Genetic Polymorphisms in Fructokinase and Aldolase B, and Biomarkers of the Metabolic Syndrome

by

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Abstract

In the absence of consistent clinical evidence, concerns have been raised regarding the potential role of fructose in the development of the Metabolic Syndrome (MetS). We determined whether common polymorphisms in the genes involved in fructose metabolism (Fructokinase (KHK), and Aldolase B (ALDOB)) modify the association between fructose intake and biomarkers of the MetS (waist circumference, blood pressure, triglyceride, HDL-cholesterol and blood glucose). Fructose intake was measured using a food frequency questionnaire and genotyping was completed using real-time PCR. We found no association between the KHK (rs2119026) and fructose intake or biomarkers of the MetS. The ALDOB (rs1929480) was associated with decreased HDL-cholesterol in East Asians, while the ALDOB (515313) modified the association between dietary fructose and serum triglyceride in South Asians. The results suggest that partial and incomplete metabolism of fructose in the liver may have adverse metabolic effects.
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# Table of Contents

Table of Contents .................................................................................................................. iv
List of Tables ....................................................................................................................... vi
List of Figures ....................................................................................................................... vii
List of Abbreviations .......................................................................................................... viii
Chapter One: Literature Review ......................................................................................... 1
  1.1 Introduction .................................................................................................................. 1
  1.2 The Metabolic Syndrome ......................................................................................... 1
  1.3 Dietary Sources of Fructose .................................................................................... 4
  1.4 Consumption of Fructose ....................................................................................... 4
  1.5 Assessment of Dietary Fructose ............................................................................ 5
  1.6 Structure of Fructose .............................................................................................. 6
  1.7 Absorption and Metabolism of Fructose ............................................................... 7
  1.8 Genetic Polymorphisms of Fructokinase ............................................................. 10
  1.9 Genetic Polymorphisms of Aldolase B ................................................................. 11
  1.10Research Gap ......................................................................................................... 12
Chapter Two: Rationale, Hypothesis and Study Design ................................................... 13
  2.1 Rationale .................................................................................................................. 13
  2.2 Hypothesis .............................................................................................................. 14
  2.3 Objective ................................................................................................................ 14
Chapter Three: Genetic Variations in *KHK* and *ALDOB*, and Habitual Fructose Consumption .......................................................... 15
  3.1 Abstract ................................................................................................................... 15
  3.2 Introduction .............................................................................................................. 16
  3.3 Methods .................................................................................................................. 18
    3.3.1 Study Population .............................................................................................. 18
    3.3.2 Dietary Assessment ......................................................................................... 18
    3.3.3 Anthropometrics and Energy Expenditure ..................................................... 19
    3.3.4 Genotyping .................................................................................................... 19
  3.4 Statistical Analysis .................................................................................................. 20
  3.5 Results ..................................................................................................................... 21
  3.6 Discussion ............................................................................................................... 27
Chapter Four: Genetic Variations in *KHK* and *ALDOB*, and Biomarkers of the Metabolic Syndrome .......................................................................................... 30
  4.1 Abstract ................................................................................................................... 30
  4.2 Introduction .............................................................................................................. 31
  4.3 Methods .................................................................................................................. 32
    4.3.1 Study Population .............................................................................................. 32
    4.3.2 Dietary Assessment ......................................................................................... 33
    4.3.3 Anthropometrics and Energy Expenditure ..................................................... 33
    3.3.3 Clinical measurements .................................................................................... 34
    4.3.4 Genotyping .................................................................................................... 34
  3.4 Statistical analysis .................................................................................................. 35
  3.5 Results ..................................................................................................................... 35
  4.4 Discussion ............................................................................................................... 41
Chapter Five: Overall Discussion ...................................................................................... 44
5.1 Overview ................................................................................................................. 44
5.2 Limitations ............................................................................................................. 45
5.3 Future Directions ................................................................................................. 47
5.4 Implications .......................................................................................................... 47
5.5 Conclusion ............................................................................................................ 48
List of Tables

Table 3-1 Genotype and allele frequency and HWE by *KHK* and *ALDOB* genotypes. ........... 22
Table 3-2 Subject Characteristics by *KHK* genotypes...................................................... 23
Table 3-3 Subject Characteristics by *ALDOB* genotypes.................................................. 24
Table 3-4 Fructose Intake by *KHK* genotypes................................................................. 25
Table 3-5 Fructose Intake by *ALDOB* genotype.............................................................. 26
Table 4-1 Subject characteristics in TNHS population........................................................ 37
Table 4-2 Biomarkers of the metabolic syndrome by *KHK* genotype................................. 38
Table 4-3 Biomarkers of the metabolic syndrome by *ALDOB* genotype............................. 39
List of Figures

Figure 1-1 U.S. per capita consumption of sugars and sweeteners. Data obtained from USDA/Economic Research Service (40) ................................................................. 5
Figure 1-2 Isomeric forms of fructose (48) ........................................................................ 7
Figure 1-3 Fructose metabolism (49) ................................................................................ 10
Figure 4-1.......................................................................................................................... 40
List of Abbreviations

AACE – American Association of Clinical Endocrinology
AHA/NHLB – American Heart Association/National Heart, Lung, and Blood Institute
ALDOB – Aldolase B
ANOVA – Analysis of variance
BMI – Body mass index
CVD – Cardio-Vascular Disease
EGIR – European Group for the study of Insulin
FFQ – Food frequency questionnaire
GHLQ – General health and lifestyle questionnaire
GLUT 2 – Glucose transport 2
GLUT 5 – Glucose transport 5
HDL – High density lipoprotein
HFI – Hereditary fructose intolerance
HFCS – High fructose corn syrup
IDF – International Diabetes Federation
KHK – Fructokinase
MET – Metabolic equivalent
MetS – Metabolic syndrome
mRNA – Messenger ribonucleic acid
NCEP:ATPIII – National Cholesterol Education Program Adult Treatment Panel III
PCR – Polymerase chain reaction
SAS – Statistical analysis software
SE – Standard error

SNP – Single nucleotide polymorphism

T2D – Type II diabetes

TNHS – Toronto Nutrigenomics and Health study

WHO – World Health Organization
Chapter One

Literature Review

1.1 Introduction

The new millennium has witnessed the emergence of a modern epidemic, the MetS, which includes a cluster of common pathologies such as obesity, insulin resistance, dyslipidemia, and hypertension (1-3). Some argue that increased consumption of a carbohydrate-rich diet especially in the form of enhanced content of fructose is responsible for the increasing incidence of the MetS (4-6). However, these assumptions are based on low-quality ecologic studies (7-9), animal models of overfeeding at levels of exposure far beyond actual fructose intake,(10) and select human interventions with methodological flaws (11). These experimental models have been grounded on simplified metabolic pathway analysis, illustrating that fructose acts as an unregulated substrate for de novo lipogenesis, depletes intracellular adenosine triphosphate and impairs satiety signaling through insulin, leptin and ghrelin (12-15). However, the clinical translation of these mechanisms remains unclear. Therefore, it is not known whether fructose-containing sugars at real-world levels of exposure in free-living people are associated with the development of type 2 diabetes (T2D) and other cardio-metabolic diseases.

1.2 The Metabolic Syndrome

In 1947, Vague et al. described the strong relationship between visceral fat distribution and the metabolic abnormalities found in cardio-vascular disease (CVD) and T2D (10). The field moved forward significantly following the 1988 Banting Lecture given by Reaven (11). He described a cluster of metabolic abnormalities including abdominal obesity, insulin resistance,
dyslipidemia, and hypertension as risk factors for diabetes and cardiovascular disease” and named it “Syndrome X” (11).

Since then, many international organizations and expert groups, such as the World Health Organization (WHO), the European Group for the study of Insulin Resistance (EGIR), the National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII), the American Association of Clinical Endocrinology (AACE), the International Diabetes Federation (IDF), and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), have attempted to develop diagnostic criteria for the diagnosis of the MetS. In 2009, a harmonized definition of the metabolic syndrome was established, with at least 3 or more following criteria required for diagnosis: Elevated waist circumference (Canada and United states: ≥102 cm in males, ≥88 cm in females; Europid, Middle Eastern, sub-Saharan African, Mediterranean: ≥94 cm in males, ≥80 cm in females; Asian, Japanese, South and Central American: ≥90 cm in males, ≥80 cm in females), Elevated Triglyceride (TG) (≥1.7 mmol/L), Reduced HDL-cholesterol (<1.0 mmol/L in males, <1.3 mmol/L in females, Elevated blood pressure (Systolic ≥130 mm Hg and/or diastolic ≥85 mm Hg), and elevated blood glucose (≥5.6 mmol/L) (12).

Insulin resistance, and increased insulin levels which is typical of type 2 diabetes, stimulate insulin like growth factor (IGF-1) and leads to inflammation, leading to oxidative stress and renal insufficiency (16). Central adiposity lead to increased secretion of pro-inflammatory cytokines, such as leptin, interleukin-6, and tumor necrosis factor-alpha (TNF-α), which leads to the production of reactive oxygen species that in turn can lead to renal endothelial cell dysfunction, mesangial expansion and fibrosis (17, 18). Visceral and ectopic adiposity also lead to elevated free fatty acids in the circulation (17, 19). Triglycerides and free fatty acids may themselves be nephrotoxic by promoting pro-inflammatory cytokine production (20). In addition,
anti-inflammatory hormones such as adiponectin, a cytokine that plays important roles in the modulation of inflammation, glycemia, and lipid metabolism, may be reduced in obesity, contributing to insulin resistance as well (21). Together, these metabolic abnormalities contribute to the development of both T2D and CVD (22).

Epidemiological studies have demonstrated that the MetS is common in adults especially among obese individuals, and that its incidence is increasing as the epidemics of obesity increases (1, 2). The estimated prevalence of metabolic syndrome in Canadian adults is 19.1% (23). There has been a heightened awareness of the metabolic syndrome and a subsequent increase in clinical attention directed towards prevention, due to its strong association with premature morbidity and mortality (19, 24). In particular, these risk factors predispose the individual to greater risk for developing CVD and T2D. Evidence shows that the development of the MetS begins early in life and persistence from childhood to adolescent/adult life (25). The symptoms of the MetS are not necessarily manifestations of age, but develop over a predisposed background established at a young age (26). This is a dangerous predisposition, with trends in modern diet and habit likely influencing health and behaviour in increasingly younger populations (6). There is growing concern that the current excessive consumption of fructose may pose a great health risk. In particular, fructose may play an important role in the development of the metabolic syndrome (27). On the other hand, data from higher-quality scientific studies from our lab, including systematic reviews and meta-analyses of randomized controlled trials and individual controlled trials, have yielded contrasting results. These studies demonstrate that fructose do not have any adverse effects when tested in isocaloric exchange for other carbohydrates; and most adverse effects, it seems, appear under conditions of hypercaloric exchange (28-34).
1.3 Dietary Sources of Fructose

Fructose exists naturally in many foods, such as fruits and vegetables. Small amounts of fructose can also be synthesized within the human body by the conversion of sorbitol by aldose reductase (E.C. 1.1.1.21). Sorbitol is found in a variety of natural fruits. Manufactured sorbitol is commonly used as a sweetener and emulsifier in a variety of food (35). However, the majority of dietary fructose comes from two sweeteners, sucrose and high fructose corn syrup (HFCS), which are commonly used in processed foods and beverages. Sucrose, a disaccharide of glucose and fructose, is manufactured from sugar cane and sugar beet. In the small intestine sucrose is hydrolyzed by sucrase (EC 3.2.1.48) and it releases equal amounts of glucose and fructose (36). HFCS is similar to sucrose in terms of calories and sweetness; however; it is made by enzymatic isomerization of glucose to fructose from cornstarch, and it provides a direct source of free fructose (37, 38). Marriott et al in 2009 summarized the most important sources of fructose in the diet. According to this paper across all gender and age groups, the highest mean percentage of added fructose intake was from nonalcoholic beverages and grain products. For naturally occurring fructose, the predominant dietary sources were fruits and fruit products. For total fructose, nonalcoholic beverages and grain products were the predominant dietary sources, overall (39).

1.4 Consumption of Fructose

Figure 1-1 shows USDA Economic Research Service per capita availability trends for sucrose and HFCS (40). Sucrose consumption increased 40% between 1910 and 1921, but then remained relatively constant for >50 y. In 1960s, HFCS was introduced to the food industry and as an alternative to sucrose, it rapidly gained market share over the next two decades at the expense of sucrose, replacing almost half of it on a nearly 1:1 basis (7, 40, 41).
Apparent consumption of added sugars, defined as sugars or syrups added to foods during processing or preparation, increased by 27% during the 30-year period from 1970 to 1999; however, the subsequent substantial decline reversed much of that gain. Therefore, the net increase in energy from added sugars over the past 4 decades was approximately 29 kcal/d (42). On the other hand, the overall increases have also not been seen in other jurisdictions. For example, the intake of total and added sugars decreased in Canada over the last 40 years (43).

Figure 1-1 U.S. per capita consumption of sugars and sweeteners. Data obtained from USDA/Economic Research Service (40).

1.5 Assessment of Dietary Fructose

Measuring the food intake of subjects is notoriously difficult to do (30). The major difficulty in estimating dietary fructose intake for the analysis of metabolic effects of fructose is the lack of a precise assessment method. So far, typically epidemiological assessments (interviews, questionnaires or dietary records) were used, which are known to be prone to measurement errors such as intentional underreporting, subjective estimations instead of objective measurements or simple disremembering of foods (31). To avoid these limitations, the
use of a biomarker for the estimation of dietary fructose intake would be generally preferred (32), especially one that can differentiate between the various types of sugar. However, self-administered and inexpensive form of the diet history, the food frequency questionnaire (FFQ), has been the dietary assessment method often used in large-scale studies to provide a rapid estimate of usual intake (33). Often the accuracy of an FFQ is evaluated by comparing its performance with more intensive recording reference methods, such as weighed-food records, food diaries, or repeat 24-h recalls. The use of an FFQ has been shown to provide comparable estimates to other methods of dietary assessment, and is considered suitable for the purpose of assessing dietary intake in population-based studies of gene-diet interactions (34). It has been difficult to accurately calculate fructose intake for an individual from FFQ questionnaire because of the wide distribution of fructose in foods and limitations in the available data describing its content in the majority of food items (44). However, the Food Commodity Intake Databases (FCID), released by the U.S. Environmental Protection Agency (EPA) in 2000 (45) and the USDA National Nutrient Database for Standard Reference (SR20), published on the website of USDA Agricultural Research Service (ARS) (46) are usually used to document the fructose contents for fructose-containing food commodities (44, 47).

1.6 Structure of Fructose

Fructose (C$_6$H$_{12}$O$_6$) is a monosaccharide and an isomer of glucose. The structure of fructose differs from glucose at carbon 1 and 2 by the location of the carbonyl group. Fructose can form both five-membered (furanose) and six-membered (pyranose) rings. Nevertheless, fructose predominantly exists as a five-member ring (Figure 1-2). The pyranose form predominates in fructose free in solution, and the furanose form predominates in many fructose derivatives. There are two different forms of cyclic sugars, alpha and beta. In fructose, alpha and
beta refer to the hydroxyl groups attached to C-2, which is an asymmetric center. Alpha means that the hydroxyl group attached to C-2 is below the plane of the ring; β means that it is above the plane of the ring (48).

![Isomeric forms of fructose](image)

**Figure 1-2** Isomeric forms of fructose (48).

### 1.7 Absorption and Metabolism of Fructose

The normal roles of fructose in human metabolism are circumscribed by the amounts of fructose found in a meal, the time course of absorption from the gut, and the concentrations of these sugars and their metabolic intermediates found in different parts of the bloodstream throughout the body (36, 49-51). These parameters together with the sugar transporters, receptors and metabolic enzymes in tissues govern the fate of dietary fructose. Fructose enters the bloodstream more slowly than glucose and its levels are much lower, but they persist longer in the circulation (36, 49, 52). Fructose enters the cell from the intestinal lumen via facilitated
diffusion and is transported across the apical membrane of enterocytes by glucose transporter 2 (GLUT2/SLC2A2) and glucose transporter 5 (GLUT5/SLC2A5). Glucose transporter 5 appears to be a principal apical transporter of fructose due to its higher affinity for fructose, and its expression is upregulated by increased fructose intake (53). Fructose, glucose and galactose they all pass basolateral membrane of enterocyte and enter the bloodstream by glucose transporter 2. Studies have shown that, intake of fructose together with either glucose or galactose, increase the intestinal absorption of fructose, primarily through the increased utilization of d glucose transporter 2 (35, 52, 53).

The majority of ingested fructose is extracted at first pass in the liver where it is rapidly metabolized into fructose-1-phosphate (P) by the enzyme fructokinase (ketohexokinase, KHK), which is highly specific for fructose. KHK is characterized by a low $K_m$ for fructose [~0.5 mM] and a high $V_{max}$ (estimated at ~3 µmol/min per gram rat or human liver at 25°C) (54). Fructose-1-P is further cleaved into dihydroxyacetone phosphate and glyceraldehyde through the action of aldolase B (ALDOB). Triokinase converts glyceraldehyde into glyceraldehyde-3-phosphate. These metabolite and dihydroxyacetone phosphates are further metabolized in the glycolytic-gluconeogenic pathway which leads to production of Glycogen, glucose, lactate, and small amount of lipids (35, 55).

In addition to liver, extrahepatic fructose metabolism by fructokinase in kidney and small intestine can be expected to be small. Meanwhile, whitin the adipose tissue, fructose is broken down by a hexokinase into fructose-6-phosphate, which is also shunted into the glycolytic-gluconeogenic pathway (35, 55, 56). Fructose in the liver may also be metabolized by glucokinase; however, the $K_m$ for fructose is much higher than glucose, and hence minimal amounts of fructose are metabolized via this pathway (Figure 1-3) (55).
Fructokinase exists in two alternatively spliced isoforms consisting of fructokinase C (KHK-C) and fructokinase A (KHK-A), which differ in exon 3 (54, 57, 58). KHK-C is expressed primarily in the liver, kidney, and intestines, whereas KHK-A is more ubiquitous in body tissues (59). Although both KHK-C and KHK-A can metabolize fructose, KHK-C is considered the primary enzyme involved in fructose metabolism due to its lower $K_m$ (54, 60). Since KHK-A has a high $K_m$, it is not considered to be actively involved in fructose metabolism in non-hepatic tissues (55).

KHK has received much attention since it is uniquely different from other hexokinases such that it lacks feedback inhibition resulting in transient ATP depletion in the cell when fructose is consumed in excessive amounts. The mechanism is that KHK phosphorylates fructose to fructose-1-phosphate rapidly, resulting in marked ATP depletion. It has been proposed that the ATP depletion is associated with intracellular phosphate depletion and AMP generation, with stimulation of AMP deaminase and the stepwise degradation of AMP to purine products including uric acid (61, 62). Recent studies indicate that elevated serum uric acid levels may be an independent causal factor for the predisposition of hypertension, and metabolic syndrome. Thus, fructose-induced hyperuricemia may be a mechanism driving the development of the metabolic syndrome (27).

Moreover, it has been hypothesized that dietary fructose may also potentially lead to augmented $de$ $novo$ lipogenesis in the liver (63). Limited tracer studies have looked at the metabolic conversion from labeled fructose carbons to triglycerides. These studies revealed that only a small percent of fructose carbons enter the pathway of liponeogenesis after fructose ingestion. However, the hyperlipidemic effect of dietary fructose observed in some studies may involve other metabolic mechanisms and this could relate to energy source shifting and lipid
De novo lipogenesis may also occur in adipose tissue or muscles, but there are no adequate methods available to quantify it (49, 56, 64).

Figure 1-3 Fructose metabolism (49).

1.8 **Genetic Polymorphisms of Fructokinase**

Deficiency of fructokinase, an autosomal recessive inborn error of metabolism, results in essential fructosuria. This condition was first recognized in 1876 (60, 65). It is an anomaly rather than a disease, since it does not lead to any outward signs or symptoms. Most cases of fructosuria have been described in Jewish families (66). In a well-characterized family, in which three of eight siblings had fructosuria, all affected individuals were compound heterozygotes for the mutations Gly40Arg and Ala43Thr (60). This disorder has no metabolic or morbid manifestations other than having transient fructosuria after meals containing either sucrose or...
fructose. This condition used to be detected during routine medical examinations when tests based on reducing properties of glucose like Benedict’s solution and Clini test were used to diagnose diabetes. These tests have since been replaced by the more specific glucose oxidase method which does not react with fructose; therefore, patients with essential fructosuria are no longer being identified (66). Essential fructosuria has an estimated incidence of 1: 130,000 (65); however its incidence might be higher since, the absence of screening recommendations, and the lack of serum KHK assay makes it difficult to identify subjects with this anomaly (66).

Despite the interruption of the specific fructose pathway, up to 90% of the administered fructose is retained by fructokinase-deficient subjects; they only appear to excrete 10-20% percent of ingested fructose in the urine. It still remains unclear how fructosurics dispose of the 90% of a fructose bolus; it is assumed that fructose retained by fructosuric subjects is metabolized via fructose-6-phosphate in adipose tissue and skeletal muscle (35, 67). It is also plausible that KHK-A, which is more ubiquitous in non-hepatic tissues, remains preserved in fructosuric individuals. For instance, Ala43Thr mutation in fructosurics is unlikely to have a major effect on the activity of KHK-A at physiologic temperature. Therefore, the modest amounts of KHK-A in many tissues may be preserved, even though the much greater levels of KHK-C in central viscera are drastically reduced (60).

1.9  Genetic Polymorphisms of Aldolase B

Deficiency of ALDOB, an autosomal recessive metabolic disorder, results in Hereditary Fructose Intolerance (HFI). The B isoform of aldolase, the second enzyme of the fructose pathway, is critical for the metabolism of exogenous fructose by the liver, kidney, and intestine. Affected subjects suffer from gastrointestinal pain, vomiting, and severe hypoglycaemia after fructose ingestion; liver damage and growth retardation can occur in the most severe forms (68,
Many of the manifestations of HFI are attributable to the toxicity of non-degraded F-1-P. Because of the high activity and lack of feedback inhibition in fructokinase, intake of fructose results in accumulation of F-1-P and the trapping of phosphate. These results in inhibition of glucose production by blockage of complete fructose metabolism, induce a rapid drop in blood glucose and overutilization and diminished regeneration of ATP (69, 70). Over 40 different mutations associated with HFI have been described (71). Treatment consists of strict elimination of fructose, sucrose, and sorbitol from the diet immediately after HFI is suspected. This diet exclusion therapy allows for a rapid recovery of symptoms. The diagnosis of HFI is generally confirmed by intravenous fructose tolerance tests and assays of aldolase B activity in hepatic biopsies (72). The incidence of HFI is around 1/10,000 to 1/100,000 newborns and varies according to the ethnic group studied. However, it is believed that HFI is underdiagnosed because of the wide and nonspecific spectrum of symptoms (73-76).

1.10 Research Gap

The controversy has existed for the last 10 years regarding the potential harmfulness of excess fructose. Studies that associate fructose with increased risk of development of the metabolic syndrome are based on observations from excessive isolated fructose intake in animals and human subjects. These extreme experimental models that feature hyperdosing or significantly alter the usual dietary glucose-to-fructose ratio are not predictive of fructose effects in humans. Recent meta-analyses of controlled clinical trials from our lab do not show adverse effect of fructose in iso-caloric exchange for other carbohydrates; however, adverse effects are only observed when fructose provides extra calories. Accordingly, the adverse metabolic effects of fructose seem more attributable to excess energy than fructose itself. However, further research is needed to resolve the controversy surrounding fructose.
Chapter Two

Rationale, Hypothesis and Study Design

2.1 Rationale

Fructose metabolism is unique in a sense that it is not regulated, and excess fructose may result in intracellular ATP depletion, increased uric acid production, and increased lipogenesis. The initial phosphorylation of dietary fructose is largely catalyzed by fructokinase in the liver. In order to advance our understanding of the potential role of fructose as an important causal factor in the pathogenesis of several common health disorders, we investigated the impact of common single nucleotide polymorphisms (SNPs) of \( KHK \), and \( ALDOB \) on the increased susceptibility of developing adverse metabolic phenotypes. There are several common polymorphisms in each of these two genes, however, it is not known if modify the response to dietary fructose. Tag SNPs in the \( KHK \) gene with a Minor Allele Frequency (MAF) \( \geq 0.05 \) and in the \( ALDOB \) gene with a MAF \( \geq 0.15 \) were selected for examination.

KHK deficiency, characterized by the incomplete metabolism of fructose in the liver, may have some protection against the adverse effects of fructose by allowing some fructose metabolism in peripheral tissues. On the other hand, deficiency of ALDOB, the second enzyme in fructose metabolism, leads to partial metabolism of fructose in the liver. Accumulation of fructose-1-phosphate in the setting of diminished aldolase B function inhibits gluconeogenesis and glycogenolysis, causes overutilization and diminished regeneration of ATP, and impairs protein glycosylation.
2.2 **Hypothesis**

Variations in *KHK* and *ALDOB* genes are associated with differences in fructose intake and biomarkers of the Metabolic Syndrome.

2.3 **Objective**

To determine the association between variations in genes involved in fructose metabolism, *KHK* and *ALDOB*, and habitual fructose consumption. Moreover, to determine the association between *KHK*, and *ALDOB*, and biomarkers of the MetS (waist circumference, blood pressure, triglyceride, HDL and blood glucose). Also, to examine whether variations in *KHK* or *ALDOB* modify the association between fructose intake and biomarkers of the MetS.
Chapter Three

Genetic Variations in KHK and ALDOB, and Habitual Fructose Consumption

3.1 Abstract

**Background:** Increased dietary fructose intake is thought to be associated with adverse metabolic risk. Genetic polymorphisms associated with fructose metabolism might explain some differences in individual fructose consumption patterns.

**Objective:** To determine the association between common variations in genes involved in fructose metabolism, Fructokinase (KHK) and Aldolase B (ALDOB), and habitual fructose consumption.

**Method:** We studied 1334 healthy young men and women, aged 20-29 years old from the Toronto Nutrigenomics and Health Study population. The study population consisted of Caucasian, East Asian, and South Asian individuals (n = 1334). Fasting blood was used for genotyping rs2119026 in KHK, and rs515313, rs578597, rs578770, rs1929480 and rs550915 in ALDOB. Dietary intake was estimated using a one-month semi-quantitative food frequency questionnaire.

**Results:** We found no association between the KHK and ALDOB polymorphisms and fructose intake.

**Conclusion:** The KHK and ALDOB common polymorphisms were not associated with fructose intake in TNHS population.
3.2 **Introduction**

Our food intake behaviors are shaped by both environmental and biological factors (77). Today’s obesogenic environment has been blamed in driving the obesity epidemic (78). On the other hand, it is known that individuals respond differently to modern sedentary lifestyle and high-energy diets (79). Therefore, there has been considerable interest in identifying genes, which predispose individuals to obesity and adverse metabolic phenotypes.

Fructose, a natural sugar found in many fruits, is consumed in significant amounts in Western diets. Concerns has been raised regarding the metabolic effect of fructose as high fructose intake have been shown to induce insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertriglyceridemia, and hypertension in animal models (80, 81). However, there is no evidence for similar effects in humans at realistic consumption patterns (10, 28-32). Indeed, adverse effects largely appear only when fructose is consumed in excessive amounts (28).

Various factors may influence an individual’s exposure and response to fructose. Previous studies from our lab have shown that individual’s sugar consumption pattern is partially determined by genetic variation in genes encoding a glucose transporter (GLUT2) (82), a dopamine receptor (DRD2) (83), and sweet taste receptor (TAS1R2) (84). Studies have also shown that genetic polymorphisms in genes involved in metabolisms of foods may impact the individual response to diet (85). Fructose metabolism in humans and animals occurs mainly in the liver, kidney, and small intestine (50). The predominance of liver, kidney, and small intestine in fructose metabolism is based on the presence of the three enzymes—fructokinase (KHK), aldolase type B (ALDOB), and triokinase—which convert fructose into intermediates of the
glycolytic–gluconeogenic pathway (35). Fructose is first phosphorylated to fructose-1-phosphate (F-1-P) by KHK. Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate, an intermediate in glycolysis, by ALDOB. Glyceraldehyde is then phosphorylated to glyceraldehyde 3-phosphate, a glycolytic intermediate, by triose kinase (51). KHK deficiency, known as essential fructosuria, is an anomaly rather than the disease, and does not lead to any outward sign or symptom (57, 67). On the other hand, ALDOB deficiency, known as hereditary fructose intolerance, a disorder requiring a lifetime fructose-restricted diet, is a serious condition in which affected subjects suffer from gastrointestinal pain, vomiting, and severe hypoglycaemia after fructose ingestion; liver damage and growth retardation can occur in the most severe forms (35, 86, 87).

This study examines two candidate genes involved in fructose metabolism. A variant in the KHK gene and five variants in the ALDOB gene were examined to determine whether common genetic variations in genes involved in fructose metabolism contribute to differences in consumption of fructose. This area of investigation may provide new insight into the physiological role of each gene in humans and identify individuals who may be genetically predisposed to consuming more/less foods containing fructose. Together this may progress the understanding of fructose and genes in their contribution to the pathophysiology of obesity and diabetes and may help researchers and clinicians assess and plan effective dietary strategies tailored to each individual. No published study to date has examined the role of genes involved in fructose metabolism on habitual fructose consumption.
3.3 Methods

3.3.1 Study Population

Subjects participating in the Toronto Nutrigenomics and Health study (TNHS) were used for the present investigation. The TNHS is a cross-sectional analysis that was approved by the Ethics Review Board of the University of Toronto. The TNHS recruited 1650 healthy Canadians between October 2004 and December 2010. All participants were between the ages of 20–29 years and were recruited from the University of Toronto campus. Subjects are representative of three major ethnic groups in Toronto: Caucasian, East Asian, and South Asians. Of the 1639 participants enrolled in the TNH study, we excluded 11 participants with no blood values and 3 participants with incomplete FFQ records or physical activity questionnaires. We excluded 125 participants who were likely to be under-reporters (<800 kcal/d) or over-reporters (>3500 kcal/d for women or >4000 kcal/d for men) on the FFQ. Also, those who follow diets that restrict carbohydrates, fat or protein (n=73) as well as smokers (n=92) were excluded from the analysis. The final number of participants used for the current study was 1334, consisting of both men (n=484) and women (n=850). Individuals in the study population corresponded to three self-reported ethnic backgrounds: Caucasians (n=626), East Asian (n=462) and South Asian (n=148).

3.3.2 Dietary Assessment

A 196-item Toronto-modified Willett food-frequency questionnaire (FFQ) was used to assess habitual dietary intake over the past month. Each subject was instructed on how to complete the FFQ using visual aids of portion sizes to improve the accuracy of self-reported food intake. Subject responses to each food item were converted to daily number of servings for each item.
The nutrient database that is used to assign grams of fructose per portion of each food is based on the United States Department of Agriculture’s Nutrient Database for Standard Reference, which is the source of 86% of non-zero nutrient data in Health Canada’s Canadian Nutrient Files. Average daily energy intake was calculated by dividing monthly energy intake by 30 days.

3.3.3 Anthropometrics and Energy Expenditure

Anthropometric measurements were recorded by a research assistant. Sociodemographic data and information on ethnicity were obtained by using a general health and lifestyle questionnaire (GHLQ). Subjects completed GHLQ, including a 196-item Toronto-modified Willett food frequency questionnaire (FFQ). Subjects indicated how many times in the past month they consumed a specified portion of each food or beverage, and responses were converted to average daily intake for each item. Moreover, the GHLQ included questions about physical activity, special diets, medication, dietary supplements, age, sex, education, place of birth, and ethnocultural group. Subjects self-reported their physical activity in the GHLQ by estimating the amount of time they spent sleeping and engaging in light, moderate, and vigorous activity. Values were subsequently converted into metabolic equivalent (MET) levels. One MET is equal to 1 kcal expended per kg body weight per hour sitting at rest (88). Subjects also provided their smoking history. Anthropometric measurements including height, weight, waist circumference, and blood pressure were also measured and recorded by trained personnel.

3.3.4 Genotyping

Tag SNPs in the KHK and ALDOB genes were selected for examination with the Haploview Tagger software using the 1000 genome project under the following parameters: gene
boundaries (KHK position 2, 27309611-27323619) and (ALDOB position 9, 104182842-104198062), pairwise comparisons >500-kb pairs apart were ignored. KHK SNPs with a Minor Allele Frequency (MAF) < 0.05, and ALDOB SNPs with a MAF < 0.15 were excluded. Blood samples were processed at the Clinical Genomics Centre at Princess Margaret Hospital, University Health Network and were genotyped for Four KHK SNPs: rs2119026, rs7573066, rs2075862, rs62130544 and five ALDOB SNPs: rs515313, rs578597, rs578770, rs1929480 and rs550915. Genotyping were completed for each subject using the iPLEX Gold assay with MS-based detection (Sequenom MassARRAY platform; Sequenom, Inc). All the KHK genotypes except rs2119026 had MAF < 0.05 in TNHS population and were excluded from the analyses. Two of the ALDOB SNPs: rs578770 and rs1929480 were in linkage disequilibrium in TNHS population (0.98), therefore, only the results of the rs1929480 SNP is presented in this paper.

3.4 Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC). The GLM procedure in SAS was used to perform a one-way analysis of variance to test for differences in the characteristics between genotypes. \( \chi^2 \) test was used to analyze categorical variables. Non-normally distributed variables (BMI, and fructose intake) were loge-transformed for analysis, and the median and interquartile range values for this variable are given. Analyses of fructose intake were adjusted for age, sex, BMI, physical activity and calorie intake. The results were stratified to look for differences between ethno-cultural groups. Departure of genotype distributions from Hardy-Weinberg equilibrium was assessed using a \( \chi^2 \) test with one degree of freedom using R software. Significant P values are two-sided and \( \leq 0.05 \).
3.5 Results

Genotype frequencies, allele frequencies and HWE for the KHK SNP and ALDOB SNPs are presented in Table 3-1 for the total population and stratified by Caucasian, East Asian, South Asian, and other ethno-cultural groups.

Subject characteristics by KHK genotypes are presented in Table 3-2, and subject characteristics by ALDOB genotypes are reported in Table 3-3. In South Asians, there were significant associations between the KHK genotypes and age (P=0.005), and the ALDOB rs515313 genotypes and physical activity (P=0.02). There were no other significant differences in subject characteristics by the KHK genotypes or the ALDOB genotypes.

The median and interquartile range values for fructose intake are given in Table 3-4, 3-5. There were no significant effects of either genotype on fructose intake. In the KHK rs2119026, the p-value for the association of the KHK genotypes and fructose intake was p=0.088. Carriers of the ALDOB rs578597 had significantly higher fructose intake compared to the TT homozygotes (p = 0.05), although the difference was small and not likely to be biologically significant in this population.

The frequency of the KHK and ALDOB genotypes by fructose consumption category were tested (data not shown). There was no association between the KHK and ALDOB genotypes and fructose consumption category in the entire sample. Moreover, polymorphisms in the KHK and ALDOB genes were tested in combination (data not shown). However, this resulted in lower statistical power and no differences were found between combined genotype groups.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (n %)</th>
<th>Allele Frequency (%)</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KHK</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2119026</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>1334 (100)</td>
<td>0.64</td>
<td>0.36</td>
</tr>
<tr>
<td>Caucasian</td>
<td>626 (100)</td>
<td>0.58</td>
<td>0.42</td>
</tr>
<tr>
<td>East Asian</td>
<td>462 (100)</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>South Asian</td>
<td>148 (100)</td>
<td>0.68</td>
<td>0.32</td>
</tr>
<tr>
<td>Others</td>
<td>98 (100)</td>
<td>0.48</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>ALDOB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs515313</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>1334 (100)</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>Caucasian</td>
<td>626 (100)</td>
<td>0.70</td>
<td>0.30</td>
</tr>
<tr>
<td>East Asian</td>
<td>462 (100)</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td>South Asian</td>
<td>148 (100)</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td>Others</td>
<td>98 (100)</td>
<td>0.66</td>
<td>0.34</td>
</tr>
<tr>
<td>rs578597</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>1334 (100)</td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td>Caucasian</td>
<td>626 (100)</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>East Asian</td>
<td>462 (100)</td>
<td>0.56</td>
<td>0.44</td>
</tr>
<tr>
<td>South Asian</td>
<td>148 (100)</td>
<td>0.41</td>
<td>0.59</td>
</tr>
<tr>
<td>Others</td>
<td>98 (100)</td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td>rs1929480</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>1334 (100)</td>
<td>0.77</td>
<td>0.23</td>
</tr>
<tr>
<td>Caucasian</td>
<td>626 (100)</td>
<td>0.63</td>
<td>0.47</td>
</tr>
<tr>
<td>East Asian</td>
<td>462 (100)</td>
<td>0.94</td>
<td>0.06</td>
</tr>
<tr>
<td>South Asian</td>
<td>148 (100)</td>
<td>0.77</td>
<td>0.23</td>
</tr>
<tr>
<td>Others</td>
<td>98 (100)</td>
<td>0.82</td>
<td>0.18</td>
</tr>
<tr>
<td>rs550915</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population</td>
<td>1192 (100)</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td>Caucasian</td>
<td>568 (100)</td>
<td>0.84</td>
<td>0.16</td>
</tr>
<tr>
<td>East Asian</td>
<td>400 (100)</td>
<td>0.99</td>
<td>0.01</td>
</tr>
<tr>
<td>South Asian</td>
<td>135 (100)</td>
<td>0.77</td>
<td>0.23</td>
</tr>
<tr>
<td>Others</td>
<td>89 (100)</td>
<td>0.91</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Subjects were classified based on self-identified ethno-cultural group. HWE was assessed using R software.
Table 3-2 Subject Characteristics by *KHK* genotypes.

<table>
<thead>
<tr>
<th></th>
<th>AA (n=203)</th>
<th>AG (n=317)</th>
<th>GG (n=106)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose Intake (g/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>23.1±0.2</td>
<td>23.4±0.1</td>
<td>23.1±0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Female (%)</td>
<td>128 (63)</td>
<td>220 (69)</td>
<td>77 (73)</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
<td>22.6±4.6</td>
<td>22.8±3.8</td>
<td>22.6±3.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Physical Activity (MET.hrs/week)</td>
<td>8.3±0.2</td>
<td>8.1±0.2</td>
<td>8.3±0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>1993±46</td>
<td>2098±37</td>
<td>2082±63</td>
<td>0.2</td>
</tr>
<tr>
<td>East Asians</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>22.2±0.1</td>
<td>22.0±0.2</td>
<td>22.3±0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Female (%)</td>
<td>199 (75)</td>
<td>115 (69)</td>
<td>21 (70)</td>
<td>0.4</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
<td>21.4±3.5</td>
<td>21.3±3.5</td>
<td>22.1±3.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Physical Activity (MET.hrs/week)</td>
<td>7.1±0.2</td>
<td>6.7±0.2</td>
<td>6.8±0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>1857±39</td>
<td>1885±49</td>
<td>1897±115</td>
<td>0.9</td>
</tr>
<tr>
<td>South Asians</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>21.9±0.3(^a)</td>
<td>22.3±0.3(^b)</td>
<td>24.4±0.7(^b)</td>
<td>0.005</td>
</tr>
<tr>
<td>Female (%)</td>
<td>39 (59)</td>
<td>44 (64)</td>
<td>7 (54)</td>
<td>0.7</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
<td>22.3±3.9</td>
<td>22.8±5.5</td>
<td>22.6±5.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Physical Activity (MET.hrs/week)</td>
<td>8.2±0.4</td>
<td>7.5±0.4</td>
<td>7.6±0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>1958±81</td>
<td>1835±79</td>
<td>1787±182</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Values shown are mean ± SE for normally distributed continuous variables, *median ± quartile range for continuous variables that are not normally distributed and n (%) for categorical variables. Differences between KHK groups were compared using an analysis of variance for continuous variables, and a Pearson chi-square test for categorical variables. Analyses are adjusted for age, sex, BMI, and physical activity. Means with different letters are significantly different following a Tukey correction (P<0.05).
Table 3-3 Subject Characteristics by ALDOB genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Caucasians</th>
<th>East Asians</th>
<th>South Asians</th>
<th>Other</th>
<th>(rs515313)</th>
<th>(rs578597)</th>
<th>(rs1929480)</th>
<th>(rs550915)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=306)</td>
<td>(n=220)</td>
<td>(n=43)</td>
<td>(n=66)</td>
<td>(n=223)</td>
<td>(n=235)</td>
<td>(n=235)</td>
<td>(n=235)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>23.2±0.2</td>
<td>22.3±0.1</td>
<td>22.1±0.2</td>
<td>22.2±0.2</td>
<td>23.2±0.2</td>
<td>23.3±0.2</td>
<td>23.3±0.2</td>
<td>23.3±0.2</td>
</tr>
<tr>
<td>Female (%)</td>
<td>215 (70)</td>
<td>156 (34)</td>
<td>107 (72)</td>
<td>100 (58)</td>
<td>151 (68)</td>
<td>163 (74)</td>
<td>169 (65)</td>
<td>187 (64)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8±3.9</td>
<td>21.7±3.4</td>
<td>21.2±3.5</td>
<td>20.9±3.8</td>
<td>22.7±3.9</td>
<td>21.7±3.5</td>
<td>20.9±3.5</td>
<td>22.6±3.9</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>8.3±0.2</td>
<td>6.9±0.2</td>
<td>7.3±0.2</td>
<td>7.0±0.0</td>
<td>8.1±0.2</td>
<td>6.7±0.2</td>
<td>6.7±0.3</td>
<td>8.1±0.2</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>2039±37</td>
<td>2076±41</td>
<td>2073±44</td>
<td>2040±43</td>
<td>2075±50</td>
<td>2040±38</td>
<td>2059±70</td>
<td>2060±33</td>
</tr>
<tr>
<td>Female (%)</td>
<td>86 (29)</td>
<td>97 (37)</td>
<td>107 (72)</td>
<td>106 (58)</td>
<td>107 (68)</td>
<td>106 (74)</td>
<td>109 (65)</td>
<td>121 (69)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4±6.2</td>
<td>23.4±6.2</td>
<td>23.6±6.2</td>
<td>23.8±6.2</td>
<td>23.4±6.2</td>
<td>23.6±6.2</td>
<td>23.8±6.2</td>
<td>23.8±6.2</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>8.2±0.4</td>
<td>7.1±0.4</td>
<td>7.2±0.4</td>
<td>7.1±0.4</td>
<td>8.1±0.4</td>
<td>7.7±0.4</td>
<td>7.7±0.4</td>
<td>8.1±0.4</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>1789±100</td>
<td>1890±81</td>
<td>1885±75</td>
<td>1895±105</td>
<td>1767±110</td>
<td>1895±93</td>
<td>1873±83</td>
<td>1896±69</td>
</tr>
</tbody>
</table>

Values shown are mean ± SE for normally distributed continuous variables, *median ± quartile range for continuous variables that are not normally distributed and n (%) for categorical variables. Differences between ALDOB groups were compared using an analysis of variance and a Pearson chi-square test for categorical variables. Analyses are adjusted for age, sex, BMI, and physical activity. Means with different letters are significantly different following a Tukey correction (P<0.05).
Table 3-4 Fructose Intake by $KHK$ genotypes.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>AA (n=203)</th>
<th>AG (n=317)</th>
<th>GG (n=106)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>24.2±15.9</td>
<td>26.7±17.0</td>
<td>27.3±20.1</td>
<td>0.088</td>
</tr>
<tr>
<td>East Asians</td>
<td>20.8±14.8</td>
<td>19.3±14.0</td>
<td>23.2±20.3</td>
<td>0.6</td>
</tr>
<tr>
<td>South Asians</td>
<td>23.2±18.2</td>
<td>20.4±15.4</td>
<td>22.4±11.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Fructose intake is not normally distributed. Values shown are median ± quartile range. Differences between KHK groups were compared using an analysis of variance. Analyses are adjusted for age, sex, BMI, calorie, and physical activity. Medians with different letters are significantly different following a Tukey correction (P<0.05).
Table 3-5 Fructose Intake by ALDOB genotype.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>(rs515313)</th>
<th>(rs578597)</th>
<th>(rs1929480)</th>
<th>(rs550915)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>P</td>
</tr>
<tr>
<td>Caucasians</td>
<td>(n=306)</td>
<td>(n=259)</td>
<td>(n=61)</td>
<td>0.5</td>
</tr>
<tr>
<td>Fructose Intake (g/day)</td>
<td>24.6±17.9</td>
<td>25.5±18.9</td>
<td>24.9±18.4</td>
<td>24.9±18.3</td>
</tr>
<tr>
<td>East Asians</td>
<td>(n=124)</td>
<td>(n=220)</td>
<td>(n=118)</td>
<td>0.7</td>
</tr>
<tr>
<td>Fructose Intake (g/day)</td>
<td>20.8±14.9</td>
<td>19.1±16.7</td>
<td>19.5±14.0</td>
<td>20.6±13.9</td>
</tr>
<tr>
<td>South Asians</td>
<td>(n=43)</td>
<td>(n=66)</td>
<td>(n=39)</td>
<td>0.3</td>
</tr>
<tr>
<td>Fructose Intake (g/day)</td>
<td>20.2±13.6</td>
<td>22.6±17.3</td>
<td>25.2±19.8</td>
<td>24.6±14.3</td>
</tr>
</tbody>
</table>

Fructose intake is not normally distributed. Values shown are median ± quartile range. Differences between ALDOB groups were compared using an analysis of variance. Analyses are adjusted for age, sex, BMI, calorie, and physical activity. Medians with different letters are significantly different following a Tukey correction (P<0.05).
3.6 Discussion

The objective of this study was to determine whether polymorphisms in \textit{KHK} and \textit{ALDOB} are associated with habitual fructose consumption. Fructose occurs naturally, along with similar amounts of glucose, in many fruits and vegetables and in the sweetener high-fructose corn syrup (HFCS). The prevalence of obesity and diabetes has been increased worldwide during the past three decades. Some investigators have suggested a causal role of fructose intake in the etiology of the global obesity and diabetes (89). However, various factors may influence an individual’s exposure and response to fructose. Studies have shown that genetic polymorphisms in genes involved in metabolisms of foods may impact the individual response to diet (85). Previous studies from our lab have shown that genetic variation in genes encoding a glucose transporter (\textit{GLUT2}) (82), a dopamine receptor (\textit{DRD2}) (83), and sweet taste receptor (\textit{TAS1R2}) (84) are associated with habitual consumption of sugars.

In the present study, we found no association between the \textit{KHK} and \textit{ALDOB} common polymorphisms and intake of dietary fructose. Previous study has been showed that subject with hereditary fructose intolerance have lower dietary sucrose consumption pattern (90). There is currently no previous published data on the role of \textit{KHK} gene and fructose consumption behaviors. One study on the \textit{KHK} gene found no association between \textit{KHK} genetic variations and metabolic phenotypes (91) whereas the association between the \textit{KHK} polymorphisms and fructose intake has not been explored in the study.

It is recommended that individuals with the \textit{ALDOB} deficiency undergo dietary restriction of fructose, sucrose, and sorbitol to prevent primary manifestations of HFI (92). However, we found no associations between \textit{ALDOB} polymorphisms in SNPs investigated in
this study and fructose intake. While the potential functional consequences of these polymorphisms is not clear, literature has been showed that a large number of children and adults likely are living undiagnosed in the general population (87). Moreover, we hypothesized that people may naturally modulate their fructose intake based on the activity of their fructose metabolism enzymes. However, we found no significant associations between the KHK and ALDOB genotypes and fructose intake.

The present study had a number of limitations. The method of SNP selection excluded any SNPs that occurred at a low frequency within the population (≤ 5% MAF for KHK, and ≤ 15% MAF for ALDOB). It is possible that an SNP that is less common may affect the fructose consumption behavior to a greater extent than those identified here. As such, additional studies that look at the effect of a greater number of variants in KHK and ALDOB on fructose intake are required. Moreover, the use of a FFQ can introduce error or recall bias by asking the participants to remember and estimate the amount and type of foods they have consumed. However, our study population was comprised on young adults in good health, with no known reason to have impaired ability to recall food intake. Also, any errors in estimating fructose intake in this study would have been non-differentially distributed between genotypes and would only have attenuated our ability to detect differences. Finally, although the analyses of this study adjusted for a number of potentially important confounders, the observational design of these studies precludes the inference of causation due to the possibility of residual confounders that remain unaccounted.

In conclusion, we found that polymorphisms in KHK and ALDOB are not associated with biomarkers of the metabolic syndrome in a young, healthy population. Replication and further
investigation will be needed to confirm these findings in other populations and to identify the specific role each of these polymorphisms play in fructose’S effects.
Chapter Four

Genetic Variations in KHK and ALDOB, and Biomarkers of the Metabolic Syndrome

4.1 Abstract

**Background:** In the absence of consistent clinical evidence, concerns have been raised regarding the potential role of fructose in the development of the Metabolic Syndrome (MetS).

**Objective:** To determine the association between variations in genes involved in fructose metabolism (Fructokinase, and Aldolase B) and biomarkers of the MetS (waist circumference, blood pressure, triglyceride, HDL and blood glucose). We also examined whether variations in KHK or ALDOB modify the association between fructose intake and biomarkers of the MetS.

**Method:** We studied 1353 healthy young men and women, aged 20-29 years old from the Toronto Nutrigenomics and Health Study population. Fasting blood was used for genotyping rs2119026 in KHK, and rs515313, rs578597, rs578770, rs1929480 and rs550915 in ALDOB, and biomarker measurements. Dietary intake was estimated using a one-month semi-quantitative food frequency questionnaire.

**Results:** We found no association between rs2119026 in KHK and biomarkers of the MS. In ALDOB gene (rs515313), South Asians with the TT genotype had a mean triglyceride level of 1.11 ± 0.06 mmol/L, which was significantly greater (p=0.01) than that of the CC group (0.82 ± 0.06 mmol/L). Also, a significant diet-gene interaction was observed for the ALDOB polymorphism (p = 0.04) and fructose intake on serum triglyceride levels. Moreover, in East
Asians, there was a significant trend toward reduced HDL levels in the GG homozygotes in ALDOB rs1929480 (p=0.05).

**Conclusion:** Our findings suggest that *KHK* gene does not modify the associations between biomarkers of the MS. However, the *ALDOB* (rs1929480) polymorphism is associated with reduced HDL-cholesterol in East Asians. Also, variation in the *ALDOB* gene (rs515313) appears to mediate the triglyceride response to fructose consumption in South Asians.

### 4.2 Introduction

Fructose-containing sugars are a focus of attention as a public health target for the development of obesity (93), cardio-metabolic disease (5) including metabolic syndrome (15) and diabetes (94). Fructose, a component of added sugars such as sucrose and high fructose corn syrup, is considered to be the primary driver for the harms of sugars due to its unique metabolic and endocrine response. However, there are misrepresentation of the data by placing undue emphasis on Low-quality ecologic studies (7-9), animal models of overfeeding at levels of exposure far beyond actual fructose intake (10), and select human interventions with methodological flaws (11), assessed in isolation. It also ignored important biological mechanisms by which fructose may assist in the metabolic handling of glucose (95). On the other hand, a series of carefully conducted systematic reviews and meta-analyses from our lab on the effect of fructose on cardio-metabolic risk factors have suggested that fructose only has adverse effects on body weight, postprandial triglycerides, glycemic control, uric acid, and markers of nonalcoholic fatty liver disease insofar as it contributes to excess calories (28-34).

Genetic mutations in, *KHK*, and *ALDOB* genes, involved in fructose metabolism, can cause known, but rare, inherited disorders in humans (57, 67, 87). Mutations in *KHK* gene can
lead to essential fructosuria which is a benign, asymptomatic metabolic disorder caused by the lower activity of KHK, resulting in the ineffective breakdown of fructose (67). Defects in ALDOB can cause hereditary fructosuria, which results in partial breakdown of fructose-1 phosphate (87). These rare genetic defects highlight the role of these genes and their encoded proteins on the regulation of fructose levels. Therefore, because of the role of KHK and ALDOB in fructose metabolism, common polymorphisms in these genes can potentially lead to inter-individual variability in fructose metabolism and the development of fructose-induced adverse metabolic effects.

This study examines a variant in the KHK gene and five variants in the ALDOB gene to determine whether common genetic variations in genes involved in fructose metabolism are associated with biomarkers of the MetS. In addition, we investigated whether these polymorphisms modify the associations between fructose intake and biomarkers of the MetS.

4.3 Methods

4.3.1 Study Population

Subjects participating in the Toronto Nutrigenomics and Health study (TNHS) were used for the present investigation. The TNHS is a cross-sectional analysis that was approved by the Ethics Review Board of the University of Toronto. The TNHS recruited 1650 healthy Canadians between October 2004 and December 2010. All participants were between the ages of 20–29 years and were recruited from the University of Toronto campus. Subjects are representative of three major ethnic groups in Toronto: Caucasian, East Asian, and South Asians. Of the 1639 participants enrolled in the TNH study, we excluded 11 participants with no blood values and 3 participants with incomplete FFQ records or physical activity questionnaires. We excluded 125
participants who were likely to be under-reporters (<800 kcal/d) or over-reporters (>3500 kcal/d for women or >4000 kcal/d for men) on the FFQ. Also, those who follow diets that restrict carbohydrates, fat or protein (n=73) as well as smokers (n=92) were excluded from the analysis. The final number of participants used for the current study was 1334, consisting of both men (n=484) and women (n=850). Individuals in the study population corresponded to three self-reported ethnic backgrounds: Caucasians (n=626), East Asian (n=462) and South Asian (n=148).

4.3.2 Dietary Assessment

A 196-item Toronto-modified Willett food-frequency questionnaire (FFQ) was used to assess habitual dietary intake over the past month. Each subject was instructed on how to complete the FFQ using visual aids of portion sizes to improve the accuracy of self-reported food intake. Subject responses to each food item were converted to daily number of servings for each item.

The nutrient database that is used to assign grams of fructose per portion of each food is based on the United States Department of Agriculture’s Nutrient Database for Standard Reference, which is the source of 86% of non-zero nutrient data in Health Canada’s Canadian Nutrient Files. Average daily energy intake was calculated by dividing monthly energy intake by 30 days.

4.3.3 Anthropometrics and Energy Expenditure

Anthropometric measurements were recorded by a research assistant. Sociodemographic data and information on ethnicity were obtained by using a general health and lifestyle questionnaire (GHLQ). Subjects completed GHLQ, including a 196-item Toronto-modified Willett food frequency questionnaire (FFQ). Subjects indicated how many times in the past
month they consumed a specified portion of each food or beverage, and responses were converted to average daily intake for each item. Moreover, the GHLQ included questions about physical activity, special diets, medication, dietary supplements, age, sex, education, place of birth, and ethnocultural group. Subjects self-reported their physical activity in the GHLQ by estimating the amount of time they spent sleeping and engaging in light, moderate, and vigorous activity. Values were subsequently converted into metabolic equivalent (MET) levels. One MET is equal to 1 kcal expended per kg body weight per hour sitting at rest (88). Subjects also provided their smoking history. Anthropometric measurements including height, weight, waist circumference, and blood pressure were also measured and recorded by trained personnel.

3.3.3 Clinical measurements

Overnight 12-hour fasting blood samples were collected to measure serum biomarkers of the metabolic syndrome including triglycerides, glucose, and HDL cholesterol, as described previously (96).

4.3.4 Genotyping

Tag SNPs in the *KHK* and *ALDOB* genes were selected for examination with the Haploview Tagger software using the 1000 genome project under the following parameters: gene boundaries (*KHK* position 2, 27309611-27323619) and (*ALDOB* position 9, 104182842-104198062), pairwise comparisons >500-kb pairs apart were ignored. *KHK* SNPs with a Minor Allele Frequency (MAF) < 0.05, and *ALDOB* SNPs with a MAF < 0.15 were excluded. Blood samples were processed at the Clinical Genomics Centre at Princess Margaret Hospital, University Health Network and were genotyped for Four *KHK* SNPs: rs2119026, rs7573066, rs2075862, rs62130544 and five *ALDOB* SNPs: rs515313, rs578597, rs578770, rs1929480 and
rs550915. Genotyping were completed for each subject using the iPLEX Gold assay with MS-based detection (Sequenom MassARRAY platform; Sequenom, Inc).

3.4 Statistical analysis

All statistical analyses were carried out with the use of SAS version 9.4 (SAS Institute, Inc.). To improve normality, nonnormally distributed continuous variables were loge-transformed before analysis and the median and interquartile range values for these variables are given with P values obtained from models using transformed variables. The GLM procedure in SAS was used to perform a one-way analysis of variance to test for differences in the characteristics between genotypes. $\chi^2$ test was used to analyze categorical variables. Analyses were adjusted for age, sex, BMI and physical activity. The results were stratified to look for differences between ethno-cultural groups. Departure of genotype distributions from Hardy-Weinberg equilibrium was assessed using a $\chi^2$ test with one degree of freedom using R software. Significant P values are two-sided and ≤ 0.05. Tukey’s post-hoc test was used to correct for multiple comparisons when appropriate.

3.5 Results

As described in chapter 2, all the KHK genotypes except rs2119026 had MAF < 0.05 in TNHS population and were excluded from the analyses. Two of the ALDOB SNPs: rs578770 and rs1929480 were in linkage disequilibrium in TNHS population (0.98), therefore, only the results of the rs1929480 SNP are presented in this paper. Genotype frequencies, allele frequencies and HWE for the KHK SNP and ALDOB SNPs are presented in previous chapter in Table 3.1 for the total population and stratified by Caucasian, East Asian, South Asian, and other ethno-cultural groups.
Subject characteristics in relation to biomarkers of the metabolic syndrome in TNHS population are presented in Table 4-1. Subject characteristics by KHK genotypes are presented in previous chapter in Table 3-2, and subject characteristics by ALDOB genotypes are reported in Table 3-3. In South Asians, there were significant associations between the KHK genotypes and age (P=0.005), and the ALDOB rs515313 genotypes and physical activity (P=0.02). There were no other significant differences in subject characteristics by the KHK genotypes or the ALDOB genotypes.

Biomarkers of the MetS by KHK and ALDOB genotypes are presented in table 4-2, 4-3. There were no significant effects of KHK genotype on biomarkers of the metabolic syndrome. The ALDOB rs515313 polymorphism was associated with differences in triglyceride levels (p=0.004) in South Asians. Moreover, a significant diet–gene interaction was found between fructose intake and the ALDOB rs515313 polymorphism on serum triglyceride levels (p = 0.04) such that the elevated serum triglyceride levels are only observed in individuals who have higher average intake of fructose (figure 4-1). Carriers of the ALDOB rs1929480 in Caucasians had significantly higher blood pressure levels compared to the GG homozygotes (p = 0.04), although the difference was small and not likely to be biologically significant in this population. Also, in East Asians, there was a significant trend toward reduced HDL levels in the GG homozygotes in ALDOB rs1929480 (p=0.05). Although the effect was small and GG homozygotes group had a very small sample size (n=1). None of the other metabolic characteristics differed between ALDOB genotypes.
| Table 4-1 Subject characteristics in TNHS population. | Men  
(n = 416) | Women  
(n = 919) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.9 ± 0.1</td>
<td>22.6 ± 0.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.6 ± 0.3</td>
<td>163.2 ± 0.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.7 ± 0.6</td>
<td>59.7 ± 0.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.8 ± 0.2</td>
<td>22.4 ± 0.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>80.6 ± 0.4</td>
<td>71.1 ± 0.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124.0 ± 0.5</td>
<td>109.4 ± 0.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71.6 ± 0.4</td>
<td>68.3 ± 0.3</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.01 ± 0.03</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.31 ± 0.01</td>
<td>1.64 ± 0.01</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.93 ± 0.02</td>
<td>4.72 ± 0.01</td>
</tr>
</tbody>
</table>

Values shown as mean ± standard error for continuous variables.
<table>
<thead>
<tr>
<th></th>
<th>AA (n=203)</th>
<th>AG (n=317)</th>
<th>GG (n=106)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caucasians</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>75.10±11.0</td>
<td>73.94±10.0</td>
<td>73.8±9.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115.2±0.6</td>
<td>116.4±0.5</td>
<td>115.5±0.9</td>
<td>0.4</td>
</tr>
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<td>Diastolic blood pressure (mmHg)</td>
<td>69.0±0.5</td>
<td>69.8±0.4</td>
<td>69.9±0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.83±0.55</td>
<td>0.90±0.53</td>
<td>0.80±0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5±0.02</td>
<td>1.6±0.02</td>
<td>1.5±0.03</td>
<td>0.7</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.7±0.02</td>
<td>4.7±0.01</td>
<td>4.7±0.03</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>East Asians</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>69.3±8.9</td>
<td>69.4±10.1</td>
<td>0.7±9.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>111.0±0.5</td>
<td>110.9±0.7</td>
<td>113.4±1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.9±0.5</td>
<td>67.8±0.6</td>
<td>70.8±1.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.84±0.42</td>
<td>0.82±0.41</td>
<td>0.78±0.40</td>
<td>0.3</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5±0.02</td>
<td>1.6±0.03</td>
<td>1.5±0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8±0.02</td>
<td>4.8±0.03</td>
<td>4.8±0.06</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>South Asians</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>72.0±10.1</td>
<td>74.1±13.8</td>
<td>73.9±16.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>112.8±1.0</td>
<td>113.4±1.0</td>
<td>116.7±2.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>69.8±0.8</td>
<td>70.7±0.8</td>
<td>70.3±2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.78±0.39</td>
<td>0.80±0.43</td>
<td>0.89±0.28</td>
<td>0.9</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±0.04</td>
<td>1.4±0.03</td>
<td>1.3±0.08</td>
<td>0.1</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.9±0.05</td>
<td>4.9±0.04</td>
<td>5.1±0.11</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Values shown are mean ± SE for normally distributed continuous variables, *median ± quartile range for continuous variables that are not normally distributed and n (%) for categorical variables. Differences between KHK groups were compared using an analysis of variance for continuous variables, and a Pearson chi-square test for categorical variables. Means with different letters are significantly different following a Tukey correction (P<0.05).
Table 4-3 Biomarkers of the metabolic syndrome by ALDOB genotype.

<table>
<thead>
<tr>
<th></th>
<th>(rs515313)</th>
<th>(rs578597)</th>
<th>(rs1929480)</th>
<th>(rs550915)</th>
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<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>P</td>
</tr>
<tr>
<td>Caucasians</td>
<td>(n=306)</td>
<td>(n=259)</td>
<td>(n=61)</td>
<td>(n=223)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>73.9±0.8</td>
<td>73.1±1.0</td>
<td>75.2±1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115.5±0.5</td>
<td>116.2±0.6</td>
<td>116.3±1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>69.4±0.4</td>
<td>69.7±0.5</td>
<td>69.5±1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.87±0.5</td>
<td>0.84±0.5</td>
<td>0.87±0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.6±0.02</td>
<td>1.5±0.02</td>
<td>1.5±0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.7±0.02</td>
<td>4.7±0.02</td>
<td>4.8±0.04</td>
<td>0.7</td>
</tr>
<tr>
<td>East Asians</td>
<td>(n=124)</td>
<td>(n=220)</td>
<td>(n=66)</td>
<td>(n=39)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>68.7±7.7</td>
<td>68.1±10.0</td>
<td>69.9±9.4</td>
<td>0.7</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>111.5±0.6</td>
<td>111.2±0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.1±0.7</td>
<td>68.0±0.5</td>
<td>68.1±0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.84±0.5</td>
<td>0.81±0.4</td>
<td>0.84±0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
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<td>1.6±0.02</td>
<td>1.6±0.03</td>
<td>0.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8±0.03</td>
<td>4.8±0.02</td>
<td>4.8±0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>South Asians</td>
<td>(n=43)</td>
<td>(n=66)</td>
<td>(n=39)</td>
<td>(n=50)</td>
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<tr>
<td>Waist circumference (cm)</td>
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<td>71.8±12.4</td>
<td>75.2±12.2</td>
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<td>Systolic blood pressure (mmHg)</td>
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<td>Diastolic blood pressure (mmHg)</td>
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<td>70.3±0.9</td>
<td>70.8±1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.70±0.4</td>
<td>0.80±0.4</td>
<td>0.97±0.6</td>
<td>0.004</td>
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<tr>
<td>HDL (mmol/L)</td>
<td>1.4±0.04</td>
<td>1.3±0.04</td>
<td>1.4±0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.9±0.06</td>
<td>4.9±0.05</td>
<td>4.9±0.06</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Values shown are mean ± SE for normally distributed continuous variables, *median ± quartile range for continuous variables that are not normally distributed and n (%) for categorical variables. Differences between ALDOB groups were compared using an analysis of and a Pearson chi-square test for categorical variables. Analyses are adjusted for age, sex, BMI, and physical activity Means with different letters are significantly different following a Tukey correction (P<0.05).
**Figure 4-1**

*ALDOB* rs515313 polymorphism modifies the association between dietary fructose intake and serum triglyceride in South Asians. Values are means ± S.E.M. adjusted for age, sex, BMI, and physical activity.

*FI: Fructose Intake (g/day)*

*P = 0.04 for interaction*
4.4 Discussion

The objective of this study was to investigate whether polymorphisms in *KHK* and *ALDOB* were associated with differences in factors of the MetS and whether it modified the association between fructose intake and these factors. The rs515313 SNP in *ALDOB* was found to modify the association between dietary fructose and serum triglyceride levels. There was a positive association between dietary fructose and serum triglyceride levels in South Asians who were TT homozygous for the polymorphism, and consuming a high dietary fructose (more than 21.7 mg/day, which is a median in South Asians). While the potential functional mechanism of our findings is not clear, the literature suggests such phenotypic associations could be consistent with impaired function of the *ALDOB* enzyme. The literature reports that in patients with *ALDOB* deficiency, the concentration of fatty acids in circulation increases more than two times after fructose intake compared to healthy subjects (76, 97-99). This can explain the higher serum triglyceride levels observed in TT homozygotes group. Moreover, it is documented that subjects that their *ALDOB* enzyme is nonfunctional may develop fatty liver in response to dietary fructose although in these individuals fructose cannot be fully metabolized to triglycerides (100). This can be explain by the notion that fructose intake by subject with *ALDOB* deficiency, results in accumulation of fructose-1 phosphate and trapping of phosphate resulting in depletion of ATP and increased production of uric acid (75). Moreover, it has been shown that increased uric acid can stimulate fat synthesis in the hepatocyte (101).

High TG levels are highly correlated with CVD and are characteristic of T2D and insulin resistance (102). Therefore, it is of importance whether the results of this study are clinically relevant and how it might be used in a preventive and therapeutic context. There is not an ethnic-specific formulation of the lipid criteria in the metabolic syndrome. However, the adult thresholds used to define hypertriglyceridemia is triglyceride level ≥ 150 mg/dL or 1.69 mmol/L
In this study, South Asians with TT homozygotes genotype in $ALDOB$ rs515313 had serum triglyceride level of 1.16 mmol/L, which is still lower than the threshold used to define hypertriglyceridemia. It is also possible that the adult thresholds used to define hypertriglyceridemia are not set at the appropriate level to identify high risk for diabetes and cardiovascular disease in South Asians.

The results of this study showed that, in East Asians, there was a significant trend toward reduced HDL-cholesterol in the GG homozygotes in $ALDOB$ rs1929480 ($p=0.05$). According to literature, ALDOB has been linked to type 2 diabetes in Japanese population (104). Although polymorphisms investigated in this study failed to be significantly associated with glucose and other diabetes risk factors including HOMA-IR, HOMA-beta and fasting insulin (data not shown here); recent studies have implicated insulin-resistance and hypertriglyceridemia with the lowering of HDL levels, potentially through the increased metabolism of apoA-I, an essential component of HDL particles (105-108). Thus, while the potential functional mechanism of our findings is not clear, the literature suggests such phenotypic associations found in our study could be consistent with impaired function of the ALDOB enzyme. We found no association between $KHK$ gene and biomarkers of the metabolic syndrome. The results are consistent with previous study, which have looked at other polymorphisms in $KHK$ gene (91).

Current recommendations from the World Health Organization indicate that free sugars (the main source of dietary fructose) should be restricted to no more than 10% of total energy intake because of potentially adverse effects on obesity and dental caries. Moreover, the American Heart Association lists reduction in fructose-containing sugars as one potential mechanism for lowering triglycerides. Our results suggest that genotype may be an important factor with regards to the effects of fructose intake on triglyceride level and account, at least in part, for some of this individual variation regarding dietary fructose and triglyceride.
No previous study has looked at the effect of ALDOB polymorphisms on metabolic outcomes. Also, this is the first study that investigated whether KHK and ALDOB polymorphisms modify the associations between biomarkers of the MetS and fructose intake. The results of this study are consistent with the notion that un-metabolized fructose in subjects with KHK deficiency is innocuous as fructose is slowly being metabolized by non-hepatic tissue and some appear in the urine. On the other hand, partial metabolism of fructose in the liver in subject with lower activity of ALDOB seems to have adverse metabolic phenotypes. Moreover, we believe that ALDOB deficiency known as hereditary fructose intolerance is underdiagnosed because of the wide and nonspecific spectrum of symptoms (76).

However, due to the limited studies conducted on KHK and ALDOB genes, more intensive studies with more extensive coverage of SNPs are needed in larger populations to better characterize the impact of polymorphisms of KHK, and ALDOB on the development of adverse metabolic effects and increased disease. Also, consideration must be given to potential limitations in the design of this study. The cross-sectional study design precludes drawing any conclusions about causality based on the observed associations. In addition, residual confounders from unidentified factors may also have confounded the result presented here.

In summary, we found that polymorphisms in ALDOB affect factors related to the metabolic syndrome in a young, healthy population. The ALDOB rs1929480 polymorphism was associated with reduced HDL-cholesterol in East Asians, while the ALDOB rs1929480 polymorphism modified the relationship between dietary fructose and triglyceride level. Further studies are needed to confirm these results in other populations as well as to establish mechanisms by which they may be occurring.
Chapter Five

Overall Discussion

5.1 Overview

The overall objective of this thesis was to determine whether genetic polymorphisms in enzymes responsible for fructose metabolism (KHK rs2119026 and ALDOB rs515313, rs578597, rs578770, rs1929480, rs550915) modify the relationship between dietary fructose and biomarkers of the MetS.

Objective 1 (Chapter 2): To determine the association between variations in genes involved in fructose metabolism, Fructokinase and Aldolase B, and habitual fructose consumption.

Results: Findings show that there were not any association between KHK and ALDOB common polymorphisms and habitual consumption of fructose.

Objective 2 (Chapter 3): To determine the association between variations in genes involved in fructose metabolism, Fructokinase, and Aldolase B, and biomarkers of the MetS (waist circumference, blood pressure, triglyceride, HDL and blood glucose). Also to examine whether variations in KHK or ALDOB modify the association between fructose intake and biomarkers of the MetS.

Results: No association was observed between rs2119026 in KHK and biomarkers of the MS. In ALDOB gene (rs515313), South Asians with the TT genotype had a higher mean triglyceride level.
compared to the CC group. Also, a significant diet-gene interaction was observed for the *ALDOB* rs515313 polymorphism and fructose intake on serum triglyceride levels in South Asians. Moreover, in East Asians, there was a significant trend toward reduced HDL levels in the GG homozygotes in *ALDOB* rs1929480 (p=0.05).

5.2 Limitations

Consideration must be given to potential limitations in the design of this study. An inherent problem in nutritional epidemiology is the difficulty in assessing usual intake given that diet constantly changes (109). Due to the impracticality of measuring daily intakes over extended periods, different methods of dietary assessment have been developed including FFQs and food records. The use of a FFQ has been shown to provide comparable nutrient intake estimates to other methods of dietary assessment and is considered suitable for the purpose of assessing dietary intake in population-based studies of gene-diet interactions (110). The TNHS uses a 196-item, self-administered, semi-quantitative FFQ, modified from the Willet FFQ to assess habitual consumption over a one-month period. However, the use of a FFQ can introduce error or recall bias by asking the participants to remember and estimate the amount and type of foods they have consumed. However, our study population was compromised on young adults in good health, with no known reason to have impaired ability to recall food intake. FFQs are also limited on the number and types of foods listed in the questionnaire (111, 112). Some foods that could be regularly consumed by subjects, such as traditional foods from different ethno-cultural groups, may not be listed in the FFQ, which could result in an under-estimation of the total caloric and nutrient intake of the participant. Assessment of dietary intake over the past month may not take into account seasonal variation in food intake. Finally, fructose intake was not
specifically recorded as a variable in the FFQ, but it was calculated by combining the recorded intake of specific foods with their fructose content. Fructose intake may have underestimated in some subjects because of the social desirability of reporting consuming less foods containing added sugars such as sugar sweetened beverages.

However, any errors in estimating fructose intake in this study would have been non-differentially distributed between genotypes and would only have attenuated our ability to detect differences. Additional studies examining the association between genetic variation in the \textit{KHK} and \textit{ALDOB} gene region and dietary fructose intake may benefit from using multiple diet records, collected seasonally, over a one-year period.

The functional significance of polymorphisms in this project is not clear. Assessing the presence of fructose in the urine or the enzymes activity on liver biopsy would have provided information on the extent of fructose metabolism, which would be beneficial to include in future studies.

Analyses within specific ethnicity may be limited by small sample size of these groups. Alternatively, given the large number of independent tests conducted in this thesis, the observed associations may have been due to chance. We attempted to minimize this possibility by using Tukey’s post hoc test whenever necessary. While, we measured a comprehensive set of biomarkers of the metabolic syndrome, it is possible that \textit{KHK} and \textit{ALDOB} polymorphisms may be associated with other cardio-metabolic disease biomarkers that are not assessed in the present thesis. Furthermore, in addition to \textit{KHK} and \textit{ALDOB} gene, it is possible that variation in other genes along the fructose uptake and metabolism pathway may affect some of the relationships examined here. Residual confounders from unidentified factors may also have confounded the result presented here. Finally, the cross-sectional study design precludes drawing any conclusions about causality based on the observed associations.
5.3 **Future Directions**

Our findings suggest an interaction between fructose intake and the ALDOB rs515313 SNP on fasting serum triglyceride level, and an association between ALDOB rs1929480 and HDL-cholesterol. This would provide a good rationale for future studies to investigate potential causal mechanisms that may be occurring. Adequately powered longitudinal studies and clinical trials that examine how *KHK* and *ALDOB* polymorphisms modify the effects of fructose intake on biomarkers of the metabolic syndrome are needed. In addition, experimental studies conducted in cell culture and animal models are needed to better understand the mechanisms through which these polymorphisms affect mRNA expression and enzyme function. These studies would provide insight into the functional consequences of these polymorphisms in cells. It would also be beneficial to determine if the polymorphisms which affected MetS components and interacted with diet to affect metabolic biomarkers actually translated into differences in risk for these diseases. While the present thesis found that *KHK* polymorphism does not modify the association of fructose intake and biomarkers of the metabolic syndrome, larger studies are needed to examine the potential effects of other variants in *KHK* and biomarkers of the MetS.

5.4 **Implications**

This study integrated genetic techniques in a traditional nutrition epidemiology design in order to assess whether genotype affected the metabolic response to dietary fructose. A growing body of evidence suggests that individuals have different levels of susceptibility to obesity and diabetes, which are likely related to genetic factors.

The parallelism between the increase in the consumption of high fructose corn syrup and dietary fructose and the rise in obesity over the past 10-20 years, linked fructose to the rise in obesity and metabolic disorders. Moreover, some animal studies found that high fructose
consumption can induce insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertriglyceridemia, and hypertension. There is no evidence for similar effects in humans at realistic consumption patterns. This is consistent with the results of this study as it was shown that \textit{KHK} polymorphism is not associated with biomarkers of the metabolic syndrome. On the other hand, the potential partial metabolism of fructose by \textit{ALDOB} may lead to deleterious metabolic consequences.

Moreover, this study serves as an example of the complexity in treatment and control of metabolic disease, illustrating how the same dietary intake could result in different individual responses due to genetics.

### 5.5 Conclusion

This thesis investigated whether genetic variation in enzymes of fructose metabolism modify the association between dietary fructose and components of the MetS. Specifically, one polymorphisms in \textit{KHK} (rs2119026) and 5 variants in \textit{ALDOB} (rs515313, rs578597, rs578770, rs1929480 and rs550915) were investigated. The findings presented in this thesis suggest that \textit{ALDOB} may be involved in the etiology of the MetS as in East Asians the rs1929480 polymorphism in \textit{ALDOB} was associated with decreased HDL-cholesterol while the rs515313 SNP in \textit{ALDOB} modified the association between dietary fructose and serum triglyceride levels in South Asians. The rs2119026 polymorphism in \textit{KHK} did not affect any of the metabolic outcomes we measured. These results suggest that partial metabolism of fructose in the liver may affect an individual risk for the development of the MetS and that genetic variation in the \textit{ALDOB} gene affect how diet influences risk for this condition.


94. Lustig RH. Response to "Metabolic improvement with fructose restriction: Is it the fructose or the weight loss?". Obesity (Silver Spring). 2016;24(3):550.
