Diet and postprandial risk markers for complications in type 2 diabetes

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DIET AND POSTPRANDIAL RISK MARKERS FOR COMPLICATIONS IN TYPE 2 DIABETES

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Stockholm 2020
Diet and postprandial risk markers for complications in type 2 diabetes
Thesis for doctoral degree (Ph.D.)

The thesis will be defended in public in the Rolf Luft Auditorium, Karolinska University Hospital, Stockholm, Sweden.

Friday the 20th of March 2020 at 9 am

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To family and friends – with love
ABSTRACT

The period after a meal is complex with fluctuation in blood glucose, lipids and other metabolic responses. This may induce and/or increase inflammation and contribute to future development of diabetic complications. Prevention of complications include well-controlled disease management, including diet. There are however gaps in the literature for dietary recommendations in diabetes, and whether those with type 2 diabetes (T2D) make dietary changes.

The aims of this doctoral thesis were: 1) To study the acute effects of fructose loading on levels of serum uric acid, metabolic and inflammatory markers using isocaloric drinks; Coca-Cola (17.5 g fructose), blueberry drink (18 g fructose) and a pure fructose drink (35 g fructose), without and with a pizza. 3) To study the acute effect of meals with different compositions of high carbohydrate (HC) (52E%), HC & fibers (50E%, 15 g), low carbohydrate (LC, 32E%)+high fat (HF) (50E%) and LC (28E%)+high protein (HP) (41E%) on metabolic and inflammatory markers 4) Examine possible changes in fruits and vegetables consumption.

The effects of acute fructose loading on levels of serum uric acid were examined in T2D (n=7), chronic kidney disease (n=3) and healthy subjects (HS) (n=6). Serum uric acid increased over time following fructose loading. The highest response was observed following fructose drink, and the lowest following the blueberry drink (p<0.05). The effect of acute fructose loading on glucose, insulin and inflammatory markers were examined in T2D and HS. The response in glucose and insulin was greater following Coca-Cola (p<0.05). MCP-1 decreased in both groups following blueberry drink and Coca-Cola (T2D; p=0.02, HS; p=0.03), probably secondary to the insulin response. The results suggest that drinks with added fructose should be avoided, and that blueberry is protective on uric acid and glucose response.

The effect of meal composition on metabolic and inflammatory markers were examined in T2D and HS. HC meals induced the highest response in glucose and insulin, and LC+HF in triglycerides (p<0.05). The inflammatory marker VCAM-1 decreased following LC+HP meal (T2D; p=0.03, HS; p=0.003), while ICAM-1 decreased following LC+HF (p=0.02) in T2D and following LC+HP (p=0.03) in HS. PAI-1 decreased following HC (T2D; p=0.04, HS; p=0.006) and LC+HP (T2D; p=0.03, HS; p=0.01) and in T2D also following LC+HF (p=0.04). The responses did not differ between meals, probably due to the healthy composition of meals. Thus, LC meals with a healthy composition of fibers, vegetables, berries, mono and polyunsaturated fat and plant-based proteins could be recommended to subjects with T2D.

Possible changes in intake of fruits and vegetables consumption over time was explored in a prospective cohort of men using food frequency questionnaires in 1997 and 2009. 1 741 men developed T2D and 22 212 remained free from diabetes. Increased intake of fruit and vegetables was greater among those who developed T2D (1.6 servings/week, 95% CI 1.08; 2.03) compared to those remained free from diabetes (0.7 servings/week, 95% 0.54; 0.84). Although improvements in consumption were observed, only 36% of those with T2D consumed ≥5 servings per day in 2009. Thus, there is a need for nutritional education in T2D.
LIST OF SCIENTIFIC PAPERS


II. **C Olofsson**, M Eriksson, AC Bragfors-Helin, B Anderstam, N Orsini, P Stenvinkel, N Rajamand Ekberg. Effects of acute fructose loading on markers of inflammation. *Submitted manuscript*

III. **C Olofsson**, I-L- Andersson, O Torffvit, K Brismar, N Rajamand Ekberg. Effect of meal composition on metabolic and inflammatory markers in type 2 diabetes and healthy controls. *Manuscript*

### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
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<td>COSM</td>
<td>Cohort of Swedish Men</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DNL</td>
<td>De novo lipogenesis</td>
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<tr>
<td>DNSG-EASD</td>
<td>Diabetes and nutrition study group- European Association for the Study of Diabetes</td>
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<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
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<tr>
<td>FFQ</td>
<td>Food frequency questionnaire</td>
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<tr>
<td>kcal</td>
<td>Kilocalorie</td>
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<tr>
<td>HbA1c</td>
<td>Glycated haemoglobin</td>
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<tr>
<td>HC</td>
<td>High carbohydrate</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HF</td>
<td>High fat</td>
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<td>HP</td>
<td>High protein</td>
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<td>HS</td>
<td>Healthy subjects</td>
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<tr>
<td>hsCRP</td>
<td>High sensitivity C-reactive protein</td>
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<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
</tr>
<tr>
<td>ICD</td>
<td>International classification of diseases</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Insulin-like growth factor binding protein 1</td>
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<tr>
<td>IgG2</td>
<td>Immunoglobulin G2</td>
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<tr>
<td>IgG4</td>
<td>Immunoglobulin G4</td>
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<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
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<tr>
<td>IL-18</td>
<td>Interleukin 18</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
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LPS  Lipopolysaccharide
NDR  The Swedish National Diabetes Registry
NPR  National Patient Registry
MCP-1  Monocyte chemoattractant protein 1
MI  Myocardial infarction
NYHA  New York Heart Association
PAI-1  Plasminogen activator inhibitor 1
PC  Percent change
RM ANOVA  Repeated Measure Analysis of Variance
ROS  Reactive oxygen species
SBU  The Swedish Council on Health Technology Assessment
SMC  The Swedish Mammography Cohort
T1D  Type 1 diabetes
T2D  Type 2 diabetes
VCAM-1  Vascular cell adhesion molecule 1
WHO  World Health Organization
E%  Energy percentage
1 INTRODUCTION

Type 2 diabetes is associated with complications affecting the micro- and macrovascular system, causing morbidity and shorter life expectancy (1, 2). The development of diabetic complications involves complex processes where increased inflammation may play an important role. Long-term hyperglycemia is central in the process but factors that are considered parts of the metabolic syndrome, as central obesity, dyslipidemia and insulin resistance are contributing factors (3-8).

The importance in secondary prevention lies in postponing complications through well-controlled disease management. The disease management involves good control of blood glucose and lipids as well as blood pressure. Lifestyle factors as physical activity and diet are also of importance in the management of diabetes. There are however gaps in the scientific evidence for dietary recommendations in diabetes (9-11). Moreover, there are gaps in the literature whether those with type 2 diabetes make changes in diet after their diagnosis (12, 13).

The postprandial period is complex with fluctuation in blood glucose, lipids and other metabolic responses, and may thus induce and/or increase inflammation. The metabolic responses may depend on total calorie intake, composition of the meal and type of macronutrients consumed etc. Also, those with type 2 diabetes might be more vulnerable to the metabolic fluctuations after a meal as they already are in a state of hyperglycemia, dyslipidemia and low-grade inflammation (9, 14, 15).

The aim of this doctoral thesis was to examine postprandial responses of fructose loading and different meal compositions on risk markers for complications in type 2 diabetes. Further, possible changes in fruit, vegetables and juice consumption after a T2D diagnosis was explored.
2 BACKGROUND

2.1 TYPE 2 DIABETES

2.1.1 Classification, diagnosis and occurrence

Diabetes mellitus are diseases characterized by hyperglycemia, and it is commonly divided into type 1 and type 2 diabetes (T2D) (16, 17). Type 1 diabetes (T1D) is an autoimmune disease characterized by pancreatic β-cell destruction leading to complete insulin deficiency. T2D is characterized by a gradually and progressive β-cell dysfunction and insulin resistance (18), and accounts for around 90% of all cases of diabetes (17). T2D was previously seen occurring in adults, but is now seen among children and adolescents (18), and there is high heterogeneity for diabetes within the age-group 20-40 years of age (16). Further, different subtypes of T2D have been suggested (19).

The World Health Organization (WHO) recommend the diagnostic criteria for diabetes to a fasting plasma glucose of ≥ 7 mmol/L, or a plasma glucose of ≥11.1 two hours following ingestion of 75g oral glucose load (20). Also, glycated haemoglobin (HbA1c) can be used as a diagnostic test, with a cut point of 6.5% (21) (48 mmol/mol) (22).

The International Diabetes Federation (IDF) estimated the global prevalence of diabetes between the ages 20-79 to 9.3%, or 463 million people, in 2019. In Sweden the prevalence was estimated to 7.2% (4.8% age adjusted) (17). The Public Health Agency of Sweden report a prevalence of 6% in 2018 among those between 16-84 years of age (23). A Swedish study among those >20 years of age indicate that the incidence has leveled off but the prevalence, which was estimated to 6.8% in 2013, is expected to rise due to improved survival and demographic changes (24).

2.1.2 Risk factors and pathophysiology

There is a genetic predisposition of developing T2D as genes associated with both insulin resistance and, mainly, β-cell dysfunction has been identified (25). Family history of T2D can increase the risk by two to fourfold depending on closeness to and number of relatives with T2D (26). Genetic predisposition only can however not be accounted for the type 2 diabetic epidemic. Sedentary lifestyle and overeating are driving forces as increasing obesity and increasing T2D goes hand in hand (27). Quality of foods is an independent risk factor for developing T2D. Consumption of whole grains, vegetables, fruit (28) and polyunsaturated fat (29) are associated with a decreased risk, while refined grains, processed meat, red meat (28), sugar sweetened beverages (28, 30) and trans-fats (29), are associated with increased risk. Regarding food pattern, a western dietary pattern (characterized by high consumption of red meat, processed meat, high-fat dairy products, French fries, refined grains, sweets and desserts) with increased risk of T2D in men. A prudent dietary pattern (characterized by high consumption of vegetables, fruits, fish, poultry, and whole grains) was on the other hand
associated with a lower risk (31). Among women, it has been shown that a western dietary pattern (here defined as high consumption of red and processed meat, refined grains and sugar-sweetened beverages and low consumption of cruciferous vegetables, yellow vegetables, wine and coffee) was strongly associated with inflammatory markers, which in turn was associated with increased risk of diabetes (29). Further, the Mediterranean diet (rich in fruit, vegetables, legumes, grains, nuts and olive oil) has been proven protective without loss in body weight or physical activity (32). Other risk factors for T2D include physical inactivity (1, 29), smoking (29), psychological distress (1, 33) and work stress (34).

T2D develops gradually and becomes manifest when insulin secretion cannot compensate for insulin resistance. Tissues that primarily demonstrate insulin resistance include skeletal muscle, liver and adipose tissue (35).

The insulin resistance contribute to impaired glucose uptake into skeletal muscle and impaired suppression of glucose production in the liver, with subsequent rises in blood glucose levels (35). In the adipocytes, the effects are disturbed lipolysis and increased delivery of free fatty acids, which accumulates in the skeletal muscle, liver, and pancreas and will contribute to insulin resistance, increased hepatic glucose production and impaired β-cell function. These negative effects of adipose tissue are referred to as lipotoxicity (35, 36). The adipose tissue can be considered an endocrine organ as it secretes pro-inflammatory cytokines affecting glucose homeostasis with induced insulin resistance (36, 37). The inflammatory response is systemic, low grade and chronic and thus different from the classic transient immune response to an injury or infection (7, 38). As the hepatic glucose production increases and the insulin signaling pathway in the skeletal muscle are inhibited glucose level rises (36). The persistent hyperglycemia causes β-cell dysfunction and reduces β-cell mass through apoptosis, an effect referred to as glucotoxicity. One possible mechanism that induces β-cell dysfunction may be through the excess production of reactive oxygen species (ROS), as β-cell have limited defense against ROS, and inflammation (3, 39). The combination of lipotoxicity and glucotoxicity creates an environment for the downward spiral leading to β-cell failure as the deleterious effects of lipids are predominant when glucose levels are high (39).

2.2 DIABETIC COMPLICATIONS

T2D is associated with complications due to long-term hyperglycemia, but also due to risk factors that are considered parts of the metabolic syndrome, as central obesity, dyslipidemia, hypertension and insulin resistance etc. (2, 5, 6, 40). Also, there seems to be a genetic susceptibility in development of diabetic complications (41). Diabetic complications are commonly divided into macro- and microvascular complications depending on type of blood vessel involved. Macrovascular complications include cerebrovascular disease, cardiovascular disease (CVD) and peripheral vascular disease, while microvascular complications include retinopathy, nephropathy and neuropathy. The major cause of mortality and disability is due to macrovascular complications, atherosclerotic CVD (2, 40), and life expectancy can be reduced
with as much as 15 years (1). Micro- and/or macrovascular complications may be present already at the time of a T2D diagnosis (42).

### 2.2.1 Pathophysiology

The mechanisms contributing to development of diabetic complications are multifactorial and complex. Increased oxidative stress and inflammation seems to play an important role in the development (4, 5, 8, 43). The hyperglycemic milieu activates several pathways contributing not only to increased oxidative stress and inflammation, but also reduced defense thereof. There may further be an effect on vascular function including factors regulating vasoconstriction and vasodilation, and the fibrinolytic system among others (4, 8). The insulin resistant state will contribute to the pathophysiology by further delivery of free fatty acids and increased hepatic glucose production (see section 2.1.2) (41, 44), as well as T2D associated dyslipidemia (low HDL, high triglycerides and LDL, the latter also converted into small dense LDL) (6, 45). There are several markers involved in the inflammatory process and that are associated with atherosclerotic CVD and diabetic complications.

The vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) may increase and facilitate adhesion of other proinflammatory molecules and contribute to increased risk of atherosclerotic CVD (3, 46, 47). The proinflammatory monocyte chemoattractant protein 1 (MCP-1), which is produced by adipocytes and endothelial cells among others, stimulates inflammatory cells to migrate through vessel walls (3). MCP-1 is, as the adhesion molecules, associated with increased cardiovascular risk (48). ICAM-1 and MCP-1 has further been associated with inflammation in diabetic retinopathy (43). Interleukin 6 (IL-6) is involved in both acute and chronic inflammation. In chronic inflammation it will contribute to MCP-1 production (49). IL-6 is associated with CVD (43) and diabetic nephropathy (50). Plasminogen activator inhibitor -1 (PAI-1), produced by a variety of cells including adipocytes and endothelial cells, promotes thrombosis and fibrosis. It is suggested to increase cardiovascular and macrovascular risk and is associated with an inflammatory milieu (51, 52). Interleukin 18 (IL-18) may lead to production of other proinflammatory molecules (50) and is associated with diabetic nephropathy (50, 53).

Markers in urine to detect injury to the kidney include the immunoglobulin G (IgG2 and IgG4). Levels of urinary IgG has been observed to be higher among those with diabetes even before microalbuminuria develops (54). Most pores in the glomerular capillary wall has a radius of 2.9–3.1 nm, while a smaller number has the radius of 8 to 9 nm. IgG2 is neutral in charge and IgG4 negative in charge, and both have a radius of 5.5 nm. An increase in urinary IgG2 and IgG4 implies an increase of larger pores in the glomeruli and loss of charge (55).

### 2.3 Disease Management

The importance in secondary prevention lies in postponing complications through well controlled disease management, taking individual preferences into account (10, 11). As
hyperglycemia plays a central role in pathogenesis of diabetic complications, good glycemic control is of importance. The National Board of Health and Welfare recommend a cut-point 52 mmol/mol in HbA1c. An individual risk-benefit evaluation of intensive treatment may change the treatment goal, and an upper recommended limit of 70 mmol/mol is also stated. Further, good blood pressure and lipid control is of importance. The importance of diet and physical activity is also emphasized, as well as smoking cessation (10).

In a recent published Swedish study, it was observed that patients with five risk factors within the target range had little or no excess risk of death, myocardial infarction (MI) or stroke when compared to the general population. The five risk factors were elevated HbA1c, elevated LDL, albuminuria, smoking, and elevated blood pressure (56).

### 2.3.1 Dietary recommendations

There is a lack of studies concerning nutrition in T2D. The Swedish Council on Health Technology Assessment (SBU) published a systematic review in 2010 in which major gaps in the scientific evidence for dietary recommendations where identified. The lack of dietary studies applicable to Swedish conditions was also emphasized (57). The American Diabetes Association (ADA) highlight the importance of nutrition therapy in their nutritional recommendations (2019), but they conclude that research in the field is lagging behind (9). Further, the lack of high quality data on the efficacy of dietary advice for treatment of T2D has been highlighted in a Cochrane review published first in 2007, and updated in 2010 with no change to conclusions (58). The Cochrane review has not been updated since then.

The National Board of Health and Welfare published Swedish dietary recommendations in diabetes in 2011. In the recommendations, it is stated that different diets as the Mediterranean (45-50% carbohydrates) and moderately low carbohydrate (30-40% carbohydrates) diet etc. may be beneficial, while the scientific evidence for extremely low carbohydrate diet is yet too weak. Further, single foods that are stated as beneficial are fruit and vegetables (including root vegetables), whole grain, legumes, fish, and nuts. Regarding the long-term effects of juice and sodas, it is concluded that there is lack in the scientific evidence. Energy balance and personal preferences are also emphasized (59).

European and North American dietary recommendations for the management of diabetes focus on individual dietary goals, types and quality of carbohydrates and fats, and total energy balance to promote a healthy body weight and take possible co-morbidities and metabolic goals into account, etc. rather than fixed proportions of macronutrients. Thus, no ideal macronutrient distribution is presented and none of the associations suggests a specific eating pattern over another. Moreover, reducing protein intake when there is evidence of kidney disease is not recommended, as the evidence is insufficient. Fruit and vegetable intake are advised, as well as whole grains and foods with a low glycemic index. It is also recommended to eat fatty fish due to its omega-3 fatty acid content. With regards to fructose, ADA state that added fructose intake should be limited or avoided while the DNSG-EASD state that moderate intake of 30 g/day do not seem to have negative effects (9, 60, 61).
2.3.1.1 Fruits and vegetables

As described above, intake of fruits and vegetables are advised in the treatment of diabetes. The intake of fruits and vegetables, and diets rich in those, are associated with decreased risk of cardiovascular events and mortality among those with T2D (9, 59, 61). The fiber content in fruits and vegetables may facilitate weight loss and reduces HbA1c (9, 61). Further, fruits (including berries) and vegetables contains compounds with antioxidant properties (62, 63). This may also explain parts of its protective effect on all cause and cardiovascular mortality found in T2D and healthy subjects (64, 65).

2.3.2 Changes in diet after diagnosis

Behavioral changes concerning lifestyle factors as diet are recognized to be difficult (66). Longitudinal studies exploring changes in diet among those diagnosed with T2D show contradictory results. Within the Nurses’ Health Study it was observed that women began consuming high-fat and low-sucrose diets after a T2D diagnosis (12), while the Whitehall II study observed no changes in diet quality (Alternative Healthy Eating Index was used; including fruit and vegetables) (13). The English longitudinal study of ageing reported no change in intake of fruit and vegetables after a T2D diagnosis (67), and the New South Wales 45 and Up Study observed a small decrease in vegetable intake and no change in fruit intake after diagnosis (68).

Although longitudinal studies indicate that there are no major changes in diet following a T2D diagnosis, cross-sectional studies indicate that individuals with T2D differ from the general population in regards of dietary patterns (12, 69-74). Lower intake of carbohydrates and higher intake of protein (12, 69-73) have been noted among diabetics when compared to non-diabetics. It has also been observed that diabetics get more energy from fat than non-diabetics (12, 71, 73). In addition, a study conducted in different countries and ethnic groups showed that individuals with diabetes have a slightly higher intake of fruit and vegetables, fish and meat compared to those without diabetes. In Sweden, the low intake of juice among diabetics was the most prominent difference (74). Despite the indications that those with diabetes have a higher intake of fruit and vegetables, studies also show that the recommended 5 servings per day are not fulfilled (75, 76). The Look AHEAD study report that those meeting recommendations of 2 servings of fruit and 3 servings of vegetables per day are 36% and 38%, respectively (75). Information from the NHANES III survey show that 62% eat less than 5 servings of fruit and vegetables combined (76).

2.4 POSTPRANDIAL CONDITIONS

The postprandial period (the period after a meal) is complex with fluctuation in blood glucose, lipids and other metabolic responses, and may subsequently induce/increase oxidative stress and inflammation. The metabolic responses may depend on total calorie intake, composition of the meal and type of macronutrients consumed. The impact of the postprandial state may
also differ depending on disease status. Those with T2D might be more vulnerable to the postprandial metabolic fluctuations considering already being in a state of hyperglycemia, dyslipidemia and low-grade inflammation (14, 15). Thus, the postprandial period may be of importance in secondary prevention in T2D.

### 2.4.1 Meal composition

The quantity and the quality of macronutrients consumed, and their combined effect, as well as micronutrient compounds, may impact the risk of subsequent diabetic complications (9, 14). In studies of longer duration, the effect of high-fat vs. high-carbohydrate diets on the metabolic profile (blood glucose, insulin and lipids) were explored in a meta-analysis. Studies included were intervention studies among those with T2D and with a median duration of 4 weeks. The authors conclude that energy restriction and quality of fat is more important than the proportion of fat and carbohydrates, and replacing fat with carbohydrates is not recommended (77). The Mediterranean diet, supplemented with olive oil or nuts, compared to a control low fat diet has shown to be protective against cardiovascular events in populations at high risk, including those with T2D (78). Protein intake has commonly been explored through a kidney protective perspective (9). In a meta-analysis exploring the effects of a high protein diet, there were no effect on kidney function (GFR) among those with T2D. Interventions included in the sub group analysis of those with T2D were few (n=4) and the trials ranged between 3-52 weeks (79).

In the postprandial state, glucose is observed to be higher and longer lasting among those with T2D when compared to healthy subjects (HS) (14). Further, blood glucose levels have been observed to be greater and longer lasting following a high carbohydrate (HC) meal compared to a high fat (HF) meal in T2D (80). Those with T2D also have a tendency to increased hyperlipidaemia postprandially (14). The increase has been observed to be greater among T2D when compared to HS (80), and to be greater following a HF meal compared to a HC meal (81).

Postprandial responses in ICAM-1, VCAM-1, IL-18 and PAI-1 following different meal compositions are scarce, somewhat conflicting and commonly explored in HS following HC and HF meal. Nappo et al. has explored postprandial ICAM-1 and VCAM-1 in HS and T2D following HF and HC meal, with and without antioxidant vitamins (vitamin E and vitamin C). An increase in ICAM-1 and VCAM-1 was observed in T2D following both HF and HC meal, while these parameters increased only after HF meal in HS. The increase was prevented with antioxidant vitamins in both groups (80). In another population of HS subjects, no increase was observed in ICAM-1 and VCAM-1 following HF meal (82). Postprandial IL-18 among HS and T2D was observed to increase following HF meal, decrease following HC and fiber meal and had no response following HC meal (81). Gregersen et al. observed the same response in IL-18 among HS subjects following a HC meal, while a decrease was observed following HF meal (83). The response in PAI-1 among T2D following HF test meals show both an increase and a decrease (84, 85), where the observed increase was prevented with vitamin supplementation given at breakfast (84). The postprandial response among those with the metabolic syndrome,
hypertensive individuals and HS show a decrease, an increase and no response in PAI-1 (86, 87). A decrease among those with the metabolic syndrome has also been observed following a HC test meal (86). Postprandial responses in urinary IgG2 and IgG4 following different meal compositions has, to the best of knowledge, not been examined previously.

### 2.4.2 Fructose

Fructose is a monosaccharide present naturally in foods as fruit, vegetables and honey. In fruit, vegetables and table sugar it is also present as a disaccharide (sucrose), where it is joined with glucose (88, 89). The intake of fructose increased dramatically between the years 1970 and 2007, mainly due to increased intake of sugar-sweetened beverages. Fructose has a low glycemic index and thus helps maintain glycemic control, a property that led to the belief that it was beneficial as a sweetener for those with diabetes (89).

The body’s capacity of absorbing fructose is limited and varies depending on health and co-ingested foods (88). Glucose is the dietary factor that has the largest impact on fructose absorption (88, 89), but animal studies also indicate that saturated fat increase absorption (89). It has been observed that the maximum fructose absorbing capacity varies between 5 and 50 g when consumed as a single dose (88) (for comparison; a medium sized banana contains ~6 g of fructose, and a medium sized apple contains ~11 g (90)). Individuals with T2D seems to have a larger capacity to absorb fructose (91), and fructose may also be produced endogenously through the polyol pathway under diabetic conditions (92).

#### 2.4.2.1 Fructose metabolism and metabolic effects

Fructose is absorbed in the small intestine by a fructose specific transporter. It is further transported to the liver through the portal vein, where it is absorbed and metabolized by liver cells. The metabolism of fructose is independent of insulin (89). Some fructose is metabolized by the enterocytes in the small intestine, but the liver metabolize the majority of ingested fructose, in comparison to about 15-30% of ingested glucose (88). In the metabolic pathway fructose can be oxidized, converted to glucose or lactic acid, or enter de novo lipogenesis (DNL) (88) (Figure 2.1). In the first hepatic metabolic step, fructose is phosphorylated by fructokinase, a fructose specific enzyme with high activity, to fructose-1-phosphate (88, 89, 93). Fructokinase is not regulated by the energy status (ATP) of the cell, and fructose will therefore be metabolized in an unlimited way. This contrasts with steps in the glycolysis where the phosphorylation is regulated by ATP levels (88). Due to the rapid phosphorylation of fructose, levels of ATP will be depleted followed by an increase in uric acid (93, 94).
Previous studies examining effects of fructose loading on levels of serum uric acid are scarce and show conflicting results. In healthy subjects (HS), an increase in serum uric acid has been observed following intake of sodas (highest dose of fructose; 39.2 g) (95, 96). A smaller intake every hour (0.2 g fructose/kg) during a 9 hour-period did however not increase levels of serum uric acid in another population of HS (97). In a study among HS, those with metabolic syndrome and patients with CKD, levels of serum uric acid increased in all subject groups following intake of 1g/kg of fructose (98). Among those with T2D, an increase has been observed following a 75 g fructose load (99).

Elevated serum uric acid levels are associated with T2D and chronic kidney disease (100-102). It has also been associated with progression of already established nephropathy among those with T2D (100) and to predict mortality among those with CKD (103). It is further a marker of increased cardiovascular risk (104, 105).

The mechanisms by which uric acid has negative effects may include an increase in reactive oxygen species and oxidative stress (94, 106), inflammatory activity (94, 107, 108), endothelial dysfunction (94, 109), fibrosis (110), renin activity and hypertension (101, 111). With regards to inflammatory activity, associations between uric acid and inflammatory markers have been found. However, previous intervention studies exploring the effect of fructose intake on the inflammatory markers IL-6, IL-18, MCP-1, ICAM-1 and VCAM-1 are scarce and show conflicting results (112-118). Further, existing studies are performed in HS subjects and most after long-term intake of fructose. The response in MCP-1 has been explored following a 4-week and a 10-week interventions, resulting in no change and an increase (113, 115). Levels
of IL-6 among HS indicate no response following acute fructose loading (75 g, follow up time 180 min) (118), while longer intake show conflicting results (increase or no response in IL-6) (113, 114, 116, 117). No responses has been observed in ICAM-1 and VCAM-1 following acute fructose loading (118), or in ICAM-1 following longer intake (113). No studies explored IL-18 after fructose loading (112).

Fructose may contribute to dyslipidemia. This may be due to that the mitochondria capacity is exceeded and acetyl-CoA will enter DNL instead of the citric acid cycle. This metabolic effect is considered as “particularly harmful” (88). In CKD, an increase in triglycerides has been observed (119). In T2D an increase in has been observed following a month’s fructose intervention (30 g/day), but the increase was modest and triglycerides were within normal range (120).
3 HYPOTHESIS AND AIMS

Based on the hypothesis that diet is of importance in postponing complications in T2D, the present thesis includes the following aims;

Study 1 – To study the acute effect of fructose loading on levels of serum uric acid in T2D, CKD and HS

Study 2 – To study the acute effect of fructose loading on metabolic and inflammatory markers in T2D and HS

Study 3 – To study the acute effects of meal composition on metabolic and inflammatory markers in T2D and HS

Study 4 – To examine changes in fruit, vegetable and juice consumption over 12 years among men diagnosed with T2D and those who remained free from diabetes
4 MATERIAL AND METHOD

4.1 INTERVENTION STUDIES – STUDY I, STUDY II & STUDY III

4.1.1 Study populations

4.1.1.1 Study I and II – The Fructose Load Study
Study participants were recruited at two study sites, Department of Endocrinology, Diabetes and Metabolism or Department of Renal Medicine at Karolinska University Hospital, or through advertisements. Inclusions criterions were >18 years of age and controlled diabetes for those with T2D and chronic kidney disease (CKD) stage 4-5 for those with CKD. Exclusion criteria’s were ongoing inflammatory disease or infection, treatment with uric acid lowering agents, signs of fluid overload and inability to understand information provided about the study. Study II included 6 HS and three patients with CKD and 7 with T2D, and Study III included 6 HS and 7 patients with T2D. Study participants were between the ages 47-76. Five were treated with non-insulin releaser oral antidiabetic drug among those with T2D. Participants who completed all interventions were included in the study.

4.1.1.2 Study III – The Standardized Meal Study
Study participants were recruited at the Department of Endocrinology, Metabolism and Diabetes at Karolinska University Hospital, Solna, or through advertisements. Criteria’s for inclusion were >20<70 years of age for all, and for those with T2D a BMI between 25-33 kg/m² and a disease duration > five years. Exclusion criteria’s were heart failure (NYHA class III & IV), renal failure (s-creatinine >200 μmol/L), liver disease (ALAT >2 μKat/L) and current treatment with pioglitazone as it may affect lipids postprandially. The study includes 21 T2D and 21 healthy subjects (HS) between the ages 20-74. Among those with T2D, 16 were treated with Metformin, of which 8 in combination with other oral agents (DDP4-inhibitor n=3, sulfonylurea n=3, akarbos n=1, GLP1-analog n=1) and five in combination with insulin (NPH-insulin n=3, long-acting insulin n=1, fast-acting n=2). Those on insulin releaser (n=4) were instructed not to take the medication the night before or on the day of the intervention. Two received insulin during the interventions (see sensitivity analysis for insulin where these are excluded). There were 15 T2D and three HS receiving lipid lowering treatment.

4.1.1.3 Ethics
The studies were approved by the Regional Ethical Review Board in Stockholm and all study participants signed informed consent.
4.1.2 Interventions

4.1.2.1 Study I and II – The Fructose load study

Study participants, who were fasting overnight, ingested three different drinks, with and without a pizza slice, thus resulting in six interventions. Also, study participants were instructed not to take their diabetic medication in the morning. The sugar content, and associated information, of the drinks are presented in Table 4.1. The pizza slice was a 425 kcal Billy’s pan cheese pizza (containing 10% pork, 170 g [17 g protein, 15 g fat and 51 g carbohydrate], G. Dafgård AB, Källby, Sweden). The caloric distributions of macronutrients were approximately 32% fat, 51% carbohydrates, 17% protein. Study participants consumed drinks or drink and pizza during 15 minutes, supervised by a study nurse.

<table>
<thead>
<tr>
<th>Table 4.1. Sugar content and additional information of drinks (140 kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose (g)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Blueberry drink, 54 cl</td>
</tr>
<tr>
<td>Fresh Swedish blueberries; Saxhyttegubben Blåbär 100% [&lt;0.5 g protein, &lt;0.5 g fat and 10 g carbohydrate/dL], Saxhytte Gård AB, Grythyttan, Sweden</td>
</tr>
<tr>
<td>Coca-Cola, 33 cl</td>
</tr>
<tr>
<td>European formula, 35g sucrose/33cL. Stored at room temperature for &gt;4 months during which all sucrose spontaneously decomposed into equal amounts of glucose and fructose (121)</td>
</tr>
<tr>
<td>Fructose drink, 20cl</td>
</tr>
<tr>
<td>Prepared by dissolving 35 g of pure fructose (&gt;99% pure, Sigma Aldrich, St Louis, MO, USA) in tap water.</td>
</tr>
</tbody>
</table>

4.1.2.2 Study III – The Standardized Meal Study

Study participants ate an isocaloric meal (600 kcal) on four different occasions and with different compositions of high carbohydrates (HC), HC and fiber, low carbohydrate (LC) + high protein (HP) and LC + high fat (HF). See Table 4.2. for the macro- and micro distribution of the meals and Table 4.3 for the menus and ingredients of the meals. A small amount of soy sauce and rapeseed oil was served with the vegetables for all meals, and spices as salt, pepper and sambal oelek etc. was used. The meals were randomly distributed, and the study participants received no prior information on the composition of the served meal. The meals were cooked and served at a restaurant in connection to the Karolinska University Hospital in Solna. The starter to the LC+HP meal and the main course of the HC+fiber meal is shown in Figure 4.1. Study participants ate a standardized breakfast approximately four hours before the interventions. The breakfast constituted of 400-450 kcal and had the following macronutrient distribution: 56-66 energy percent (E%) from carbohydrates (8-10 grams of fiber), 21-24 E% from protein, and 13-20 E% from fat.
### Table 4.2. Macro- and micronutrient distribution of the intervention meals, 600 kcal

<table>
<thead>
<tr>
<th></th>
<th>HC meal</th>
<th>LC+HP meal</th>
<th>LC+HF meal</th>
<th>HC+fiber meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (E%)</td>
<td>52</td>
<td>28</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>Protein (E%)</td>
<td>19</td>
<td>41</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>Fat (E%)</td>
<td>30</td>
<td>31</td>
<td>50</td>
<td>27</td>
</tr>
<tr>
<td>Saturated (E%)</td>
<td>10</td>
<td>6</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Monounsaturated (E%)</td>
<td>11</td>
<td>17</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Polyunsaturated (E%)</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Ascorbic acid, mg</td>
<td>122</td>
<td>112</td>
<td>48</td>
<td>118</td>
</tr>
<tr>
<td>Tocopherols, mg</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Carotenoids, µg</td>
<td>3866</td>
<td>556</td>
<td>2940</td>
<td>3991</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>0.6</td>
<td>0.7</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Selenium, µg</td>
<td>9</td>
<td>28</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table 4.3. The menus and ingredients of the meals

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>LC+HP</th>
<th>LC+HF</th>
<th>HC+fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter</td>
<td>-</td>
<td>Avocado and shrimp</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Main course</td>
<td>Roast beef, stir fried vegetables and boiled potatoes</td>
<td>Roast beef, stir fried vegetables and boiled potatoes</td>
<td>Prime rib, stir fried vegetables, avocado and French fries</td>
<td>Roast beef, stir fried vegetables, red beans, green beans and boiled potatoes</td>
</tr>
<tr>
<td>Dessert</td>
<td>Sugared blueberries and strawberries with cream (not whipped)</td>
<td>-</td>
<td>-</td>
<td>Sugared blueberries and strawberries with dairy ice cream</td>
</tr>
<tr>
<td>Ingredients</td>
<td>Roast beef 75 g, Boiled potatoes 160 g, Broccoli 75 g, Carrot 50 g, Onion 30 g, Leek 30 g, Sugared strawberries 80 g, Sugared blueberries 80 g, Cream 20 g</td>
<td>Shrimp 50 g, Avocado 50 g, Roast beef 150 g, Boiled potatoes 150 g, Corn 30 g, Broccoli 100 g, Onion 35 g, Leek 35 g</td>
<td>Prime rib 75 g, French fries 100 g, Corn 30 g, Broccoli 40 g, Carrot 40 g, Onion 30 g, Avocado 60 g</td>
<td>Roast beef 80 g, Boiled potatoes 100 g, Broccoli 75 g, Carrot 50 g, Onion 30 g, Leek 30 g, Read beans 70 g, Green beans 70 g, Sugared strawberries 75 g, Sugared blueberries 75 g, Dairy ice cream 30 g</td>
</tr>
</tbody>
</table>
4.1.3 Blood sampling and laboratory analysis

Blood samples in Study I, Study II and Study III were drawn by experienced study nurse through an intravenous catheter that was put in place before the intervention and kept in place throughout the follow-up period. Serum samples were allowed to clot for about 30 min and then centrifuged, while plasma samples were centrifuged immediately. Specimens were thereafter pipetted into designated tubes and stored shortly in -20 °C before long-term storage in -80 °C freezers. Samples were also sent for analysis immediately after being drawn. Analysis were carried out at the Clinical Research Center at Karolinska University Hospital, at the research laboratories that are part of the Department of Endocrinology, Diabetes and Metabolism, Solna, and Department of Renal Medicine, Huddinge, or at the SciLife Laboratory in Solna. Laboratory analysis were carried out by trained personnel using assays intended for the markers, and by following manuals provided by manufacturer. ICAM-1, VCAM-1, IL-18, PAI-1 and LPS were analyzed in plasma, others in serum.

Urine samples collected in Study III were put in a -20 freezer immediately after collection and thereafter stored long-term in -80 °C. Immunoglobulins in urine were analyzed at the Department of Nephrology, Lund University.

4.1.3.1 Study I and Study II – The Fructose Load Study

Blood samples were collected at -15 min (fasting and before intake) and at 30 min, 60 min, 90 min and 120 min following drinks, and at an additional 240 min following drinks and pizza. In Study I postprandial fructose, uric acid and triglycerides were explored, and the study includes HS, T2D and CKD patients. Study II includes HS and T2D patients and postprandial glucose,
insulin, IGFBP-1, LPS and markers of inflammation were explored. The study population and the postprandial markers, and their time-points, in **Study I/II** are summarized in **Figure 4.2**.

**Figure 4.2.** The study population and postprandial markers explored in Study I and Study II

<table>
<thead>
<tr>
<th>Male/female, n</th>
<th>CKD: n=3</th>
<th>T2D: n=7</th>
<th>HS: n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61 (52, 72)</td>
<td>67 (58, 76)</td>
<td>59 (47, 71)</td>
</tr>
</tbody>
</table>

**Postprandial markers explored in blood**

<table>
<thead>
<tr>
<th>Study I</th>
<th>Marker</th>
<th>Time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinks</td>
<td>Fructose</td>
<td>-15, 30, 60, 90, 120</td>
</tr>
<tr>
<td></td>
<td>Uric acid</td>
<td>-15, 30, 60, 90, 120</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>-15, 30, 60, 90, 120</td>
</tr>
<tr>
<td>Drinks &amp; pizza</td>
<td>Fructose</td>
<td>-15, 30, 60, 90, 120, 240</td>
</tr>
<tr>
<td></td>
<td>Uric acid</td>
<td>-15, 30, 60, 90, 120, 240</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>-15, 30, 60, 90, 120, 240</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study II</th>
<th>Marker</th>
<th>Time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinks</td>
<td>Glucose</td>
<td>-15, 30, 60, 90, 120</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>-15, 30, 60, 120</td>
</tr>
<tr>
<td></td>
<td>IGFBP-1</td>
<td>-15, 120</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>-15, 60, 120</td>
</tr>
<tr>
<td></td>
<td>MCP-1</td>
<td>-15, 60, 120</td>
</tr>
<tr>
<td>Drinks &amp; pizza</td>
<td>Glucose</td>
<td>-15, 30, 60, 90, 120, 240</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>-15, 30, 60, 120, 240</td>
</tr>
<tr>
<td></td>
<td>IGFBP-1</td>
<td>-15, 240</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>-15, 60, 120, 240</td>
</tr>
<tr>
<td></td>
<td>MCP-1</td>
<td>-15, 60, 120, 240</td>
</tr>
<tr>
<td></td>
<td>ICAM-1</td>
<td>-15, 240</td>
</tr>
<tr>
<td></td>
<td>VCAM-1</td>
<td>-15, 240</td>
</tr>
<tr>
<td></td>
<td>IL-18</td>
<td>-15, 240</td>
</tr>
<tr>
<td></td>
<td>LPS (Coca-Cola)</td>
<td>-15, 240</td>
</tr>
</tbody>
</table>

**4.1.3.2 Study III – The Standardized Meal Study**

Blood samples were collected before intake and up until 240 min after intake, and urine samples before intake and at 180 min after intake. The postprandial markers, and their time-points, explored in **Study III** are summarized in see **Table 4.4**.

**Table 4.4.** Postprandial markers explored in Study III

<table>
<thead>
<tr>
<th>Marker</th>
<th>Time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Before intake, 30, 60, 90, 120, 150, 180, 210 and 240</td>
</tr>
<tr>
<td></td>
<td>Before intake, 30, 60, 120, 180 and 240</td>
</tr>
<tr>
<td>Triglycerides, HDL, LDL, cholesterol</td>
<td>Before intake, 60, 120, 180 and 240</td>
</tr>
<tr>
<td>PAI-1, ICAM-1, VCAM-1, IL-18</td>
<td>Before intake and 180</td>
</tr>
<tr>
<td>Urine</td>
<td>Before intake and 180</td>
</tr>
</tbody>
</table>
4.1.4 Statistical analysis

In Study I, postprandial serum fructose, serum uric acid and triglycerides were analyzed using RM ANOVA for within group analysis. The RM ANOVA explored possible differences between the interventions over time for each group. As there were variability in levels of serum uric acid between the group it was expressed as percent change (PC), where each time-point was related to the baseline value in the RM ANOVA. Possible differences in area under the curve (AUC) and PC (here calculated from the baseline to the endpoint value) for serum fructose and serum uric acid were for each group also tested using Friedmans ANOVA. Postprandial responses between the three groups were not tested statistically due to differences in etiology. See Table 4.5 for a summary of statistical analysis performed in Study I.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Analysis within each group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>RM ANOVA, absolute values</td>
</tr>
<tr>
<td></td>
<td>AUC and PC (baseline to endpoint value), Friedmans ANOVA</td>
</tr>
<tr>
<td>Uric acid</td>
<td>RM ANOVA, percent change</td>
</tr>
<tr>
<td></td>
<td>AUC and PC (baseline to endpoint value), Friedmans ANOVA</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>RM ANOVA, absolute values</td>
</tr>
</tbody>
</table>

Postprandial glucose and insulin were in Study II explored using AUC and tested with Friedmans ANOVA for within group analysis. Postprandial responses in IL-6, MCP-1, IL-18, ICAM-1, VCAM-1, IGFBP-1 and LPS were for each intervention and group examined as possible difference between the absolute baseline and the endpoint value and tested using Wilcoxon matched pair test. Postprandial differences in inflammatory markers and IGFBP-1 between the interventions were for each group (within group analysis) explored as PC (from the baseline to the endpoint value) and tested using Friedmans ANOVA. Further, differences between the groups in inflammatory, IGFBP-1 and LPS responses were tested using Mann Whitney U test. Statistical analysis of glucose, insulin, IL-6 and MCP-1 were complemented with RM ANOVA as more time-point were available. See Table 4.6 for a summary of statistical analysis performed in Study II.
Table 4.6. Statistical analysis performed for each marker in blood in Study II

<table>
<thead>
<tr>
<th>Markers</th>
<th>Analysis within each group</th>
<th>Analysis between the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, insulin</td>
<td>AUC, Friedmans ANOVA</td>
<td>No statistical analysis between groups</td>
</tr>
<tr>
<td></td>
<td>RM ANOVA, absolute values (as supplementary material in article)</td>
<td></td>
</tr>
<tr>
<td>IL-6, MCP-1</td>
<td>Difference between absolute baseline and endpoint value for each intervention, Wilcoxon's</td>
<td>PC (baseline to endpoint value), Mann Whitney U test</td>
</tr>
<tr>
<td></td>
<td>matched pair test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC (baseline to endpoint value), Friedmans ANOVA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM ANOVA, absolute values (as supplementary material in article)</td>
<td></td>
</tr>
<tr>
<td>IL-18, ICAM-1, VCAM-1, IGFBP-1</td>
<td>Difference between absolute baseline and endpoint value for each intervention, Wilcoxon's</td>
<td>PC (baseline to endpoint value), Mann Whitney U test</td>
</tr>
<tr>
<td></td>
<td>matched pair test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC (baseline to endpoint value), Friedmans ANOVA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM ANOVA, absolute values (as supplementary material in article)</td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>Difference between absolute baseline and endpoint value for Coca-Cola and pizza, Wilcoxon's</td>
<td>PC (baseline to endpoint value), Mann Whitney U test</td>
</tr>
<tr>
<td></td>
<td>matched pair test</td>
<td></td>
</tr>
</tbody>
</table>

In Study III, postprandial glucose, insulin and blood lipids were analyzed using Repeated Measure (RM) ANOVA for within group analysis. The RM ANOVA explored possible differences between the interventions over time for each group.

Postprandial PAI-1, IL-18, ICAM-1, VCAM-1 in blood and IgG2 and IgG4 in urine were for each intervention and group examined as possible difference between the baseline and the endpoint value and tested using Wilcoxon matched pair test. Possible postprandial differences between the interventions were for each group (within group analysis) explored using the absolute change between the baseline and the endpoint value (delta Δ) and tested using Friedmans ANOVA. See Table 4.7 for a summary of statistical analysis performed in Study III.
Table 4.7. Statistical analysis performed for each marker in blood and urine in Study III

<table>
<thead>
<tr>
<th>Markers</th>
<th>Analysis within each group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, insulin, triglycerides</td>
<td>RM ANOVA, absolute values (as supplementary material in the article)</td>
</tr>
<tr>
<td>HDL, LDL, cholesterol</td>
<td>RM ANOVA, absolute values (as supplementary material in the article)</td>
</tr>
</tbody>
</table>
| PAI-1, IL-18, ICAM-1, VCAM-1, IgG2, IgG4     | Difference between absolute baseline and endpoint value for each intervention, Wilcoxon’s matched pair testry 
|                                              | Delta Δ for differences between the interventions for each group, Friedmans ANOVA |

In Study I, Study II and Study III, the level of statistical significance was set to p<0.05, unadjusted for multiple testing. P-values for the RM ANOVA interaction term is presented if not otherwise indicated. AUC was calculated using the trapezoidal rule (122), taking basal value into account. Post hoc analysis were performed for significant results in the Friedmans ANOVA. Statistical analysis and figures were made in the analytic software Statistica (Dell).

4.2 COHORT STUDY – STUDY IV

4.2.1 Study population

The Cohort of Swedish Men (COSM) was established in late 1997 when all men born between 1918 and 1952 (between 45 and 79 years of age), living in Västmanland and Örebro counties, received a questionnaire regarding diet (food frequency questionnaire; FFQ) and other lifestyle factors. A total of 100,303 men were invited to participate and 48,850 men returned a completed questionnaire, yielding a response rate of 49%. From the baseline cohort the following exclusions were made; men with incomplete/incorrect personal number (n=352), men with prevalent cancer (n=2592) and men with implausible energy intakes (±3sd from the log-transformed mean energy intake) (n=567), leaving 45,339 men. In 2008 and 2009 a second wave of questionnaires was sent out to men still alive. The 2008 questionnaire included general health questions and updated anthropometric measures, and the response rate was 78%. The 2009 questionnaire included questions on diet (FFQ), alcohol consumption, smoking and physical activity, and the response rate was 90% of the 2008 responders. From the 2008/2009 cohort the following exclusions were made; those with incomplete/incorrect personal number (n=56) and implausible energy intakes (n=223), leaving 25,697 men eligible for Study IV (Figure 4.3).

The baseline cohort represents the Swedish population well with regards to age distribution, educational level and BMI (123). Further, the proportion of men with diabetes in the COSM (7%) is similar to the proportion in the corresponding age group in the general Swedish population (124).
From the 25,697 men that were eligible for the study, further exclusions were made based on diabetes status; prevalent diabetes in 1997, those who developed diabetes other than T2D, and self-reported diabetes that could not be verified in registries. Also, as the 2009 questionnaire was distributed for an extended time period, men who developed diabetes during that year was also excluded. The final cohort included 23,953 men, out of which 1,741 men developed T2D between the FFQ. The flow chart of the final analytical cohort in Study IV is presented in Figure 4.4.

**Figure 4.4.** The final analytical cohort; exclusions based on diabetes status

4.2.1.1 **Ethics**

The study was approved by the Regional Ethical Review Board in Stockholm. The return of the questionnaire was considered as consent of participation.
4.2.2 Ascertainment of type 2 diabetes and other diseases

The COSM was linked to registries to identify T2D diabetes cases and other diseases. T2D was identified through the Swedish National Diabetes Registry (NDR) and the National Patient Registry (NPR). The NDR is a quality registry that was established in 1996, with the purpose to improve medical care for those with diabetes. The coverage of the NDR has improved since its establishment. At the time of linkage, in year 2013, it was almost complete in the study area based on that 4% of the adult population had diabetes. The coverage was somewhat lower, approximately 90%, when validated against the Prescribed Drug Registry. The NPR is a mandatory registry to report to and contain information from inpatient care since 1987 and specialized outpatient care since 2001.

The NDR uses an epidemiological (type 1 diabetes if age at onset <30 and insulin treatment, T2D if treated with diet or oral agents, or insulin treated with or without oral agents and age of onset ≥ 40) and a clinical classification of diabetes, while the NPR uses the ICD-10 system (E11 for T2D). The priority order for classification of type of diabetes was as followed: 1) the epidemiological classification from the NDR, 2) the clinical classification from the NDR and 3) the ICD-classification from the NPR. The first available date in the two registries, or self-reported diabetes in any of the two questionnaires, was used to classify diabetes status in 1997 and 2008. Self-reported diabetes had to be verified in any of the two registries as type of diabetes was not identified in the questionnaire. Other diseases than diabetes were identified in the NPR using ICD-codes.

4.2.3 Assessment of dietary intake and covariates

Dietary intake was assessed using questions from a 96-item FFQ in 1997 and a 132-item FFQ in 2009. The expanded 2009 FFQ are due to separation of clustered items and added items. In the FFQs, study participants indicated how often, on average, they consumed the dietary items during the past year using predefined frequency categories. The FFQ has been validated against diet records with correlations ranging from 0.4 to 0.8 for a selection of fruits, vegetables and juice (A Wolk, unpublished data). It has also been validated for nutrients using 24-hour recall interviews with correlations of 0.65 and 0.62 for macro- and micronutrients, respectively (125).

In Study IV, possible changes in diet was examined using questions for fruits (five questions), vegetables (five questions) and orange and grapefruit juice (one question). The predefined frequency categories and foods included are marked and presented in Figure 4.5.
Figure 4.5. The predefined questions in the 1997 and 2009 FFQ used to examine possible changes in fruits, vegetables and juice intake.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fruits and juice</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>![Image of pre-defined questions for 1997 FFQ]</td>
<td>![Image of pre-defined questions for vegetables in 1997 FFQ]</td>
</tr>
<tr>
<td>2009</td>
<td>![Image of pre-defined questions for 2009 FFQ]</td>
<td>![Image of pre-defined questions for vegetables in 2009 FFQ]</td>
</tr>
</tbody>
</table>
When exploring proportions of men consuming ≥5 servings of fruit and vegetables per day, 18 questions were included from the 1997 FFQ (five for fruits and 13 for vegetables) and 23 questions were included from 2009 FFQ (five for fruits and 18 for vegetables). Pea soup, beans, lentils, chickpeas and soybean products were not included in the vegetable category.

Covariates BMI, physical activity, smoking habits and alcohol consumption were reported in 1997 and 2008/2009, and education only in 1997 (the baseline questionnaire). BMI was calculated by dividing the reported weight by the square of reported height. Physical activity, smoking habits, alcohol consumption and education were reported using predefined options.

### 4.2.4 Statistical analysis

In **Study IV**, linear mixed models were used to explore changes in diet over time and differences in mean intake of fruits, vegetables and juice. The two groups, those who developed T2D between the FFQs and those who remained diabetes free, were treated as separate groups from baseline. This allowed for possible differences in intake at baseline. The multivariable linear mixed model was adjusted for age, education, BMI, physical activity, smoking status, alcohol consumption, CVD and/or cancer. An indicator variable was used when covariates had missing data. Sensitivity analysis was performed where those with cancer and CVD were excluded, and possible statistical interaction were examined.

The level of statistical significance was set to p<0.05. Statistical analysis was carried out in Stata 13 (Stata Corp, College Station, TX).
5 RESULTS

5.1 STUDY I AND STUDY II

Baseline characteristics of the study population in Study I/II are presented in Table 5.1, and in respectively paper where it is accompanied by p-values. Briefly, there were differences in levels of uric acid between the three groups with the highest median level in CKD. Baseline levels of inflammatory markers did not differ between T2D and HS.

Table 5.1. Characteristics of the study population in Study I and Study II

<table>
<thead>
<tr>
<th></th>
<th>CKD; n=3</th>
<th>T2D; n=7</th>
<th>HS; n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61 (52, 72)</td>
<td>67 (58, 76)</td>
<td>59 (47, 71)</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>0/3</td>
<td>3/4</td>
<td>3/3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28 (25, 35)</td>
<td>28 (26, 33)</td>
<td>25 (23, 26)</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>2 (1.2, 8.2)</td>
<td>0.8 (0.5, 3.4)</td>
<td>0.6 (0.4, 1.8)</td>
</tr>
<tr>
<td>HbA1c, mmol/L</td>
<td>41 (33, 42)</td>
<td>50 (43, 57)</td>
<td>38 (33, 47)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.1 (4.7, 5.4)</td>
<td>6.9 (5.8, 7.7)</td>
<td>5.4 (4.2, 6.0)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.1 (1.6, 2.8)</td>
<td>1.1 (0.9, 1.7)</td>
<td>1.0 (0.8, 1.3)</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73m²</td>
<td>16 (10, 31)</td>
<td>75 (57, 112)</td>
<td>82 (75, 104)</td>
</tr>
<tr>
<td>Fructose, µmol/L</td>
<td>101 (63, 163)</td>
<td>107 (75, 124)</td>
<td>72 (50, 88)</td>
</tr>
<tr>
<td>Uric acid, µmol/L</td>
<td>530 (422, 563)</td>
<td>339 (241)</td>
<td>317 (185, 404)</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>-</td>
<td>300 (243, 445)</td>
<td>319 (231, 462)</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>-</td>
<td>1.4 (0.5, 2.7)</td>
<td>0.9 (0.1, 2.3)</td>
</tr>
<tr>
<td>IL-18, pg/mL</td>
<td>-</td>
<td>12 (10, 33)</td>
<td>16 (10, 25)</td>
</tr>
<tr>
<td>ICAM-1, pg/mL</td>
<td>-</td>
<td>552 (457, 618)</td>
<td>685 (443, 782)</td>
</tr>
<tr>
<td>VCAM-1, pg/mL</td>
<td>-</td>
<td>1639 (1472, 2154)</td>
<td>1704 (1261, 1916)</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>-</td>
<td>18 (10, 48)</td>
<td>33 (27, 55)</td>
</tr>
<tr>
<td>Diabeteduration, years</td>
<td>-</td>
<td>10 (8, 14)</td>
<td>-</td>
</tr>
</tbody>
</table>

5.1.1 Study I

In Study I, levels of serum fructose increased over time with differences between drinks and drinks+pizza, respectively, for all three groups (T2D; p<0.001, HS; p<0.001 CKD; p<0.001; RM ANOVA interaction term). Interventions with fructose drink resulted in the highest peaks of serum fructose, followed by Coca-Cola and blueberry drink.

Change in levels of serum uric acid are presented in Figure 5.1. Levels of serum uric acid increased over time with differences between drinks and drinks+pizza for all three groups (RM ANOVA interaction term). Patients with CKD attained the highest levels of serum uric acid.
Figure 5.1. Postprandial percent change in serum uric acid among T2D, HS and CKD*

Drinks for T2D; p<0.001

Drinks and pizza for T2D; p=0.003

Drinks for HS; p<0.001

Drinks and pizza for HS; p=0.009

Drinks for CKD; p=0.02

Drinks and pizza for CKD; p=0.004

*Mean values with 95% confidence interval. Solid line = blueberry drink/blueberry drink+pizza, dashed line = Coca-Cola/Coca-Cola+pizza, dotted line = fructose drink/fructose drink+pizza.

When exploring AUC for uric acid, there was a difference between drinks and drinks+pizza, respectively, for HS and T2D. For T2D, the AUC was greater following interventions with fructose drink compared to both sucrose drinks (p=0.004 for both, post hoc analysis). For HS, the AUC was greater following interventions with fructose drink compared to blueberry drink (p=0.016 for drinks, p=0.006 for drinks+pizza).
Postprandial triglycerides did not change over time for HS and CKD following pure drinks (p=0.2 and p=0.6, respectively; RM ANOVA main effect of time). For T2D, there was a difference over time between the interventions (p=0.01, RM ANOVA interaction term). Triglycerides increased over time following drinks+pizza for HS and CKD (p< 0.001 for both; RM ANOVA main effect of time). For T2D there was a difference between interventions in levels of triglycerides over time (p< 0.001; RM ANOVA interaction term). See Figure 5.2 for postprandial triglycerides following drinks and drinks+pizza for T2D and HS.

**Figure 5.2.** Postprandial change in triglycerides (mmol/L) for T2D and HS. Mean values with 95% confidence interval*

Drinks for T2D; p=0.01

Drinks and pizza for T2D; p<0.001

Drinks for HS; p=0.2**

Drinks and pizza for HS; p<0.001**

*Solid line = blueberry drink/blueberry drink+pizza, dashed line = Coca-Cola/Coca-Cola+pizza, dotted line = fructose drink/fructose drink+pizza. **Main effect of time.
5.1.2 Study II

There were no postprandial changes in IL-6 following pure drinks for the subject groups. When pizza was added to the drinks, an increase was observed for HS following interventions with blueberry drink and fructose drink (p=0.03). Postprandial MCP-1 are shown in Figure 5.3. For HS, there was a greater PC decrease in MCP-1 following Coca-Cola compared to pure fructose drink (p=0.03). When examining postprandial changes in ICAM-1, VCAM-1 and IL-18 after drink and pizza, a decrease in ICAM-1 was observed for HS only following blueberry drink and pizza (p=0.03).

**Figure 5.3.** Levels of MCP-1 (median pg/mL [min, max]) 15 min before interventions, and 120 minutes after drink interventions and 240 min after drink + pizza interventions

<table>
<thead>
<tr>
<th>MCP-1 after drinks for T2D</th>
<th>MCP-1 after drinks for HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry drink</td>
<td>p=0.06</td>
</tr>
<tr>
<td>Coca-Cola</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Fructose drink</td>
<td>p=0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCP-1 after drinks and pizza for T2D</th>
<th>MCP-1 after drinks and pizza for HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry drink &amp; pizza</td>
<td>p=0.18</td>
</tr>
<tr>
<td>Coca-Cola &amp; pizza</td>
<td>p=0.03</td>
</tr>
<tr>
<td>Fructose drink &amp; pizza</td>
<td>p=0.03</td>
</tr>
</tbody>
</table>

*Possible differences between baseline and endpoint levels of MCP-1, tested using Wilcoxon matched pairs test. Square= 15 min, circle=120 min/240 min respectively.
Postprandial glucose and insulin from the RM ANOVA are presented in Figure 5.4. Interventions with The Coca-Cola induced the highest peak in glucose and insulin. Postprandial glucose AUC was greater following Coca-Cola as compared to fructose drink for T2D (p=0.002, post hoc analysis). Changes in IGFBP-1 was observed for both groups after blueberry drink, Coca-Cola and all interventions including pizza (all p<0.05). For HS, the decrease was greater following blueberry drink compared to fructose drink (p=0.002).

**Figure 5.4.** Postprandial glucose and insulin among patients with type 2 diabetes (T2D) and healthy subjects (HS) after drinks and drinks + pizza. Mean values with 95% confidence interval*
Insulin after drinks for T2D

Insulin after drinks for HS

Insulin after drinks and pizza for T2D

Insulin after drinks and pizza for HS

*For a-h, Solid line=blueberry drink, dashed line=Coca-Cola, dotted line=fructose drink. **RM ANOVA main effect of intervention.
5.2 STUDY III

Baseline characteristics of the study population in Study III are presented in Table 5.2. Those with T2D had higher levels of hsCRP compared to HS (p=0.004). Other inflammatory markers, or immunoglobulines in urine, did not differ between the groups.

Table 5.2. Baseline characteristics. Values are median (min, max), unless otherwise indicated

<table>
<thead>
<tr>
<th></th>
<th>T2D</th>
<th>HS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>64 (55, 74)</td>
<td>57 (20, 74)</td>
<td>0.02</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>10/11</td>
<td>9/12</td>
<td>1.00</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29 (25, 33)</td>
<td>24 (19, 32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>102 (91, 113)*</td>
<td>85 (68, 112)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>52 (40, 84)</td>
<td>37 (29, 42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.2 (0.6, 3.2)</td>
<td>0.8 (0.4, 2.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.2 (0.8, 2.8)</td>
<td>1.5 (0.8, 2.2)</td>
<td>0.008</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>2.9 (1.6, 5.7)</td>
<td>3.3 (1.5, 5.1)</td>
<td>0.78</td>
</tr>
<tr>
<td>Lipoprotein(a), nmol/L</td>
<td>186 (50, 1227)</td>
<td>79 (50, 691)</td>
<td>0.08</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>1.2 (0.5, 7.2)</td>
<td>0.5 (0.2, 6.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-18, pg/mL</td>
<td>11 (7, 24)</td>
<td>10 (3, 21)</td>
<td>0.78</td>
</tr>
<tr>
<td>PAI-1, pg/mL</td>
<td>1545 (974, 3189)</td>
<td>1460 (760, 7032)</td>
<td>0.26</td>
</tr>
<tr>
<td>ICAM-1, pg/mL</td>
<td>546 (176, 837)</td>
<td>586 (415, 822)</td>
<td>0.14</td>
</tr>
<tr>
<td>VCAM-1, pg/mL</td>
<td>1390 (772, 2192)</td>
<td>1457 (1013, 2348)</td>
<td>0.17</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73²</td>
<td>88 (51, 96)</td>
<td>90 (68, 137)</td>
<td>0.16</td>
</tr>
<tr>
<td>U-albumin, mg/L</td>
<td>11 (4, 85)*</td>
<td>9 (3, 156)</td>
<td>0.36</td>
</tr>
<tr>
<td>IgG2, µg/mL</td>
<td>0.97 (0.11, 9.40)</td>
<td>0.67 (0.07, 18.17)</td>
<td>0.21</td>
</tr>
<tr>
<td>IgG4, µg/mL</td>
<td>0.84 (0.19, 9.07)</td>
<td>0.56 (0.05, 1.86)</td>
<td>0.12</td>
</tr>
<tr>
<td>IgG2/IgG4</td>
<td>1.44 (0.89, 12.68)</td>
<td>1.47 (0.66, 12.11)</td>
<td>0.94</td>
</tr>
<tr>
<td>Diabetesduration, yrs</td>
<td>9 (5, 26)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Tested with Mann-Whitney U test or Fishers exact test, as appropriate. *One missing value.

Postprandial serum glucose, insulin and triglycerides increased over time with differences between interventions for T2D (p<0.001, p<0.001 and p=0.006, respectively [RM NOVA interaction term]) and HS (p<0.001, p=0.002 and p=0.001, respectively [RM ANOVA interaction term]). The highest peaks in glucose and insulin followed intake of HC meals, while the highest peak in triglycerides followed HF meal. See Figure 5.5. Postprandial HDL, LDL and cholesterol is presented as supplementary material in the article.
Figure 5.5. Postprandial serum glucose, insulin and triglycerides among patients with type 2 diabetes (T2D) and healthy subjects (HS) after interventions. Mean values with 95% confidence interval*.
Postprandial changes in PAI-1, IL-18, ICAM-1 and VCAM-1 are presented in Figure 5.6. There were no differences in the inflammatory postprandial response between the interventions for any of the groups (within-group differences).

*High carb meal; solid line w circle, high protein meal; dashed line w square, high fat meal; dotted line w diamond, high carb & fiber meal; semi-dashed line w triangle. Glucose; two T2D not included due to missing values. Insulin; three T2D not included due to missing values (n=2) or extreme values (n=1). Triglycerides; four T2D and three HS not included due to missing values.

$p=0.006$

$p=0.001$
Figure 5.6. Levels, median (box; 25%-75%, whisker; non-outlier range) of PAI-1, IL-18, ICAM-1 and VCAM-1 (pg/mL) before intake and 180 min after interventions*
Urinary IgG4 decreased following all interventions for both groups (all p<0.05), while IgG2 decreased following HC meal (p=0.009) and HC & fiber meal (p=0.005) for T2D, and for HS following HF meal (p=0.02). There were no differences in the postprandial response of IgG2 and IgG4 between the interventions for any of the groups (within-group differences).
5.3 STUDY IV

The study population consisted of 23,953 men, out of which 1,741 (7%) developed T2D between the years 1997 and 2008. Baseline characteristics of the study population in Study IV is presented in Table 1 of the paper. Briefly, men who developed diabetes during follow-up had a higher BMI, lower level of education, were less physically active and more likely to be smokers than those who did not develop T2D.

Baseline intake and the multivariable adjusted model of change in fruit, vegetable, and orange and grapefruit intake was observed among the university educated as compared to those with primary school education. Further, for T2D a greater decrease in orange and grapefruit intake was observed among those without diabetes. Exclusions of those with incident cancer and/or prevalent CVD strengthened the results slightly.

Table 5.3. Baseline intake, change in intake and difference in change between 1997 and 2009 among men who developed type 2 diabetes and among those who remained free from diabetes.

<table>
<thead>
<tr>
<th>Foodstuff, mean intake servings/week (95% CI)</th>
<th>Baseline intake*</th>
<th>Multivariable adjusted‡</th>
<th>Difference in change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-T2D T2D</td>
<td>T2D Non-T2D</td>
<td></td>
</tr>
<tr>
<td>Total fruits &amp; vegetables</td>
<td>13.82 14.19</td>
<td>1.55 (1.08; 2.03)</td>
<td>0.69 (0.54; 0.84)</td>
</tr>
<tr>
<td>Root- &amp; cruciferous vegetables</td>
<td>4.96 5.09</td>
<td>0.92 (0.68; 1.15)</td>
<td>0.35 (0.28; 0.42)</td>
</tr>
<tr>
<td>- Root vegetables§</td>
<td>2.70 2.74</td>
<td>0.44 (0.29; 0.58)</td>
<td>0.23 (0.19; 0.28)</td>
</tr>
<tr>
<td>- Cruciferous vegetables</td>
<td></td>
<td></td>
<td>2.34 2.44</td>
</tr>
<tr>
<td>Fruits¶</td>
<td>8.99 9.20</td>
<td>0.64 (0.27; 1.02)</td>
<td>0.33 (0.21; 0.44)</td>
</tr>
<tr>
<td>-Orange/citrus</td>
<td>1.60 1.56</td>
<td>0.59 (0.45; 0.72)</td>
<td>0.52 (0.48; 0.57)</td>
</tr>
<tr>
<td>Juice, orange/grapefruit</td>
<td>1.72 1.74</td>
<td><strong>-0.55 (-0.71; -0.39)</strong></td>
<td><strong>0.10 (0.05; 0.15)</strong></td>
</tr>
</tbody>
</table>

*Standardized to the age (<50, 50-54, 55-59, 60-64, 65-69, ≥70 years) of the study population. ‡ Linear mixed model. Adjusted for age (<50, 50-54, 55-59, 60-64, 65-69, ≥70 years) and education (primary school, high school, university) in 1997, and BMI (<25, ≥25 - <30, ≥30), smoking (never, former, current), physical activity (seldom-20 min/day, > 20 min/day), alcohol consumption non-drinkers/quartiles, cardiovascular disease (yes/no) in 1997 and 2009, and cancer (yes/no) in 2009. §Carrots and beet roots, ||Cabbage, cauliflower, broccoli/Brussel sprouts, ¶Orange/citrus, apple/pear, banana, other fruit, berries.

The changes in consumption of fruit and vegetable were dependent on age, level of physical activity and level of education. For both subject groups, men who were in the youngest age category and men with the lowest level of physical activity increased their intake more than their respective comparison group/s. For T2D, those with the lowest educational level increased their intake to a greater extent than those with higher educational level while men with the highest level of education had the greatest increase among those without diabetes. Further, for T2D a greater decrease in orange and grapefruit intake was observed among the university educated as compared to those with primary school education.
In the 2009 FFQ, 36% of those with T2D reported consuming the recommended \( \geq 5 \) servings of fruits or vegetables/d, and the corresponding proportion for HS was 35%.
6 DISCUSSION

6.1 STUDY I, STUDY II AND STUDY III

6.1.1 Main findings Study I and Study II

The main findings in Study I were that serum uric acid increased following fructose loading, and the blueberry drink induced the lowest increase. Those with CKD attained the highest levels of serum uric acid, followed by those with T2D.

In Study II, a decrease in MCP-1 was observed in both subject groups following fructose loading. The baseline levels of inflammatory markers and the postprandial inflammatory response following fructose loading did not differ between T2D and HS.

6.1.2 Main findings Study III

The main findings in Study III were that HC meals resulted in higher postprandial glucose and HF meal in higher triglycerides, suggesting increased inflammation. There were decreased responses in PAI-1, ICAM-1, VCAM-1 and immunoglobulins for the groups but the markers were not modulated by meal composition within the groups.

6.1.3 Common general and methodological considerations

6.1.3.1 General considerations and discussions

As described in the background, postprandial metabolic responses may depend on several factors as total calorie intake, composition of the meal and type of micronutrients consumed among others. And it may not only depend on nutrients consumed, but also on what is not consumed. The metabolic responses may further differ depending on disease status.

With regards to macronutrients, quality rather than quantity is emphasized in dietary recommendations (9). The meal in Study I/II consisted of easily accessible carbohydrates (pizza and a fructose containing drinks) with the purpose of representing a common Western meal. In Study III the different meals were home cooked meals consisting of red meat, potatoes (for the HF meal the potatoes were served as French fries) and vegetables. There were discussions on serving fatty fish, with beneficial n-3 polyunsaturated fatty acid, instead of red meat and that might have given other results than those observed. Fatty fish is also recommended in the dietary treatment for diabetes (9).

It has been observed that those with T2D have a delayed response in insulin and higher and longer lasting blood glucose levels after a meal compared to HS. Further, those with T2D has a greater postprandially hyperlipidemia (14). This is supported by results in Study III, see Figure 5.3. Thus, when examining postprandial responses follow-up time might be of importance. The effect on other markers, as inflammatory markers, might be delayed as well.

In Study III, inflammatory and urinary markers were explored 180 minutes after intake of meal. Following fructose loading (Study I and Study II), follow-up was only 120 minutes and when pizza added to the drinks, 240 minutes, which should be kept in mind.
The postprandial glucose following Coca-Cola and pizza in the fructose load study was greater compared to the postprandial glucose following HC meals in the standardized meal study. The easy accessible carbohydrates in the pizza compared to the home-cooked meals and the carbonic acid in the Coca-Cola might explain this finding. The effect of carbonic acid on gastric emptying is however discussed (126).

Postprandial responses may be affected by factors other than those associated with a single test meal. Regular consumption of certain nutrients may have an effect on the single test meal, hypothesised to be explained by modulation of immune response (14). Also, antioxidant vitamins given before breakfast has shown to prevent a rise in PAI-1 following a HF dinner with a four hour follow-up (84). There is no information on dietary habits of study participants prior to the interventions included in this thesis. Study participants were not given any prior dietary restrictions except for fasting prior Study I/II and a standardized breakfast prior to the lunches in Study III. Also, there might be differences between women and men. For example, there has a tendency to lower serum concentrations of vitamin E been found among men compared to women (127). Separate analysis of men and women has not been performed in Study I-III.

Study participants ingested the same amount of calories in the intervention studies independent on gender, body size and level of physical activity etc. For some, the calorie intake was greater than they would normally consume, for others lower. As portions sizes were not individualized, some results might be different from what would be observed in a more natural setting. Further, the intervention studies included in this thesis only examined acute effects of different meal compositions and fructose loading. Thus, no conclusions on long-term effects can be drawn.

There are some factors concerning risk markers of inflammation worth mentioning. Circulating levels of inflammatory markers may not reflect inflammation in a specific organ or within a specific tissue (7). Only circulating and urinary markers were examined in the intervention studies. Also, markers may be age dependent. An age dependent association for levels of VCAM-1 among those with different risk of atherosclerosis has been found, while the associations between age and levels of ICAM-1 was found only among HS (128). In Study III, those with T2D were older than HS subjects. However, no differences in baseline levels of inflammatory markers were observed in any of the intervention studies included in this thesis. Levels of urinary IgG2 and IgG4 did not differ between groups either.

6.1.3.2 Blood and urine specimens

The collection and handling of specimen may have an impact on the test results, and thus subsequently the results presented. The blood specimen collection process was following a protocol (presented in the method section), meaning it was collected and handled in a standardized way and by experienced personnel. However, there are still steps in the process that should be acknowledged.

Blood was sampled through an intravenous catheter that was in place throughout the follow-up time. It has been observed that a venous catheter in place for a longer time increased levels of IL-6 over time as compared to samples obtained through single needle stick (129, 130).
Other markers (IL-8 and hsCRP examined) were not affected by sampling method (130). Thus, there might be a local inflammatory effect and not a systemic effect for some markers (129, 130). An increase in IL-6 was observed for HS following drinks and pizza (4 hours follow-up time) in Study II. No increase in IL-6 was observed among T2D after drinks and pizza, and there are no indications that the sampling method would affect T2D differently than HS. The sampling method was the same the same for T2D and HS in the studies, and other inflammatory markers decreased postprandially.

Further, storage and freeze-thaw cycles may affect stability of cytokines. The inflammatory markers explored in Study II and Study III were analyzed in samples not previously thawed. Samples have however been stored for a longer time in -80°C before being analyzed. In a review examining stability of some cytokines, serum and EDTA plasma of IL-6, IL-18 and MCP-1 show stability when stored in -80°C for various time periods (131). ICAM-1 and VCAM-1 has shown to be stable after freezing (132). Storage of samples was the same for the subject groups in the studies and it is unlikely that it would have a major impact on observed results.

Urine samples were stored in -80°C. As IgG has shown to be sensitive to storage in -20°C IgG, and -70°C has been recommended for longer storage (133).

6.1.3.3 Statistical analyses

Parametric and non-parametric methods were used to explore postprandial markers. Some of the markers were not normal distributed and therefore analysed using non-parametric methods. The number of participants in each group in Study I and Study II are very small, and the choice of using RM ANOVA can be discussed. Study I was however complemented with non-parametric tests for serum fructose %/AUC and serum uric acid %/AUC for within group analysis, and median (min, max) values are presented. In Study II, the main results are analysed using non-parametric methods and the RM ANOVA for glucose, insulin and IL-6 and MCP-1 are presented as supplementary material. The small number of participants in Study I and Study II may have had an impact on observed results, as differences that exist were not found.

Statistical analyses are unadjusted for multiple testing, which is accounted for in each article. The choice of not adjusted for multiple testing may have resulted in chance associations (type 1 error; the rejection of a true null hypothesis). However, as T Perneger state, the decrease in type 1 error will inevitably result in an increase in type II error (accepting a false null hypothesis). The author further states that the best approach is to describe what has been done, and discuss the results, instead of adjusting for multiple testing (134).

6.1.4 Discussions and interpretations Study I and Study II

In Study I, levels of serum uric acid increased following fructose loading. The highest levels of serum uric acid at baseline and after fructose loading were observed among those with CKD, followed by those with T2D. Those with CKD had however, a smaller percent increase compared to T2D and HS. This response has been observed previously in CKD (98). In T2D, an increase in serum uric acid following 75 g of fructose has been observed (99), which
supports findings in Study I. When fructose (30 g/d) was isocaloric replaced with starch for two months, there was no effect on levels of uric acid. The total daily calorie intake in the study, also the intake prior to the study, was 1400-1600 kcal (120). In HS, an increase was observed in serum uric acid following Coca-Cola and pure fructose drink, supported by some previous observations in HS (95, 96). However, when fructose was ingested in smaller servings for a longer time (0.2 g/kg every hour for 9 hours), no increase was observed (97).

In Study I, an increase in triglycerides was observed only following intake of fructose containing drinks and pizza. Among those with CKD, an increase in triglycerides has previously been observed following intake of fructose only. The intake of fructose was 70 g and the follow-up time 240 min (119). Fructose contributes to hepatic de novo lipogenesis to a greater extent than glucose, but the pathway is reported to represent a minor part of the overall fructose disposal (135).

In Study II, decreased levels of IGFBP-1 were observed following blueberry drink and Coca-Cola for both HS and T2D. For HS, a difference between the drinks was also observed were the decrease was greater following blueberry drink compared to the pure fructose drink. The observed results may be explained by the lower observed increase in insulin following fructose drink. Worth noting is that a reduced suppression in IGFBP-1 has previously been associated with abnormal glucose metabolism, that is future development of T2D (136).

An increase in IL-6 was observed only in HS following intake of drinks in combination with pizza (Study II). Previous studies show conflicting results following HC meal in HS and T2D (80, 83, 137, 138). The lack of response in IL-6 among those with T2D might be explained by a delayed response in glucose. Responses in MCP-1 has previously been explored following longer interventions (4 and 10 weeks, respectively) (113, 115). In Study II, a decrease or no response was observed in MCP-1 among HS and T2D. In HS, a greater decrease following Coca-Cola compared to the fructose drink was also observed. These observations of decreased MCP-1 might partly be explained by the increase in insulin (139).

In Study II, responses of fructose on levels of IL-18, ICAM-1 and VCAM-1 was explored only in combination with pizza. There was no response in IL-18 for any of the groups. These results are supported by previous studies following HC meal (81, 83), and by results in Study III. ICAM-1 decreased only following blueberry drink+pizza in HS, while no responses were observed in VCAM-1 for any of the groups. A previous study examining postprandial ICAM-1 and VCAM-1 following HC meal, observed an increase in T2D only. The increase was prevented with vitamins (80). There was no response in ICAM-1 or VCAM-1 following HC meal in Study III.

The excessive doses of fructose used in some studies, not reflecting a normal intake, are discussed in the literature. In the general US-population, intake of fructose has been observed to be 49 g/d (50th percentile) (140). The mean intake of monosaccharides (mainly glucose and fructose) in the adult population in Sweden is about 30 g/d (141). The fructose dose used in the fructose drink in Study I/II is thus above the mean intake of fructose in the general population
in Sweden. Whether the intake of fructose is consumed as excessive calories is also a matter of discussion, as it may confound possible negative health effects found (135, 140).

Fruits and berries contain fructose, although lower amounts (88, 89). However, they also contain fibers and phytochemicals with a positive effect on health (142). The results in Study I suggests that blueberry drink is protective as it gave the lowest concentrations of serum uric acid. Against the results in Study I, a protective effect of the blueberry drink on inflammatory markers, as compared to the other drinks, was hypothesized in Study II. However, there was only a greater postprandial decrease in MCP-1 for HS following Coca-Cola compared to fructose drink. No other postprandial difference in IL-6, MCP-1, ICAM-1, VCAM-1 or IL-18 between the interventions was observed. The blueberry juice used had been pasteurized and it has been observed that treatment, as increased temperature, has a negative effect on the anthocyanin’s (143). Also, the fiber content of the juice might be reduced as compared to blueberry’s (142). The postprandial response of fructose loading on ICAM-1, VCAM-1 and IL-18 was only explored in combination with pizza. Thus, no conclusion can be drawn on the effect of fructose alone on these markers.

The choice of using isocaloric drinks (140 kcal) in Study I/II resulted in that the fructose drink contained 35 g fructose, while the Coca-Cola and blueberry drink contained ~18 g. This makes comparisons between the pure fructose drink and the other two drinks limited when it comes to dosage. Further, there is no consideration taken to gender in Study I/II. In a population-based study, associations between intake of added sugar or sugar sweetened drinks and uric acid levels were found among men only (144).

6.1.5 Discussions and interpretations Study III

As expected, HC meals gave the highest responses in glucose and insulin, and HF meal gave the highest response in triglycerides. These results suggest increased inflammation but explored inflammatory and urinary markers were not modulated by meal composition within subject groups.

IL-18 showed no response in the two groups in. Comparing with previous studies, the lack of responses has been observed following HC meal in HS and T2D. When the HC meal was accompanied with fiber (17 g, as compared to 15 g in Study III), a decrease has been observed. Following HF meal, IL-18 has been observed to increase in T2D, while results among HS are mixed (increase and decrease) (81, 83). Postprandial IL-18 was also explored in Study I, and no responses was observed following intake of pizza and drinks in T2D and HS.

In Study III PAI-1 decreased following HC, LC+HP and LC+HF meal among T2D. Previous studies following HF meal show conflicting results in this population, with an increase and a decrease (84, 85). The observed increase was prevented (baseline levels at the end of follow up) with vitamins (84). A decrease following a HF and a HC meal has further been observed among those with the metabolic syndrome, which supports the results among those with T2D.
in Study I (86). A previous study exploring postprandial PA-1 following HF meal in HS show no response (87), which is supported by findings among HS in Study III.

VCAM-1 decreased following LC+HP meal in both groups. In HS, an increase and no response following HF meal has previously been observed, where the increase was prevented with vitamins (80, 82). Previous observations of no response following HC meal in HS support findings in Study III. In T2D, the no response following LC+HF and HC meal is supported by previous observations only when meal was accompanied by vitamins (80). Also, no responses in VCAM-1 were observed in Study II following intake of pizza and drinks.

ICAM-1 decreased postprandial following LC+HF meal in T2D and LC+HP meal in HS. In HS, the lack of response following HF meal is supported by Tsai et al (82). Nappo et al observed a no response only when vitamins accompanied the meal. They further observed a lack of response following HC meal (80), which is supported by findings among HS in Study III. An increase in ICAM-1 following HF and HC meal was also prevented with vitamins in T2D (80). In Study III, there was no response in T2D following HC meal, thus only supporting previous results when meal was accompanied by vitamins (80). Postprandial ICAM-1 was also explored in Study II, and a decrease was observed only following pizza and blueberry drink for HS.

Urinary IgG4 decreased following all meals for both groups, and IgG2 following the both HC meals in T2D and the HF meal in HS. Postprandial IgG2 and IgG4 was not modulated by meal composition for any of the two groups. To the best of knowledge, no other studies explored postprandial IgG2 and IgG4 following different meal compositions.

Comparing results from Study III with previous studies is challenging. The definition of high carbohydrate meal vs high fat meal is one of the challenging factors. For example, in the study exploring postprandial IL-18 the carbohydrate content was higher in the HC meal (~70% and ~76%, compared to ~50% in Study III) and the fat content was higher in the HF meal (60% and ~77%, compared to 50% in Study III) (81, 83). Further, in Study III the meals were home cooked. Some previous studies used test meals of fast food character, hamburgers, shakes and/or muffins, and with and/or without vitamins etc. (80, 84, 86, 87), thus making comparisons difficult. Also, some responses are explored fasting, others non-fasting. Further, disease status among those with T2D might vary. In Study III, median HbA1 was 52 mmol/mol which is acceptable.

6.2 STUDY IV

6.2.1 Main findings

In Study IV, an increased intake of fruit and vegetables and a decreased intake of juice were observed among those who developed T2D. The changes observed among T2D was greater
than changes observed among men who remained diabetes free. The proportion of those with T2D who consumed the recommended daily servings of fruits and vegetables was 36%.

6.2.2 Methodological considerations

Cohort studies with self-reported questionnaire and register data makes it possible to perform large studies, epidemiological observational studies, which may provide with valuable hypothesis-generating information regarding potential associations. However, several methodological issues, errors, may arise when performing epidemiological observational studies. Errors in epidemiological studies can be categorized into two broad types; systematic and random errors, see Figure 6.1.

**Figure 6.1.** Systematic and random errors

<table>
<thead>
<tr>
<th>Systematic error (validity)</th>
<th>Random error (precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection bias</td>
<td>Confounding</td>
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<tr>
<td></td>
<td>Misclassification (information bias)</td>
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<tr>
<td></td>
<td>Exposure</td>
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<td></td>
<td>Outcome</td>
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</table>

6.2.2.1 Systematic errors

Systematic errors include selection bias, confounding and misclassification. Systematic errors are errors that would remain despite increased study size.

Rothman describes selection bias as a systematic error “that stems from the procedures used to select subjects and from factors that influence study participation” (145). The error is present when the association between exposure and outcome differs between participants and eligible non-participants of the study. In the COSM, all eligible men were invited to participate in the study and the baseline cohort represents the Swedish population well with regards to age distribution, educational level, BMI (146) and proportion of men with diabetes (124, 147). However, the response rate for the COSM was 49% in 1997, and one cannot rule out bias due to study participation. Loss to follow-up is also a concern in cohort studies. In Study IV, cases of diabetes/T2D were identified through the NPR and NDR. The NPR contains information on visits in inpatient- and specialized outpatient care, thus excluding the primary care where most patients with T2D are treated. However, the NDR was used as a source as well and the coverage of the registry has improved. The loss of follow-up was minimized by using both the NDR and the NPR, but one cannot rule out bias. To introduce selection bias due to loss to follow-up, it
would however have to be related to changed consumption of fruit, vegetable and juice. There is corresponding female cohort to the COSM, the Swedish Mammography Cohort (SMC). The SMC was not included in Study IV due to lower coverage of the study area in NDR.

The word confound comes from the Latin word confundere, meaning pour together or mix. Confounding can be explained as confusion of effects and the concept is an important issue in cohort studies. When confounding is present the 1) exposure of interest and the potential confounder are associated with the outcome, 2) the exposure and the confounder are associated, and 3) the confounder is not caused by the exposure. A confounder is not in the causal pathway and should be adjusted for in the statistical analysis. In Study IV, potential confounders are included in the analysis and age-adjusted and multivariable results are presented. Covariates included in the model stems from registry data and self-reported information and residual confounding cannot be ruled out. Also, there may be additional information, as medical treatment, that may have an impact and is unknown.

Misclassification of exposure and outcome (information bias) can be divided into differential errors or non-differential errors. Differential errors refer to misclassification, that are dependent on the value of other variables, while non-differential errors refer to misclassification that are not dependent of other variables. In Study IV dietary intake is self-reported and thus may be subject to some variability due to difficulties to recall intake properly (most likely non-differential). However, in 2009 those with T2D might have been influenced by their diagnosis and inflate reported intake of fruit and vegetable. This can result in differential misclassification and greater observed change among those with T2D compared to those who remained free of diabetes. With regards to classification of T2D, there may be some misclassification on year of debut as information of visits that are recorded in the NPR does not equal date/year of disease onset. The misclassification may also apply for the NDR although quality has improved. There may also be some misclassification on type of diabetes. This should however not have an impact on result as dietary treatment is of importance for diabetes in general.

6.2.2.2 Random errors

Random errors accounts for the variability of the data that is due to chance. The variability of the data, the precision, is in present study explained by a 95% confidence intervals (CI) (if replicated, the correct value would be included in the CI 95% of times). As study size increases, the CI becomes narrower, decreasing the variability and increasing precision. Thus, large studies have the advantage to be able to detect small absolute differences as CI are getting narrower. This can be illustrated by the change in total fruit and vegetable intake (servings per week) among the subject groups: T2D; 1.55 (95% CI 1.08; 2.03) and non-T2D 0.69 (95% CI 0.54; 0.84). A difference in changed intake is observed, but the absolute difference of 0.86 (95% CI 0.38; 1.35) servings per week might be perceived as small.

The interpretation of the precision assumes no systematic errors. However, there might be systematic errors remaining, see Systematic errors e.g. confounding. Thus, this should be taken into considerations when interpreting findings.
6.2.3 General discussions and interpretations

Besides methodological considerations previously discussed, there are secular trends in diet that could affect findings in Study IV. The National Food Agency compared nutritional habits between the years 1998 to 2011. The report indicated that intake of fruit and berries, root vegetables and other vegetables has increased both among men and women, but a greater increase was observed among women (141). It has further been observed that older adults had a better dietary pattern than younger adults (148). In a study among Swedish elderly men and women (70-year olds) it was observed that there was an increase in consumption of vegetables and fruit between the years 1971 and 2000 (149). Thus, as an increase in intake of fruit and vegetables was observed also among those without diabetes, some changes among those with T2D might be attributed to secular trends in diet and not disease status. This is supported by the increased intake also among those who remained free from T2D in Study IV.

Longitudinal studies examining changes in fruit and vegetable consumption after a T2D diagnosis have shown no or decreased intake in fruit and vegetable consumption (67, 68), which is in contrast with findings of increased intake in Study IV. However, findings in Study IV is supported by a cross-sectional study, including participants from Sweden, that observed a greater increase of fruit and vegetables intake among those with T2D compared to those without diabetes. In addition, a low intake of juice among participants from Sweden was noted (74), which is supported by decreased intake observed in Study IV.
7 CONCLUSION

Dietary treatment is of importance in T2D to improve metabolic control in order to postpone complication. There are however gaps in the scientific evidence for dietary recommendations in this patient group. In this thesis, the acute postprandial responses to fructose loading and different meal compositions on risk markers for future complications in T2D were examined and compared to HS. Further, possible changes in fruit, vegetables and juice consumption after a T2D diagnosis were explored.

Levels of serum uric acid increased following fructose loading in both HS and T2D as well as in CKD. Increased levels of serum uric acid, previously associated with negative health effects, was observed even at low doses of fructose. The blueberry drink induced the lowest increase in serum uric acid and may thus be protective. Further, Coca-Cola with or without pizza induced higher responses in glucose and insulin compared to the blueberry drink, although the two drinks had similar fructose and sucrose content. This suggests a protective effect of the blueberry drink also on glucose, maybe explained by increased insulin sensitivity.

Postprandial inflammatory responses of fructose loading were examined in HS and T2D. The fructose loading resulted in a postprandial decrease in MCP-1 in both groups. In HS, the decrease was greater following Coca-Cola compared to fructose, possibly explained by a higher peak in insulin following Coca-Cola. There were no difference in baseline levels nor in postprandial inflammatory response between the two groups, which might be explained by well-controlled diabetes among those with T2D.

Postprandial responses of different isocaloric meal compositions were examined in T2D and HS. HC meals induced highest peaks in glucose, and in T2D also high levels of triglycerides. Adding of fiber to the HC meal gave lower responses in glucose and insulin for T2D. The LC+HF meal gave the highest peaks in glucose and triglycerides for both groups. The increase in glucose and triglycerides suggest a postprandial inflammatory response. The inflammatory responses explored were however not affected by meal composition, which might be explained by the content of vitamins, antioxidants and fiber etc. in the home-cooked meals.

The intervention studies suggests that drinks with high concentrations of fructose and sucrose, as Coca-Cola, should be avoided. Further, those with T2D could be recommended a diet low in carbohydrates (30E%) with focus on the quality of included macronutrients, such as fibers, fruits and vegetables, mono- and polyunsaturated fats, including plant-based protein.

Changes in fruits, vegetable and juice intake were explored among men who developed T2D and those who remained free from diabetes. Men who developed T2D increased their intake of fruits/berries and vegetables to a greater extent than those who remained free of diabetes. A decreased juice intake was also observed among men who developed T2D. Although improvements in diet were observed, the proportions of men with T2D who fulfilled the recommended ≥5 servings of fruits or vegetables day was low, 36%. Thus, there is a need for education and support amongst those with T2D.
8 FUTURE PERSPECTIVES

This thesis contributes to a small piece of the puzzle regarding acute metabolic and inflammatory responses from fructose loading and different meal compositions, as well as whether changes in fruit, vegetable and juice consumption occur after a T2D diagnosis.

Studies examining the responses of fructose loading has been criticized using high doses of fructose, not reflecting a normal intake. In today’s society, the intake of fructose, mainly through soft drinks, may however vary greatly. Also, the responses may differ depending on disease status. Long term studies of inflammatory responses in different populations, where fructose containing beverages in different amounts are ingested in combination with meals, are needed to reflect the society of today.

There are gaps in the literature regarding acute responses following home-cooked meals with different compositions and quality of macronutrients, and future studies in this area is needed. Also, in settings with more personalized portion sizes. With regards to the study included in this thesis there is further information available and thus analysis to be made. There are data on other inflammatory markers as well as urinary markers available, and that will be analyzed. The metabolic and inflammatory responses are of great importance, but personal aspects should not be forgotten. Included in the study is for example questionnaire on perceived satiety and hunger for the different meals, and that can be complemented with markers as leptin. Further, there is a need for long term studies in this area. As discussed previously, the responses might be affected by regular consumption of nutrients. Further, there is data available among those with type 1 diabetes.

Increased intake of fruit and vegetables and a decreased intake of juice was observed in men who developed T2D. The food frequency questionnaire used in the study also include other dietary information but also other lifestyle factors. Thus, there is a possibility to explore changes in other dietary factors and e.g. level of physical activity. The dietary data could further be used to explore associations to complication outcomes among those with T2D.
9 ACKNOWLEDGEMENTS

First, I would like to thank the study participants in the Fructose load study, the Standardized meal study and the COSM. Without your participation in the studies this work could not have been performed. Thank you for your contribution!

To my main supervisor, Neda Rajamand Ekberg: Always available for discussions in person and over the phone. Thank you for being positive, for standing solid through this work and for encouraging me at times of struggle. And thank you for valuable discussions where you shared your knowledge within the field, for challenging me (in a positive way!) and made me think a second round.

To my co-supervisor, Kerstin Brismar: you believed in me even when I didn’t. Without your support and encouragement, this thesis would not exist. I am grateful for everything you have done for me, professional but also personal. And thank you for sharing your great knowledge within the field of diabetes and doing it so with patience. You have a sense for details no one else has. You are a true inspiration!

My co-supervisor, Nicola Orsini: your knowledge in statistics contributed to these studies and helped me along the way. I do however have one of your more ”simple” phrases in my mind a lot; Don’t complicate things! Thank you!

To the co-authors of the papers included in this thesis: Peter Stenvinkel, Björn Anderstam, Monica Eriksson, Annki Bragfors-Helin, Bengt Lindholm, Tony Qureshi, Wieslaw Wiczkowski, Ole Torffvit, Inga-Lena Andersson. Thank you for your work with the intervention studies, for good discussions and quick responses and for being welcoming! Alicja Wolk for letting me work with data from the COSM, Agneta Åkesson for good discussions concerning the study based on the COSM-data, and Andrea Discacciati for great support with the mixed model.

Agneta Hilding, you are not only a co-author and a support, you have become a friend that I miss daily at work. Luckily, you come by occasionally for lunch! Thank you for everything!

To all of you involved in the studies in other ways: Anette Landström, my great company in the freezers! Elisabeth Norén Krog, Magnus Bentinger, Kajsa Sundqvist, Anette Härström, Sofia Geschwind among others. Thank you for being supportive and caring!

There are those whom I did not have the privilege of working together with but were involved in other ways. Daily chitchats, asking me how things are moving along, providing me with valuable vacation activities, reminding me to take a coffee break etc. Michael Alvarsson, Claes-Göran Östensson, Gustav Dallner, Ileana Botusan, Lotta Öberg and Päivi Lundvall, Kerstin Sännås among others - Thank you! And Claes-Göran, thanks for accepting being chair at my dissertation.
To other colleagues and PhD students I have met along this journey and who made life more enjoyable, many becoming close friends: Susanne, I have no idea what I would have done without your support! Meike and Tilo, for lunches/dinners and for trusting us with your beautiful children. Mimi and Heather, for dinners after work. Thanks also to: Ann Burgaz, Jinjin Selin, Josefin Löfvenborg, Bettina Julin, Alice Wallin, Alessio Crippa, Andrea Bellavia, Holly Ruth Harris, Louise Sjöholm, Johanna Falk, Elina Scheers Andersson and Afrodit Barouti.

For help with the administration concerning the PhD studies, Ann-Britt Wikström and Katarina Breitholtz. For the IT-support, Jan-Erik, Zebastian and Thomas (A cup of coffee spilled over my laptop as a highlight). Always with a smile - Thank you!

Vill också rikta ett tack till mina härliga kollegor vid Folkhälsomyndigheten. Det är roligt att gå till jobbet och det är ni starkt bidragande till! Ni har också stöttat mig under denna period av delad arbetstid, och där vill jag särskilt rikta ett tack till min chef Maria Axelsson.

Till familj och vänner:


Svärfar Omar och Eva, tack för lugn och händelser i en härlig Norrländsk blandning.


Mathilda och William, ni betyder allt för mig! Tack för att ni finns och förgyller våra liv. Det är så härligt att få spendera tid med er, med allt från intressanta samtal till minnesvärda upplevelser. Det blir aldrig tråkigt med er!

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Camilla Olofsson