, lack of knowledge on the best behaviour change strategies, inadequate tools to monitor behaviours over time and lack of motivation of the people. [36] There is also an unmet need in Bangladesh in providing adequate capacity building of the clinicians on nutrition science in relation to CHD. For this, beside postgraduate and professional training courses, importance of better nutrition for chronic NCDs should be added to medical school's curriculum in Bangladesh.

Furthermore, Bangladesh lacks an integrated public health program and a national surveillance program focused on monitoring of NCDs and their behavioral risk factors (such as healthy diet) on a regular basis.[47] The focus of these strategies could be on early detection of high-risk individuals through screening (such as hypertensive and diabetic patients) through electronic systems and early detection of people with clinical disease, and health promotion (e.g., lifestyle modification, including diet). In addition, new technological strategies such as mobile device applications can be used to give dietary advice tailored to the person which may include targeted goals, self-monitoring and

feedback. The clinicians can use the data collected form surveillance to make evidence-based decisions about the prevention and treatment of CHD in people.

### *7.6.4 Implications for the community-Health promotion strategies*

This section will discuss the possible health promotion strategies that can be used to promote healthy diets in Bangladesh. The potential to reduce the burden of CHD and its risk factors through dietary interventions is great.[50,51]

One recommendation is to focus on context specific behaviour change interventions by organising health promotion campaigns and counselling with the help of stake holders such as local politicians and religious leaders. The close-knit community of Bangladesh can be conducive to disseminate messages. In addition, public health education programmes focusing on the promotion of healthy diet (such as higher consumption of fruits and vegetables) is required. The primary healthcare (community health clinics) and social welfare activities can be used as channels to reach the people at the grass root level. Additionally, educational institutions can be used create awareness about healthy diets by adding it to the curriculum.

Dietary habits are affected by cultural beliefs and traditions. Mass media campaigns using television and radio in coordination with the government can also be utilised to create awareness of the positives and negatives effects of diet in relation to CHD. This strategy uses the Geoffrey Roses's population approach in which the whole population receives the intervention.[52] To target high risk populations, general practitioners can educate high risk individuals about the importance of diet in reducing the risk of CHD. There is evidence from RCTs that intervention of dietary advice can reduce the incidence of CHD.[50,53] A combination of population based and high risk based approaches will be best to create awareness about diet and risk of CHD. The HNPSIP (2016-2021) also has a component on health education (mass media and community level) which aims to disseminate knowledge on dietary issues, lifestyle choices and sanitation. Such measures will bring benefits for not only CHD prevention but also wide range of other chronic diseases such as diabetes, hypertension and some cancers.

However, reducing the burden of CHD is a multidimensional challenge in Bangladesh, requiring financial resources, coherent polices, political will, adequate health systems and advocacy with the public and private sector. Hence, the feasibility of conducting dietary interventions in developing countries like Bangladesh need to be tested. Furthermore, it is

also important to consider how accepting the Bangladeshi population will be to health promotion strategies, and health policies related to diet and CHD. Bangladeshis mostly follow homogenous dietary patterns; therefore, it may be difficult to ask them to change their dietary habits. Therefore, for strategies to have a large effect, they must be culturally appropriate.

### *7.6.5 Nutritional labels*

Nutritional labels use the health promotion technique of "nudging", to stimulate behaviour change in the population.[54] Nudge is based on the concept that people are not rational in making choices and therefore interventions can be formulated to alter the behaviour of people and help them make better decisions.[54] However, it is important to note that in Bangladesh people purchase fruits and vegetables mostly from the open informal food stalls. In addition, most of the milk and dairy products, are also sold through informal channels by small-scale local traders.[55] Therefore the nutritional labelling may only target those who consume pre-packaged food items and beverages available in the general stores. Nevertheless, the sale persons of the informal markets could be given brochures to those who purchase items.

### *7.6.6 Agriculture and aquaculture-based interventions*

Bangladesh has an agrarian based economy. Therefore, interventions based on agriculture may have a huge impact on improving nutrition in the country. It will be important to provide training and education to people for homestead farming for a wide variety of fruits and vegetables.

Fish is an important agriculture crop that contributes to the livelihood of many people in Bangladesh. Bangladesh the third largest producer of fish after China and India.[56] As discussed in **Chapter 4**, the possible reasons for the adverse effect of fish consumption on CHD could be due to cooking practices (i.e. prolonged frying). Contamination of fish from effluents of textile and leather industries and improper waste disposal and contamination by arsenic are the main causes of water pollution in Bangladesh which may also affect fish.[57] As fish is an important source of nutrition and an important part of diet of people in Bangladesh, it will be important to educate people about using different methods of cooking. For example, instead of deep frying they can steam or grill the fish. In addition, the people may be encouraged to start aquaculture practices using clean

water. This small-scale fish farm will make sure that the contaminants like arsenic do not enter the food chain through fish. Previously there have been projects in Bangladesh to promote aquaculture,[58,59] however there is no evidence whether this reduces the arsenic content of the fish and its effect on disease outcomes.

## *7.7 Conclusion*

In conclusion, the findings from the first detailed study from Bangladesh on diet and risk of AMI indicates that higher consumption of fruits, vegetables, yoghurt and certain spices are associated with a lower risk of AMI. In contrast, higher consumption of biryani, sweet water and sea water fish were associated with a higher risk of developing AMI. The "vegetable pattern" and "fruits and dairy pattern" were inversely associated with AMI, whereas "energy dense food pattern" had no significant association. The results of association of nutrients with AMI also complement the food group and dietary pattern analyses. Specifically, higher intake of refined carbohydrates had a weak but non-significant association with the risk of AMI, while non-refined carbohydrates had a lower risk of AMI. Plant protein showed an inverse association, on the contrary animal protein showed an increased association with the risk of AMI. As for specific fatty acids, moderate intakes of SFA from dairy and PUFA showed an inverse association. In contrast MUFA showed an increased association only in the highest quintile. Whereas, SFA from other sources had no significant association. In a resource constraint country like Bangladesh, where carrying out RCTs may not be very feasible, a large observational study may provide the best evidence.

Owing to the observational nature of the study, a causal assessment could not be conducted. This most detailed and powerful epidemiological study among a major South Asian population highlights the importance of further future research to elucidate diet-CHD associations and its determinants. Furthermore, if this evidence is replicated in future observational studies, it may hold the potential to influence local dietary guidelines specific to Bangladeshis (and broadly to other similar settings), which may in turn reduce the rising burden of CHD. It may be postulated that when examining diet and its association with disease, it is important to consider the totality of evidence by investigating the inter-connections between food groups, dietary patterns and nutrients to enhance quality of evidence and understanding. A systematic top down approach should be used in which dietary patterns should be given the most importance followed by food groups and then nutrients to formulate future dietary guidelines.

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**Figure 7.1:** Schematic diagram of the relation between food good groups, dietary patetrns and dietary nutrients and risk of disease

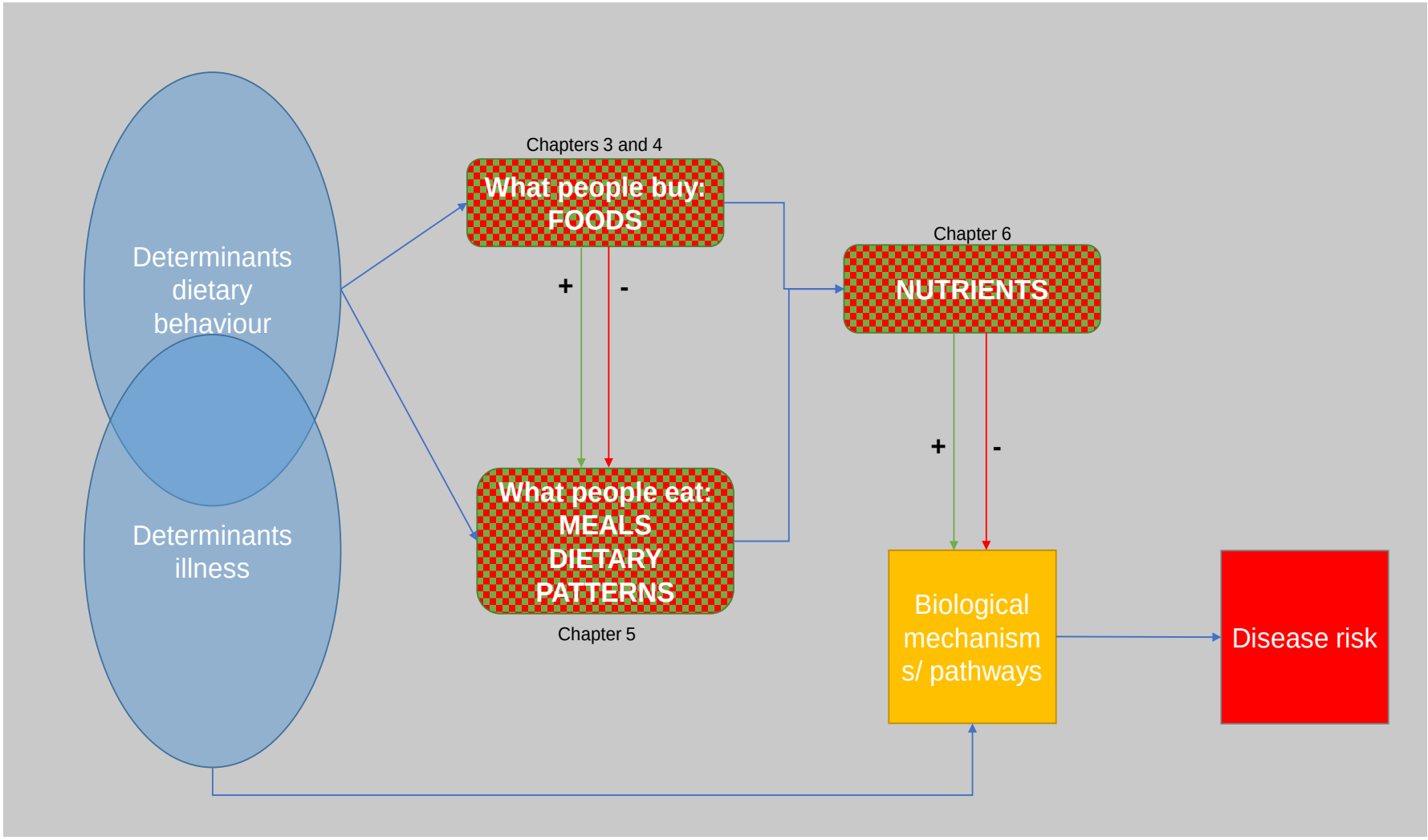
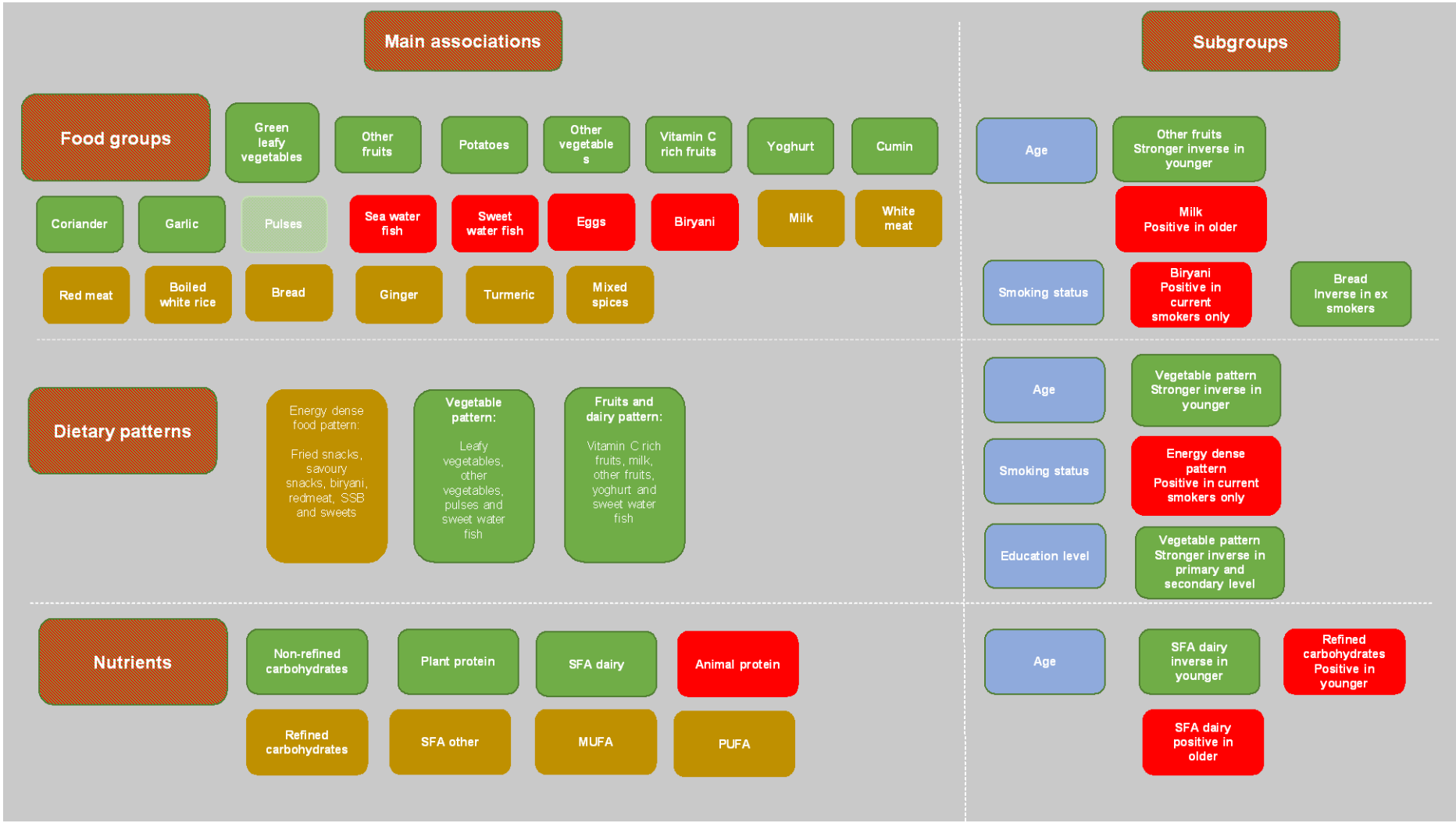


**Figure 7.2:** Summary diagram for main association of food groups, dietary patetrns and dietary nutrients with AMI



***Appendix 1 List of publications authored during PhD***

Publications, news articles and conference abstracts

1) **S Shahzad**. Dairy products and risk of heart disease in South Asians. Conference series abstract.
2) R Chowdhury, A Ramond, **S Shahzad** al. Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis. British Medical Journal. 2018
3) R Chowdhury, R Lawrence, **S Shahzad** et al. Reducing NCDs globally: the under-recognised role of environmental risk factors. Lancet. 2018
4) **S Shahzad**, M Glisic et al. Association between progestin-only contraceptive use and cardiometabolic outcomes: A systematic review and meta-analysis. European Journal of Preventive Cardiology. 2018
5) C O Willaims, M Glisic, **S Shahzad** et al. The impact of postmenopausal hormone therapy on cardiovascular outcomes in women: a systematic review and meta-analysis. Journal of Human Reproduction
6) Toxic metals tied to increased heart disease risk. Reuters Health, UK

Papers in progress:

1) R Chowdhury, **S Shahzad**, E Di Angelantonio, A Butterworth et al. Conventional and local socio-behavioural factors of first-ever myocardial infarction risk in South Asia: the BRAVE Bangladesh study
2) **S Shahzad,** R Chowdhury, M Lentjes et al. Diet and risk of coronary heart disease in South Asians: The BRAVE Bangladesh study.
3) **S Shahzad,** R Chowdhury, M Lentjes et al. Nutrients and risk of coronary heart disease in South Asians: The BRAVE Bangladesh study.
4) **S Shahzad,** R Chowdhury, M Lentjes et al. Cross-sectional correlates of dietary patterns in South Asian: The BELIEVE Bangladesh study.
5) **S Shahzad,** R Chowdhury et al. Impact of major cardiovascular drugs in the prevention of cardiovascular disease in South Asia: a systematic Review and meta-Analysis (in preparation)
6) R Chowdhury, **S Shahzad** et al. BELIEVE study protocol

***Appendix 2: Food Frequency Questionnaire of BRAVE study***

| SL No. | Food Groups | Food Items (in local terms) | Unit | Never | 1-3 Per month | Once A week | 2-4 Per Week | 5-6 per Week | Once A Day | 2-3 Per day | 4-5 Per Day | 6+ Per Day |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FF1. | Rice | Sada bhat | Plate | | | | | | | | | |
| | | Biriyani/Polau/Tehari | Plate | | | | | | | | | |
| | | Muri | Bowl | | | | | | | | | |
| | | Panta bhat | Plate | | | | | | | | | |
| FF2. | Bread | Ruti | Number | | | | | | | | | |
| | | Porotha/ Luchi | Number | | | | | | | | | |
| | | Chapati | Number | | | | | | | | | |
| | | Pauruti or Bon ruti (bread loafs or bun) | Number | | | | | | | | | |
| FF3. | Red meat | Goru | Bowl | | | | | | | | | |
| | | Khashi/ Patha | Bowl | | | | | | | | | |
| *FF4.* | *Poultry* | Deshi Murgi | Piece | | | | | | | | | |
| | | Farmer Murgi | Piece | | | | | | | | | |
| | | Haas | Piece | | | | | | | | | |
| | | Dim (egg) | Number | | | | | | | | | |
| FF5. | Drinks | Soft drinks (coke, pepsi) | Bottle-250 ml | | | | | | | | | |
| | | Foler rosh (fresh) | Glass | | | | | | | | | |
| | | Foler rosh (bottled) | Bottle-250 ml | | | | | | | | | |
| | | Lassi | Glass | | | | | | | | | |
| | | Mod (Deshi) | Peg-30 ml | | | | | | | | | |
| | | Mod (Bedeshi) | Peg-30 ml | | | | | | | | | |
| FF6. | Vegetables | Shobji (any vegetables) | Table spoon | | | | | | | | | |
| | | Kumra | Table spoon | | | | | | | | | |
| | | Shak (all) | Table spoon | | | | | | | | | |
| | | Data (all) | Table spoon | | | | | | | | | |
| | | Begun | Piece | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dherosh | Table spoon | | | | | | | | | |
| | | Korola/ Ucche | Table spoon | | | | | | | | | |
| | | Lau/ kodu/ Jali | Table spoon | | | | | | | | | |
| | | Jhinga | Table spoon | | | | | | | | | |
| | | Chichinga | Table spoon | | | | | | | | | |
| | | Kacha Pepe | Table spoon | | | | | | | | | |
| | | Potol | Table spoon | | | | | | | | | |
| | | Shapla | Table spoon | | | | | | | | | |
| | | Kochu | Table spoon | | | | | | | | | |
| | | Tomato | Number | | | | | | | | | |
| | | Shosha | Piece | | | | | | | | | |
| | | Gajor | Table spoon | | | | | | | | | |
| | | Borboti | Table spoon | | | | | | | | | |
| | | Kolar Mocha | Table spoon | | | | | | | | | |
| | | Sajna | Table spoon | | | | | | | | | |
| | | Dhundol | Table spoon | | | | | | | | | |
| | | Shim | Table spoon | | | | | | | | | |
| | | Cauliflower/ Cabbage | Table spoon | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FF7. | Pulses | Muger dal | Dal spoon | | | | | | | | | |
| | | Musurir dal | Dal spoon | | | | | | | | | |
| | | Buter dal | Dal spoon | | | | | | | | | |
| FF8. | Potato | Aloo | Number | | | | | | | | | |
| | | Misti aloo | Number | | | | | | | | | |
| FF9. | Spices | Jeera | Times | | | | | | | | | |
| | | Dhonya | Times | | | | | | | | | |
| | | Gorom masala | Times | | | | | | | | | |
| | | Ada | Times | | | | | | | | | |
| | | Holud | Times | | | | | | | | | |
| | | Roshun | Times | | | | | | | | | |
| FF 10. | Fruits | Fol (any fruits) | Number | | | | | | | | | |
| | | Kola | Number | | | | | | | | | |

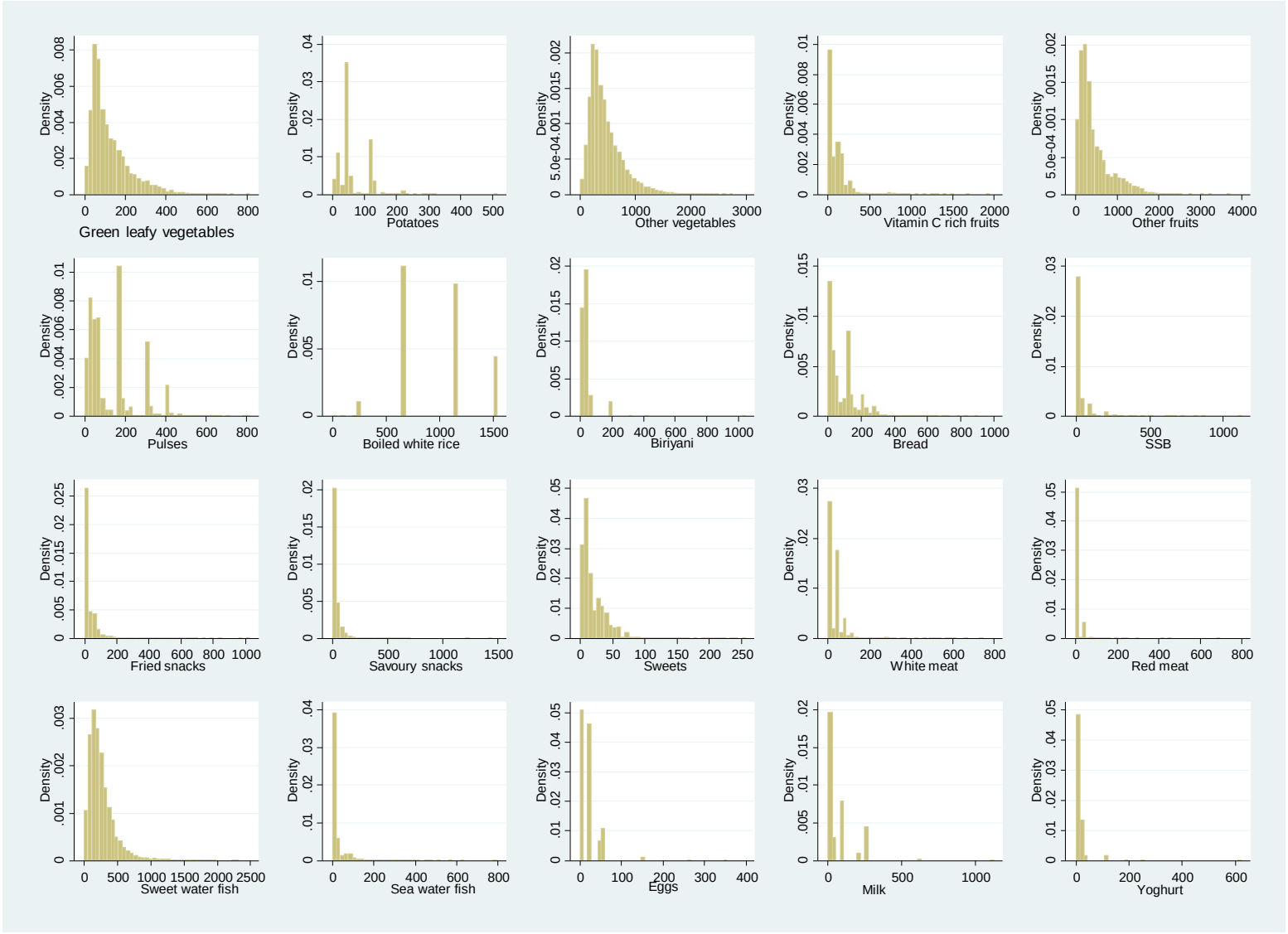
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FF 10a. | Local produce Fruits | Peyara | Number | | | | | | | | | | |
| | | Ata | Number | | | | | | | | | | |
| | | Amra | Number | | | | | | | | | | |
| | | Lotkon | Number | | | | | | | | | | |
| | | Paka pepe | Piece | | | | | | | | | | |
| | | Amlaki | Number | | | | | | | | | | |
| | | Dewa | Number | | | | | | | | | | |
| | | Kul/boroi | Number | | | | | | | | | | |
| | | Bel/Kotbel | Number | | | | | | | | | | |
| | | Jaam | Number | | | | | | | | | | |
| | | Anarosh | Number | | | | | | | | | | |
| | | Narkel/Daab | Number | | | | | | | | | | |
| | | Aam | Number | | | | | | | | | | |
| | | Kathal | Koa/piece | | | | | | | | | | |
| | | Sharifa | Number | | | | | | | | | | |
| | | Mewa | Number | | | | | | | | | | |
| | | Shofeda | Number | | | | | | | | | | |
| FF 10b. | Imported fruits | Apple | Number | | | | | | | | | | |
| | | Angur | Chora/bunch | | | | | | | | | | |
| | | Komola | Number | | | | | | | | | | |
| | | Naspati | Number | | | | | | | | | | |
| | | Mosambi | Number | | | | | | | | | | |
| FF11. | Fish | Mach (any) | Piece | | | | | | | | | | |
| FF11a. | Sweet water | Rui | Piece | | | | | | | | | | |
| | | Katla/ katol | Piece | | | | | | | | | | |
| | | Chital | Piece | | | | | | | | | | |
| | | Ilish/ hilsha | Piece | | | | | | | | | | |
| | | Pangash | Piece | | | | | | | | | | |
| | | Pabda | Number | | | | | | | | | | |
| | | Shing/ magur | Piece | | | | | | | | | | |
| | | Koi | Number | | | | | | | | | | |
| | | Telapia | Piece | | | | | | | | | | |
| | | Tengra | Number | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Puti/ shorputi | Number | | | | | | | | | |
| | | Byne | Piece | | | | | | | | | |
| | | Mola/ dhela/ kachki | Bowl | | | | | | | | | |
| | | Shol/ Gogar | Piece | | | | | | | | | |
| | | Taki | Piece | | | | | | | | | |
| | | Nola | Piece | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FF1 1b. | Saline water | Golda (lobster) | Number | | | | | | | | | |
| | | Choto chingri (shrimp & prawn) | Number | | | | | | | | | |
| | | Rupchada/ chanda | Piece | | | | | | | | | |
| | | Lutia/ Loitta | Number | | | | | | | | | |
| | | Silver curve | Piece | | | | | | | | | |
| | | Mrigel | Piece | | | | | | | | | |
| | | Koral | Piece | | | | | | | | | |
| | | Ain/ Air | Piece | | | | | | | | | |
| | | Rissha/ Topshey | Piece | | | | | | | | | |
| | | Poa | Piece | | | | | | | | | |
| | | Rita | Piece | | | | | | | | | |
| | | Churi | Piece | | | | | | | | | |
| | | Hangori | Piece | | | | | | | | | |
| | | Parshey | Piece | | | | | | | | | |
| | | Vetki | Piece | | | | | | | | | |
| | | Bata or Bele | Number | | | | | | | | | |
| F12 a. | Local recipe | Puri (eg, Dalpuri) | Number | | | | | | | | | |
| | | Singara (aloo) | Number | | | | | | | | | |
| | | Singara (koliza) | Number | | | | | | | | | |
| | | Samosa | Number | | | | | | | | | |
| | | Deshi (jhal) patties | Number | | | | | | | | | |
| | | Mughlai paratha | Number | | | | | | | | | |
| | | Kabab/ Tikka | Number | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Chop or roll (meat) | Number | | | | | | | | | |
| | | Chanachur | Bowl | | | | | | | | | |
| | | Haleem | Bowl | | | | | | | | | |
| | | Beguni/ Pakora/ Piaju | Number | | | | | | | | | |
| | | Chotpoti/ Fuchka | Bowl | | | | | | | | | |
| | | Misti (any sweets) | Number | | | | | | | | | |
| | | Deshi Pitha (any) | Number | | | | | | | | | |
| | | Faluda | Bowl | | | | | | | | | |
| | | Badam bhaji/ Jhal muri | Bowl | | | | | | | | | |
| | | Chola/ chana boot | Bowl | | | | | | | | | |
| Ff12 b. | Western recipe | Biscuits (any) | Number | | | | | | | | | |
| | | Cake/ pastries | Number | | | | | | | | | |
| | | Burger | Number | | | | | | | | | |
| | | Sandwich | Number | | | | | | | | | |
| | | Pizza | Piece | | | | | | | | | |
| | | Fried chicken | Piece | | | | | | | | | |
| | | Grilled chicken | Piece | | | | | | | | | |
| | | Chips (crisp) | Bowl | | | | | | | | | |
| | | Noodles | Bowl | | | | | | | | | |
| | | Popcorn | Bowl | | | | | | | | | |
| FF13. | Dairy products | Doodh (milk) | Glass | | | | | | | | | |
| | | Makhon (butter) | Tea spoon | | | | | | | | | |
| | | Margarine (spread) | Tea spoon | | | | | | | | | |
| | | Mayonnaise | Tea spoon | | | | | | | | | |
| | | Cheese | Tea spoon | | | | | | | | | |
| | | Doi (yogurt) | Bowl | | | | | | | | | |

***Appendix 2, Figure 1:*** Distribution of food groups in the BRAVE study



***Appendix 3: Recipes for calculation of energy intake.***

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **PURI BREAD** | **242** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Chholar Dal (1 cup) (soaked) | 182 | 02_0012 | 182 | 51.75544132 |
| Mashed potato (1/3 cup) | 70 | 05_0021 | 84 | 9.187356448 |
| Ginger (1 inch by 1/2 inch) | 6 | 07_0011 | 72 | 0.674989453 |
| Green Chillies (2-3) | 30 | 03_0010 | 45 | 2.109342042 |
| White Oil (2 tbl spn) | 22 | 17-686 | 899 | 30.90264215 |
| Jeera Powder (1 tspn) | 3 | 07_0008 | 402 | 1.884345557 |
| Green chilli (2-3) | 30 | 03_0010 | 45 | 2.109342042 |
| Red Chilli Powder (1/2 tspn) | 1 | 13-812 | 314 | 0.490617334 |
| Salt to Taste | 1 | 15_0007 | 0 | 0 |
| Sugar (1/2 tspn) | 2 | 15_0008 | 398 | 1.243730567 |
| Maida or White Flour (2 cup) | 250 | 01_0031 | 347 | 135.5447571 |
| White Oil (1-2 tspn) | 4.5 | 17-686 | 899 | 6.320994984 |
| Warm Water (3/4 cup) | 178 | 14_0010 | 0 | 0 |
| Salt (1 pinch) | 1 | 15_0007 | 0 | 0 |
| TOTAL WEIGHT | **780.5** | | | **242.223559** |
| COOKING LOSS FACTOR | 0.18 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.82 | | | |
| TOTAL WEIGHT AFTER COOKING | **640.01** | | | |

| RECIPE CODE | RECIPE NAME | AL/100G |
|---|---|---|
| - | **BISCUITS, SWEET** | **345** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Wheat flour | 84 | 01_0032 | 346 | 189.3790317 |
| Sugar | 28 | 15_0008 | 398 | 72.61354011 |
| Ghee, vegetable/Vanaspati | 14 | 13_0005 | 900 | 82.1007363 |
| Baking powder | 1 | 15_0001 | 172 | 1.12074021 |
| Water | 22 | 14_0010 | 0 | 0 |
| TOTAL WEIGHT | **149** | | | **345.2140483** |
| COOKING LOSS FACTOR | -0.03 | | RETENTION FACTOR | |
| (1 --- COOKING LOSS FACTOR) | 1.03 | | | |
| TOTAL WEIGHT AFTER COOKING | **153.47** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **JHAL MURI** | **206** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Muri | 250 | 01_0023 | 361 | 113.5220126 |
| Medium potato, boiled, peeled, chopped (1) | 167 | 05_0012 | 67 | 14.07421384 |
| Cucumber (1/2 small) | 75 | 03_0012 | 17 | 1.603773585 |
| Green chilli (4) | 60 | 03_0010 | 45 | 3.396226415 |
| Mixed sprouts (2 tbl spn) | 24 | - | 146 | 4.40754717 |
| Pinch of salt (4) | 4 | 15_0007 | 0 | 0 |
| Small onion (1) | 70 | 03_0024 | 59 | 5.194968553 |
| Medium tomato (1) | 80 | 03_0031 | 16 | 1.610062893 |
| Ginger (2 tsp) | 4 | 07_0011 | 72 | 0.362264151 |
| Raw peanuts (2 tbl spn) | 17 | 06_0007 | 585 | 12.50943396 |
| Mustard oil (4 tsp) | 44 | 13_0008 | 900 | 49.81132075 |
| TOTAL WEIGHT | **795** | | | **206.4918239** |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **SINGARA ALOO** | **270** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Maida (3 cups) | 375 | 01_0031 | 347 | 74.31637502 |
| Ghee (4 tbl spn) | 60 | 13_0005 | 900 | 30.84022479 |
| Salt (1 tspn + pinch) | 8 | 15_0007 | 0 | 0 |
| Sugar (2 tspn) | 8 | 15_0008 | 398 | 1.818431032 |
| Water | 250 | 14_0010 | 0 | 0 |
| Vegetable oil (214 g) | 214 | 17-686 | 899 | 109.8745831 |
| Medium sized potato (5) | 835 | 05_0012 | 67 | 31.951044 |
| Cauliflower florets (1 cup) | 124 | 03_0036 | 28 | 1.982912231 |
| Green peas (1/2 cup) | 80 | 13-439 | 79 | 3.609448531 |
| Roasted peanuts (1/4 cup) | 37 | 14-878 | 590 | 12.46744643 |
| Ginger, grated (1 inch) | 6 | 07_0011 | 72 | 0.246721798 |
| Green chillies (2) | 30 | 03_0010 | 45 | 0.77100562 |
| Turmeric powder (1/2 tspn) | 1 | 07_0020 | 335 | 0.191323617 |
| Red chili powder, dry (1/2 tspn) | 1 | 13-812 | 314 | 0.179330196 |
| Cumin seeds, whole (1 tspn) | 2 | 07_0008 | 402 | 0.45917668 |
| Bhaja Masala (2,5 tspn) | 5 | - | 358 | 1.021607063 |
| TOTAL WEIGHT | **2036** | | | **269.7296301** |
| COOKING LOSS FACTOR | 0.14 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.86 | | | |
| TOTAL WEIGHT AFTER COOKING | **1750.96** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **MISTI SWEETS (LADOO)** | 471 |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Besan (250 g) | 250 | 16157 | 387 | 223.9583333 |
| Sugar (1/2 cup) | 100 | 15_0008 | 398 | 92.12962963 |
| Green cardamom, ground (1 tspn) | 2 | 07_0002 | 261 | 1.208333333 |
| Ghee (4 tbl spn + extra) | 70 | 13_0005 | 900 | 145.8333333 |
| Sooji or rawa (1 tbl spn) | 10 | 01_0026 | 346 | 8.009259259 |
| TOTAL WEIGHT | **432** | | | **471.1388889** |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **DESI PITHA (IDLI)** | **129** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Urad dal (1 cup) | 140 | 02_0004 | 350 | 40.93567251 |
| Idli rava (2 cups) | 269 | - | 356 | 80.00334169 |
| Rice, cooked (1/2 cup) | 93 | 01_0037 | 109 | 8.468671679 |
| Salt (to taste) | 2 | 15_0007 | 0 | 0 |
| Cooking soda (pinch) | 1 | 17-356 | 0 | 0 |
| Water | 825 | 14_0010 | 0 | 0 |
| TOTAL WEIGHT | **1330** | | | **129.4076859** |
| COOKING LOSS FACTOR | 0.1 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.9 | | | |
| TOTAL WEIGHT AFTER COOKING | **1197** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **BEGUNI** | **276** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Eggplant (1 medium) | 450 | 13-161 | 15 | 10.9150887 |
| Besan or Gram flour (1/2 cup) | 46 | 11-896 | 353 | 26.25766078 |
| Kalo Jeery or Black Nigella seeds (1 tspn) | 2 | - | 375 | 1.212787633 |
| Rice flour (1 tbl spn) | 9 | 11-021 | 366 | 5.326563283 |
| Coconut powder (1 tbl spn) | 5 | - | 626 | 5.061367054 |
| Red chili powder (1/2 tspn) | 1 | 13-812 | 314 | 0.507753756 |
| Turmeric powder (1/2 tspn) | 1 | 07_0020 | 335 | 0.541711809 |
| Salt (to taste) | 2 | 15_0007 | 0 | 0 |
| Sugar (1 pinch) | 2 | 15_0008 | 398 | 1.287171941 |
| Water (1 cup) | 250 | 14_0010 | 0 | 0 |
| Oil (for frying) | 155 | 17-686 | 899 | 225.3278569 |
| TOTAL WEIGHT | **923** | | | **276.4379619** |
| COOKING LOSS FACTOR | 0.33 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.67 | | | |
| TOTAL WEIGHT AFTER COOKING | **618.41** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **SINGARA KOLIZA** | **278** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Plain flour (1 cup) | 125 | 01_0031 | 347 | 41.56246107 |
| Salt (1/2 teaspoon) | 3 | 15_0007 | 0 | 0 |
| Oil (2 tbl spn) | 22 | 17-686 | 899 | 18.95152404 |
| Nigella seeds (1/4 tspn) | 0.5 | - | 375 | 0.179664817 |
| Water (1/3 cup) | 83 | 14_0010 | 0 | 0 |
| Diced beef liver (1.5 cups) | 375 | 10_0001 | 130 | 46.7128525 |
| Potato, peeled, diced (1.5 cups) | 225 | 05_0012 | 67 | 14.44505131 |
| Green peas (1/2 cup) | 80 | 13-439 | 79 | 6.055902109 |
| Onion, sliced (1/2 cup) | 58 | 03_0024 | 59 | 3.279002693 |
| Turmeric powder (1/4 tspn) | 0.5 | 07_0020 | 335 | 0.16050057 |
| Chili powder (1 tspn) | 2 | 13-812 | 314 | 0.601757361 |
| Ginger paste (1 tspn) | 5 | - | 64 | 0.306627955 |
| Garlic paste (1 tspn) | 5 | - | 91 | 0.435986623 |
| Green chillies (3-5 --> 4) | 60 | 03_0010 | 45 | 2.587173369 |
| Cumin powder, roasted (1/2 tspn) | 1.5 | 07_0008 | 402 | 0.577802052 |
| Salt (to taste) | 3 | 15_0007 | 0 | 0 |
| Oil (2 tbl spn) | 22 | 17-686 | 899 | 18.95152404 |
| Oil (for deep frying) | 143 | 17-686 | 899 | 123.1849062 |
| TOTAL WEIGHT | **1213.5** | | | **277.9927368** |
| COOKING LOSS FACTOR | 0.14 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.86 | | | |
| TOTAL WEIGHT AFTER COOKING | **1043.61** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **MUGLAI PARATHA** | **174** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Whole wheat flower (1 cup) | 120 | 01_0033 | 344 | 76.62323199 |
| Salt (to taste) | 2 | 15_0007 | 0 | 0 |
| Water (1/2 cup) | 125 | 14_0010 | 0 | 0 |
| Ground beef (85% lean) | 150 | 23567 | 215 | 59.86189999 |
| Olive oil (2 tspn) | 6 | 17-038 | 899 | 10.01225081 |
| Medium onion (1) | 110 | 03_0024 | 59 | 12.04662732 |
| Garlic cloves (2) | 6 | 03_0014 | 147 | 1.637153358 |
| Coriander powder (1/2 tspn) | 1 | 13-818 | 279 | 0.517875042 |
| Cumin powder (1/2 tspn) | 1.5 | 07_0008 | 402 | 1.119278316 |
| Curry powder (1/2 tspn) | 1.5 | 13-876 | 233 | 0.648735939 |
| Black pepper powder (1/4 tspn) | 1 | 07_0017 | 302 | 0.56056725 |
| Garam masala powder (1/4 tspn) | 1 | 13-829 | 379 | 0.703493336 |
| Green coriander, chopped (1 tbl spn) | 1 | 07_0006 | 30 | 0.055685488 |
| Boiling water (1/2 cup) | 125 | 14_0010 | 0 | 0 |
| Oil (2 tspn) | 6 | 17-686 | 899 | 10.01225081 |
| TOTAL WEIGHT | **657** | | | **173.7990496** |
| COOKING LOSS FACTOR | 0.18 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.82 | | | |
| TOTAL WEIGHT AFTER COOKING | **538.74** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **ALUR CHOP** | **192** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Boiled potato (2 cups) | 420 | 05_0012 | 67 | 49.31910195 |
| Onion, finely sliced (1.5 tspn) | 5 | 03_0024 | 59 | 0.517026833 |
| Green chili (2) | 30 | 03_0010 | 45 | 2.366054998 |
| Fresh coriander, finely chopped (1 tspn) | 0.5 | 07_0006 | 30 | 0.0262895 |
| Salt (to taste) | 4 | 15_0007 | 0 | 0 |
| Vegetable oil (for deep fry) | 40 | 17-686 | 899 | 63.0246946 |
| Besan flour (1 cup) | 92 | 16157 | 387 | 62.40075714 |
| Egg (1/2) | 22 | 11_0002 | 158 | 6.092153461 |
| Rice flour (1 tbl spn) | 9 | 11-021 | 366 | 5.773174194 |
| Chili powder (1/2 tspn) | 1 | 13-812 | 314 | 0.550326866 |
| Chat masala (1 tspn) | 3 | - | 260 | 1.367053999 |
| Turmeric powder (1/4 tspn) | 0.5 | 07_0020 | 335 | 0.293566083 |
| TOTAL WEIGHT | **627** | | | **191.7301996** |
| COOKING LOSS FACTOR | 0.09 | | | |
| (1 --- COOKING LOSS FACTOR) | 0.91 | | | |
| TOTAL WEIGHT AFTER COOKING | **570.57** | | | |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **HALEEM** | **151** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Beef (boneless) | 1000 | I141 | 244 | 60.45589693 |
| Mung daal (1 cup) | 125 | 02_0005 | 351 | 10.87091179 |
| Masur daal (1 cup) | 125 | 02_0008 | 317 | 9.817888999 |
| Channa daal (1 cup) | 125 | 02_0001 | 375 | 11.614222 |
| Mash daal (1 cup) | 125 | 02_0004 | 350 | 10.83994054 |
| **Water (to cook daal, 2*weight in water uptake)** | 1000 | | 0 | 0 |
| Wheat grains (crushed) dalia (1 cup) | 125 | A021 | 342 | 10.59217047 |
| Oat grains (crushed) (1 cup) | 125 | 11-792 | 352 | 10.90188305 |
| Rice (1/2 cup) | 50 | A8 | 360 | 4.459861249 |
| **Water (for grains)** | 300 | 0 | | 0 |
| Ghee/oil (8 tbl spn) | 80 | L173 | 874 | 17.32408325 |
| Onion (chopped) (3 medium) | 150 | D70 | 44 | 1.635282458 |
| Tomatoes (chopped) (3 medium) | 150 | C63 | 21 | 0.780475719 |
| Garlic (10 cloves) | 10 | D68 | 121 | 0.299801784 |
| Ginger (crushed) (2 piece) | 10 | D69 | 53 | 0.131318137 |
| Mint leaves | 8 | C57 | 38 | 0.075322101 |
| Iodized salt (2 tspn) | 8 | 15_0007 | 0 | 0 |
| Red chili powder (1.5 tspn) | 6 | 13-812 | 314 | 0.466798811 |
| Coriander powder (2 tspn) | 8 | E81 | 327 | 0.648166501 |
| Turmeric powder (1/2 tspn) | 2 | E80 | 365 | 0.180872151 |
| Garam masala (1/2 tspn) | 4 | 13-829 | 379 | 0.375619425 |
| **Water (2 cups)** | 500 | 14_0010 | 0 | 0 |
| TOTAL WEIGHT | **4036** | | | **151.4705154** |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **CHOTPOTI** | **76** |

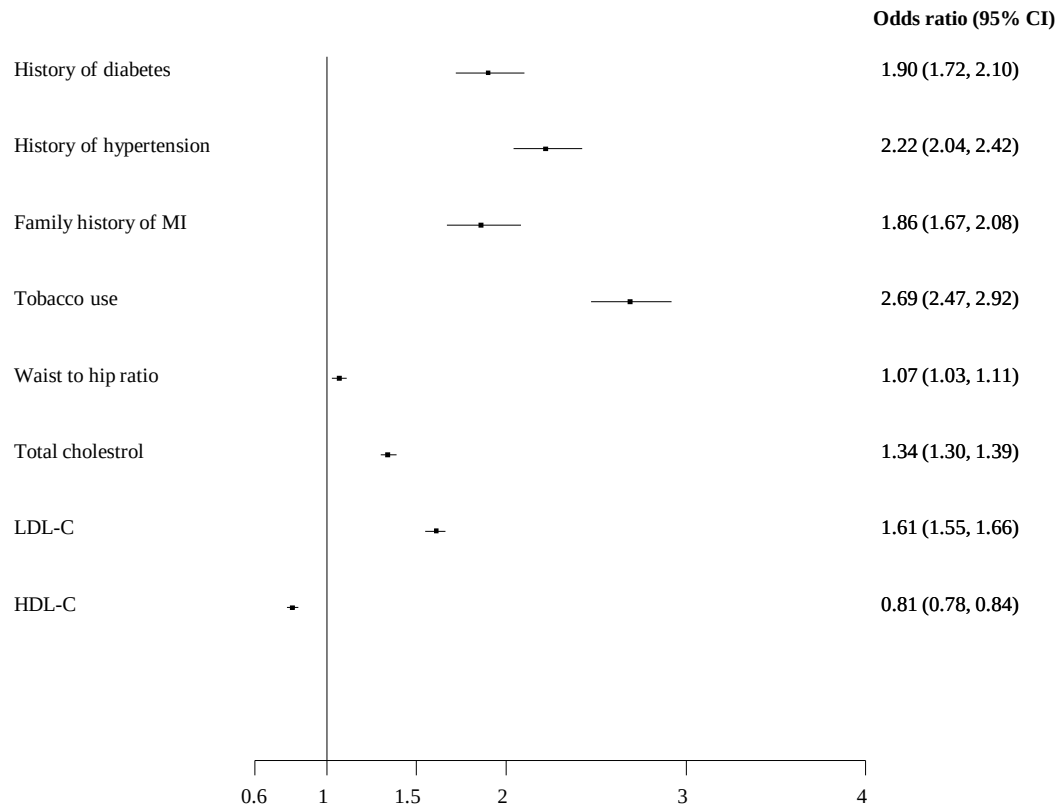
| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Dried peas (1.5 cups) | 218 | 03_0026 | 91 | 11.50696056 |
| Large potato, boiled (1) | 300 | 05_0012 | 67 | 11.65893271 |
| Egg, boiled (6) | 300 | 11_0006 | 179 | 31.14849188 |
| Chat masala (3 tbl spn) | 27 | - | 260 | 4.071925754 |
| Medium onion, finely sliced (1/2) | 55 | 03_0024 | 59 | 1.88225058 |
| Green chillies (4) | 60 | 03_0010 | 45 | 1.56612529 |
| Cucumber, chopped (1/2 cup) | 52 | 03_0012 | 17 | 0.512761021 |
| Coriander leaves, chopped | 1 | 07_0006 | 30 | 0.017401392 |
| Turmeric (1/2 tspn) | 1 | 07_0020 | 335 | 0.194315545 |
| Salt (to taste) | 2 | 15_0007 | 0 | 0 |
| **Water (for soaking + boiling)** | 371 | 14_0010 | 0 | 0 |
| Tamarind (50 g) | 50 | 08_0041 | 270 | 7.83062645 |
| Cumin powder, roasted (1 tbl spn) | 6 | 07_0008 | 402 | 1.399071926 |
| Dry chillies, roasted (4) | 7 | 07_0003 | 313 | 1.270881671 |
| Sugar (1 tbl spn) | 12 | 15_0008 | 398 | 2.770301624 |
| Salt (2 tspn) | 12 | 15_0007 | 0 | 0 |
| **Water (1 cup)** | 250 | 14_0010 | 0 | 0 |
| TOTAL WEIGHT | **1724** | | | **75.8300464** |

| RECIPE CODE | RECIPE NAME | KCAL/100G |
|---|---|---|
| - | **CHANA CHAAT** | **130** |

| INGREDIENTS | WEIGHT | FOODCODE | KCAL/100G | KCAL/INGREDIENT |
|---|---|---|---|---|
| Chana, boiled (3 cups) | 492 | B027 | 187 | 103.6664789 |
| Onion, finely chopped (1 cup) | 160 | 03_0024 | 59 | 10.63661972 |
| Tomatoes, finely chopped (1 cup) | 180 | 03_0031 | 16 | 3.245070423 |
| Green chili (1) | 15 | 03_0010 | 45 | 0.76056338 |
| Cumin seeds powder (1/2 tspn) | 1 | 07_0008 | 402 | 0.452957746 |
| Red chili powder (1/4 tspn) | 1 | 13-812 | 314 | 0.353802817 |
| Chaat masala (1/2 tspn) | 1.5 | - | 260 | 0.43943662 |
| Dry mango powder (1/2 tspn) | 2 | 45099951 | 360 | 0.811267606 |
| Black salt (to taste) | 2 | 45209996 | 0 | 0 |
| Lime juice (2 tspn) | 10 | 14-279 | 36 | 0.405633803 |
| Pani pooris/papis, crushed (5-6) | 22 | 11-911 | 366 | 9.072676056 |
| Coriander leaves, chopped | 1 | 07_0006 | 30 | 0.033802817 |
| TOTAL WEIGHT | **887.5** | | | **129.8783099** |

## *Appendix 4: Association of conventional risk factors with AMI using multiple imputation.*

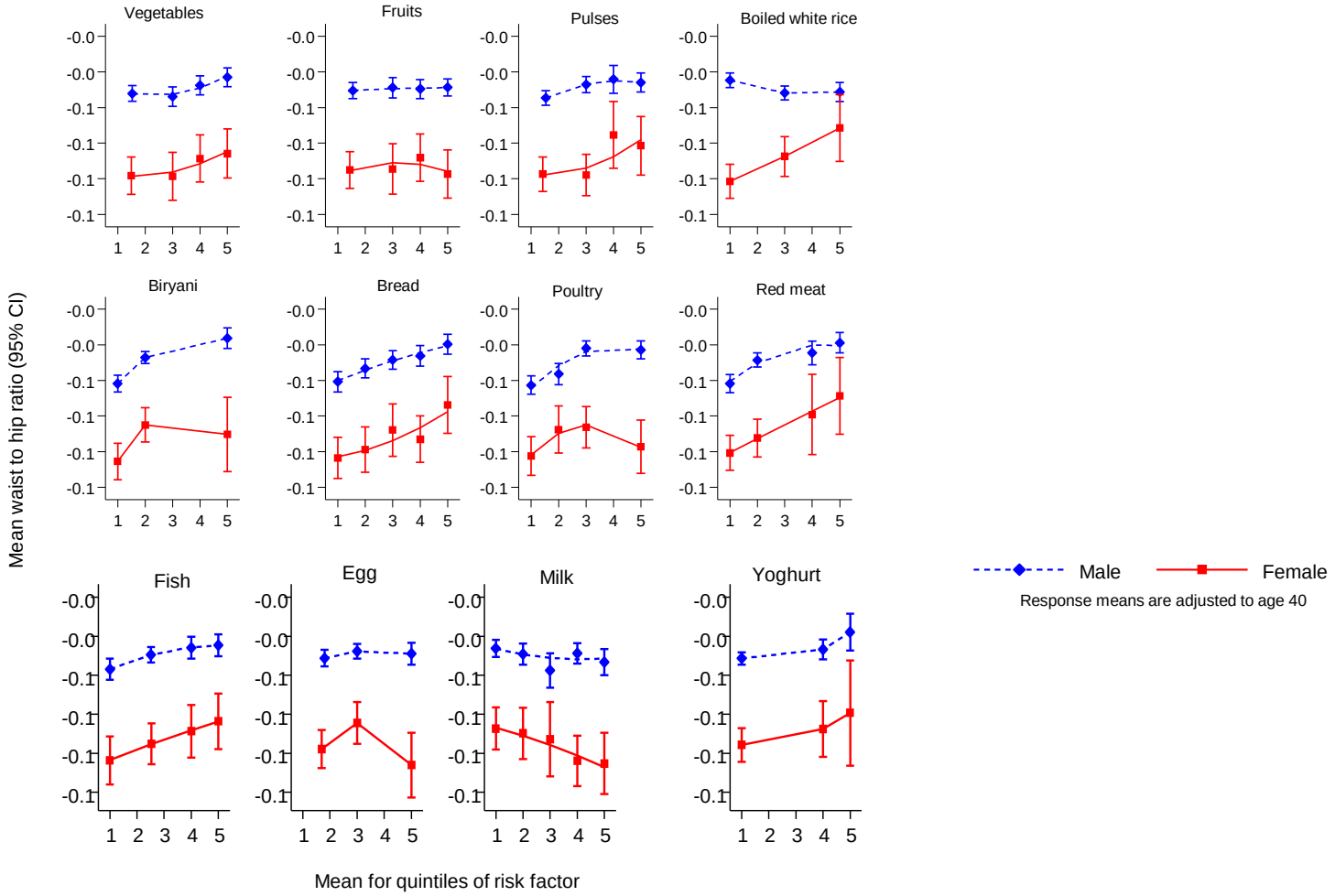
Associations of family history and conventional risk factors with risk of acute MI in Bangladesh using multiple imputation



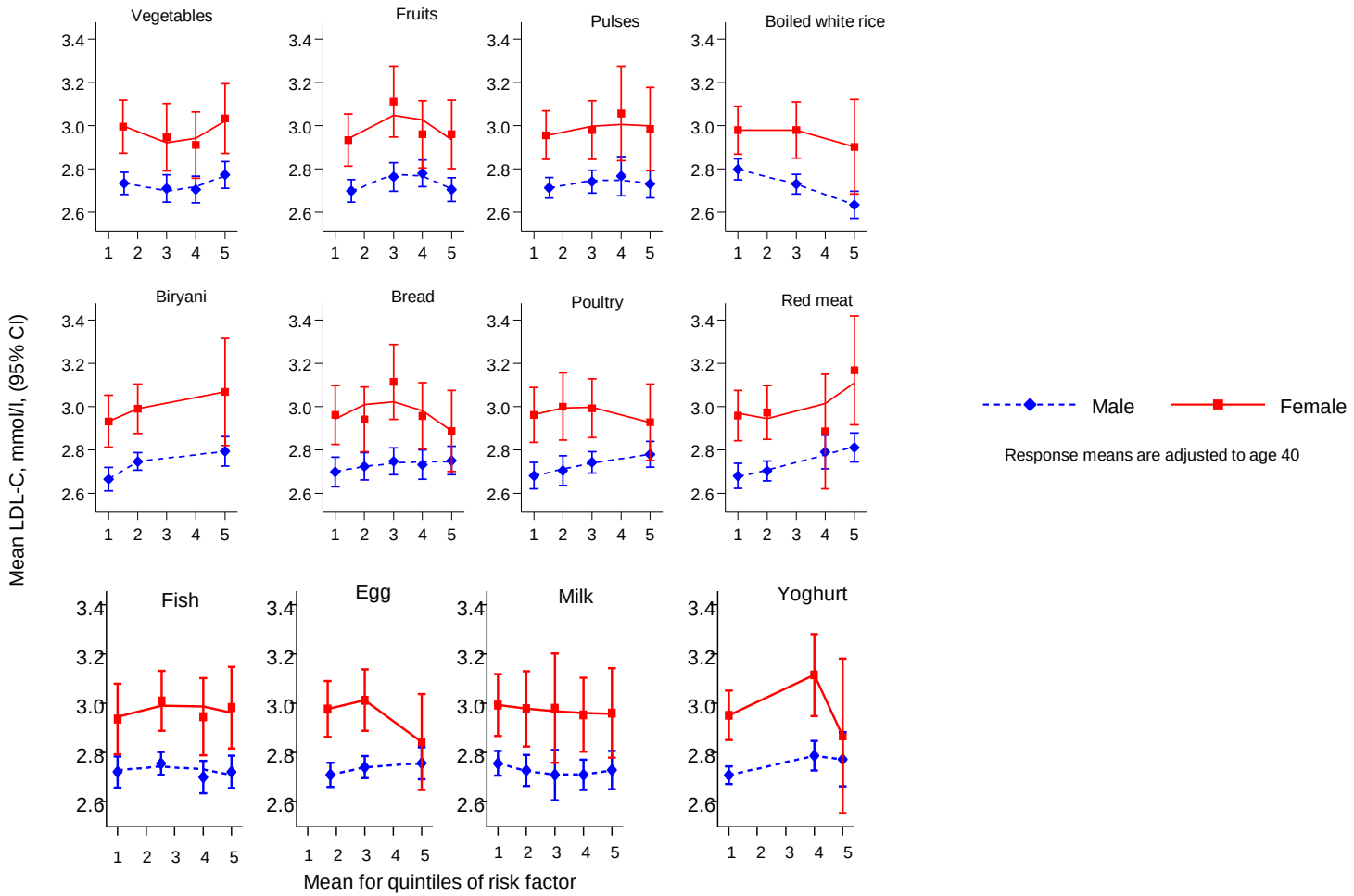

Adjusted for: age, sex, tobacco use and (where appropriate) for LDL and HDL cholesterol, history of diabetes, history of hypertension, and family history of MI.
Waist/ hip ratio is adjusted for age, sex and tobacco use only.
*Tobacco use includes both smoked/inhaled and chewed tobacco; reference category is never users

## *Appendix 5: Cross-sectional correlates of food groups*

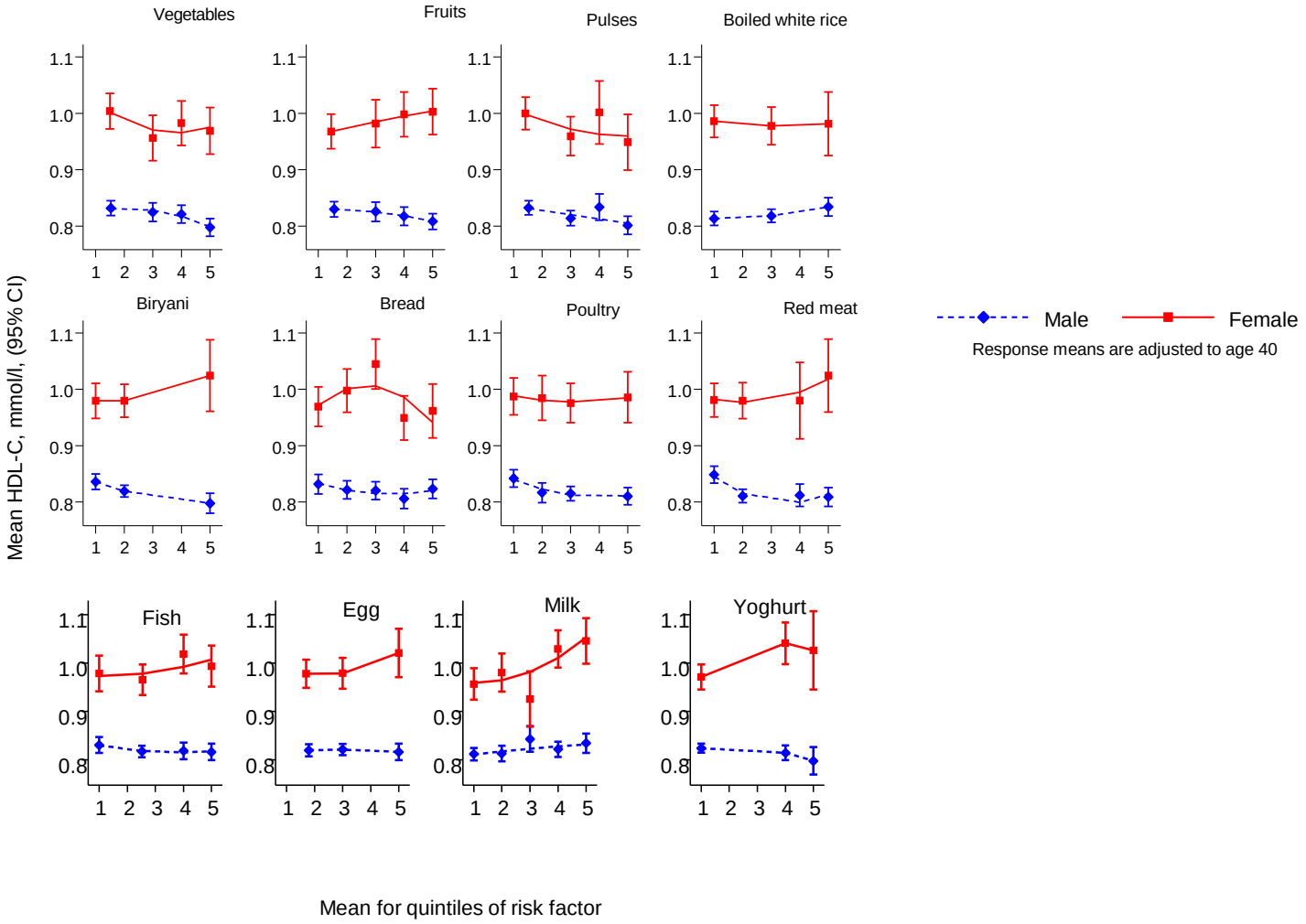
### Cross-sectional correlates of food groups with waist-to-hip ratio



***Appendix 5: Cross-sectional correlates of food groups with LDL-C***

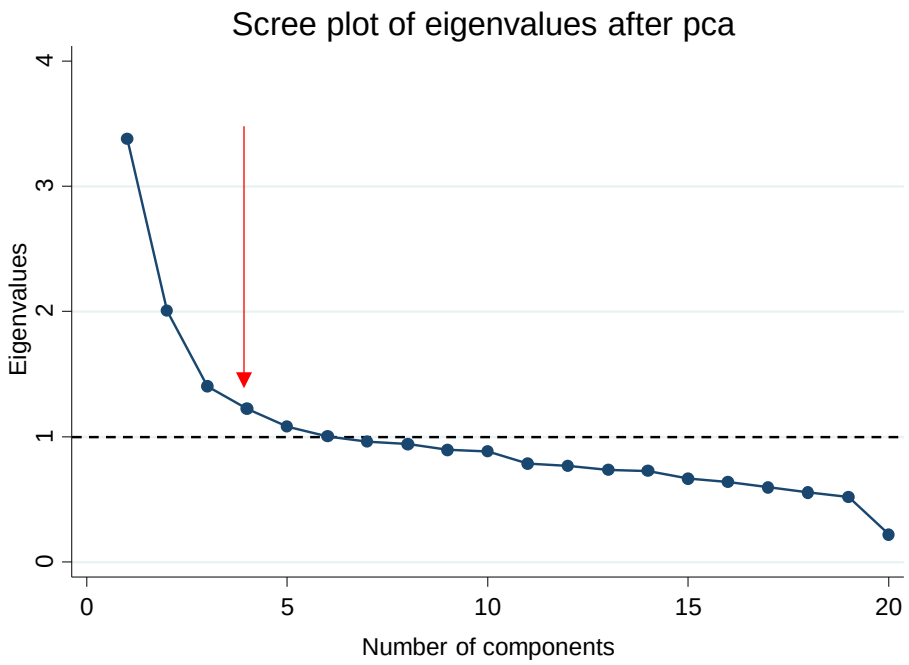


***Appendix 5: Cross-sectional correlates of food groups with HDL-C***



***Appendix 6: Scree plot of principal component analysis***

**Figure 1:** Scree plot of principal component analysis




The elbow of the scree plot as indicated by the red arrow is used to retain three dietary patterns