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Dietary Intake, Fatty Acid Biomarkers, and Abdominal Obesity

Population-Based Observational Studies

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Abstract

Alsharari, Z. 2017. Dietary Intake, Fatty Acid Biomarkers, and Abdominal Obesity. Population-Based Observational Studies. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1349. 47 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0024-5.

The aim of this thesis was to investigate the associations between fatty acid (FA) biomarkers, carbohydrate intake, and abdominal obesity (AO) and related anthropometric measures in a population-based cohort of men and women in Stockholm County. The overall hypothesis was that dietary fat quality assessed by serum and adipose tissue FA composition, and dietary intake of especially carbohydrates is associated with AO. FA composition was assessed by liquid gas chromatography, and AO was measured as waist circumference (WC), waist hip ratio (WHR) and sagittal abdominal diameter (SAD). Dietary intake was assessed by 7-day food records.

Papers I, II, III, and IV were all observational studies based on a Swedish population in Stockholm County (n=5460). A sub-cohort of only men (n=301) was included in Papers II, III, and IV.

In Paper I, serum proportions of the polyunsaturated FA (PUFA), linoleic acid (LA) (18:2n6), was inversely associated with AO in both men and women, whereas a positive association was observed between the saturated FA (SFA), palmitic acid (PA) (16:0) and AO measures. These findings support recent interventional studies suggesting that a higher relative intake of PUFA (LA) from vegetable oils as compared with 16:0 is associated with decreased abdominal adiposity.

In Paper II, we investigated whether biomarkers of dietary fat quality were related to the corresponding FA intake from fat-rich foods reported in a short food frequency questionnaire (FFQ). Serum proportions of the long-chain n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) were higher among men with higher total fish intake. Serum LA was higher among men who reported a consumption of more than 5 g/d of margarine. Absolute agreement between intakes assessed with FFQ of 60YO and 7-day food record of "Kost och Metabola syndromet"/"Diet and the Metabolic syndrome" (KOMET) was highest for alcohol, total fish, and eggs. Weighted Kappa statistics revealed the strongest agreement for alcohol, margarine, and fruits.

In Paper III, carbohydrate intake was inversely associated with 16:0 in serum phospholipids (PL). Disaccharide and alcohol intake was positively and non-linearly associated with palmitoleic acid (16:1) and stearoyl-CoA-desaturase (SCD) activity in PL. Alcohol was consistently associated with higher SFA and monounsaturated FA (MUFA).

Results of Paper IV indicated that total carbohydrate intake was inversely associated with measures of AO and central fat distribution, WHR and SAD, respectively. Likewise, monosaccharide intake was associated with lower AO. In contrast, alcohol intake was associated with AO prevalence and all anthropometric measurements.

In conclusion, serum SFA (palmitic acid) was positively associated with AO, whereas n-6 PUFA (linoleic acid) was associated with lower AO. High intake of total carbohydrate and monosaccharides were associated with lower AO. Overall, these results support a beneficial role on adiposity of diets that are higher in polyunsaturated fat (vegetable oils) and total carbohydrates compared with saturated fat.

Keywords: abdominal obesity, fatty acid biomarker, dietary intake

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Alsharari ZD**, Risérus U, Leander K, Sjögren P, Carlsson AC, Vikstrom M, Laguzzi F, Gigante B, Cederholm T, De Faire U, Hellénius M-L, Marklund M. Serum Fatty Acids, Desaturase Activities and Abdominal Obesity - A Population-Based Study of 60-Year Old Men and Women. PLoS One 2017;12.

II **Alsharari ZD**, Marklund M, Leander K, Risérus U, Vikstrom M, Laguzzi F, Gigante B, Cederholm T, De Faire U, Hellénius M-L, Sjögren P. Comparison of a 21-item food questionnaire with a 7-day dietary registration and biomarkers of fat intake in a Swedish cohort of 60-year-old adults. 2017 (*Submitted manuscript*).

III **Alsharari ZD**, Leander K, Sjögren P, Carlsson AC, Cederholm T, De Faire U, Hellénius M-L, Marklund M, Risérus U. Association between carbohydrate intake and fatty acids in the de novo lipogenic pathway in serum phospholipids and adipose tissue among 63-year old men. (*Manuscript*).

IV **Alsharari ZD**, Risérus U, Leander K, Sjögren P, Cederholm T, De Faire U, Hellénius M-L, Marklund M. Carbohydrate Intake and Abdominal Obesity in Swedish Men. (*Manuscript*).

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Contents

Abbreviations

ALA	Alpha-linolenic acid
AO	Abdominal obesity
AT	Adipose tissue
BMI	Body mass index
CE	Cholesterol esters
CFR	Carbohydrate to fiber ratio
CVD	Cardiovascular disease
D5D	Δ5-desaturase
D6D	Δ6-desaturase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FFQ	Food frequency questionnaire
LA	Linoleic acid
MUFA	Monounsaturated fatty acid
PA	Palmitic acid
PL	Phospholipid
PUFA	Polyunsaturated fatty acid
SAD	Sagittal abdominal diameter
SFA	Saturated fatty acid
SCD	Stearoyl-CoA-desaturase
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-hip ratio

Introduction

Obesity epidemic

The worldwide prevalence of obesity was more than doubled between 1980 and 2014(1). About 13% of the world's adult population were obese in 2014 (11% of men and 15% of women) according to global estimates by World Health Organization (WHO)(1). In European countries, obesity is responsible for 10% to 13% of the annual death toll. The prevalence of obesity has increased worldwide(2). According to WHO, overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health(1). There is a wealth of epidemiologic evidence linking obesity with an increased risk of metabolic syndrome(3, 4) and non-communicable diseases (NCDs), such as cardiovascular disease (CVD)(5, 6), cancer, and diabetes(7).

Globally, a fundamental cause of obesity and overweight is an energy imbalance between energy consumed and energy expended(1). Mechanisms responsible for increasing visceral fat storage include dietary fructose, local cortisol production in abdominal adipose tissues, endocannabinoids, and sex or growth hormones(8). Features of abdominal adipose tissues that increase the cardiometabolic risk associated with visceral obesity include lipid storage capacity, adipocyte size and number, lipolytic responsiveness, and inflammatory cytokine production(8). General obesity is usually estimated by body mass index (BMI) expressed as kg/m^2, whereas regional adiposity can be estimated using anthropometric measurements of WC, WHR, and SAD for AO (9). WC and WHR provide indirect measures of central adiposity, and demonstrate the incremental prognostic value of AO compared to BMI alone(10). Therefore, WC is the recommended measure for AO rather than BMI(9).

Both peripheral obesity (accumulation of subcutaneous adipose tissue) and AO (accumulation of visceral adipose tissue) are associated with increased risk of metabolic syndrome and consequences that include dyslipidemia, diabetes, hypertension, and atherosclerosis(11-13). Visceral adipose tissue has been suggested to have a stronger metabolic activity than subcutaneous adipose tissue due to the release of more free fatty acid, adipocytokines, hormones, and inflammatory factors, which flux into the liver directly via the hepatic portal vein(14, 15). The portal vein importantly contributes to the development of hepatic insulin resistance and hepatic steatosis

through high free fatty acid flux (15). These features indicate that accumulation of visceral adipose tissue might be a clinical target for prevention and treatment of AO. AO is the most prevalent manifestation of metabolic syndrome, is a marker of dysfunctional adipose tissue, and is fundamental in clinical diagnosis (9). AO is defined by WC, using the National Cholesterol Education Program-adult Treatment Panel (ATP) III cut-off points >102 cm for men and >88 cm for women (Table 1.)(16)

Table 1. Abdominal anthropometric measures

Abdominal obesity measures	Males	Females
Waist circumference (WC)[1]	WC >102 cm	WC >88 cm
Waist-Hip Ratio (WHR)[1]	WHR >0.9	WHR >0.85
Sagittal abdominal diameter SAD)[2]	<25 cm for persons of normal BMI	

1. *NCEP-ATP III and WHO* ***cut-off points and risk of metabolic complications(17)***.
2. ***National Center for Health Statistics (2013) Anthropometry Procedures Manual - National Health and Nutrition Examination Survey (NHANES)****(18)*.

Dietary habits

Dietary habits and environmental factors have a strong impact on health and development of NCDs. In the Swedish population, food consumption has changed from predominantly animal-based foods to more plant-based foods(19). The dietary emphasis on vegetables, whole grains, lean dairy products, fish, and vegetable oil has decreased the risk of NCDs including CVD, over-weight/obesity, and type 2 diabetes(20, 21). Currently, half of Swedish adults are overweight (i.e., BMI >25 kg/m^2)(22). The increased prevalence of obesity in the past few decades(23) has likely been driven by increased food supply and total energy intake(24). The main cause for overweight is exceeding the energy intake need of the body. A balance of energy intake and physical activity is important to achieve weight stability.

Macronutrients

Estimation of macronutrient dietary intakes is generally obtained from dietary surveys using various assessment methods. Nutrient availability in food composition databases can be determined by validated dietary assessment(25). The diet of Nordic countries is characterized by a higher consumption of animal, processed, and sweetened foods, including non-

alcoholic beverages and soft drinks(21, 26). Added fats, spreads and dairy products are common in Nordic populations(27). Nordic populations are characterized by the high intake of PUFA and SFA, a relatively high intake of sugar, but low intake of MUFA(21, 27).

In Sweden, the dietary supply of SFA and added sugars is higher than the values of the Nordic Nutrition Recommendations, while total PUFA and dietary fiber are lower(25, 28). Focusing on the quality and quantity of fat and carbohydrates in food sources that can help to replace foods high in SFA and added sugars by food rich in fiber and fruit may protect against AO(29).

Dietary fats

Dietary fat is an essential component in the human diet. An imbalance of fat can have negative effects on health and well-being (30, 31). Fats in the typical Swedish diet contribute to approximately 34% of the energy intake(21). The main sources of fat intake are spreads, butter, and oils; milk and milk products; meat and meat products(21). About 14% of the energy intake in the Swedish diet is saturated fat derived mainly from hard margarines, meat and dairy products(28, 32). The proportion of SFA is exceeds the Nordic nutrition recommendation(21). Excessive consumption of SFA may promote lipid storage and inflammation. In contrast, dietary PUFA could play a protective role in the development of CVD(33-35). Improved blood lipid profile and lowered hepatic fat content have resulted from the replacement of SFA with PUFA(33, 36, 37).

Dietary carbohydrates

Total carbohydrate intake in the range of 45-60 percent of total energy (E%) has been associated with reduced risk of chronic diseases(21). The influence of carbohydrates on lipid profile, glucose and insulin levels depends on several factors that include food sources, physical activity, and type of macronutrients that are replaced. In general, when total dietary carbohydrate intake is increased from 30-40 E% up to 60-70 E%, a transient increase in fasting triglycerides and decreased High density-lipoprotein (HDL)-cholesterol levels is noted in subjects(38). The main sources of carbohydrates in the Swedish diet that relate to the increased risk of obesity are disaccharides and monosaccharides (19, 39-41). High dietary fiber intake has been associated with lower risk of developing CVD and colorectal cancer in Nordic populations(42). Moreover, foods rich in fiber can maintain a healthy body weight(43). High intake of refined grains is associated with increased obesity(29). Refined grain products usually have a high glycemic index (GI), high insulin response, and a fast glucose decline. These properties could increase

hunger and enhance lipogenesis(29). Carbohydrate intake could provide a potentially important source of excess levels of liver FAs through the process of de novo lipogenesis (DNL)(44).

Intake of added refined sugars and alcohol should be restricted to support a healthy dietary pattern. In short-term human studies, a high intake of refined sugars (>20 E% sucrose or >5 E% fructose) resulted in increased triglyceride levels(38, 45). Also, Sonestedt et al. find a positive association between intake of sugar-sweetened beverages and dyslipidaemia, (i.e., elevated triglycerides and reduced HDL-cholesterol(45). The consumption of sugar-sweetened beverages has been associated with increased WC, more adverse abdominal adipose deposition, and an increased risk of type-2 diabetes(46, 47). According to the Nordic nutrition recommendation, alcohol consumption should be limited and not exceed approximately 10 g/day for women and 20 g/day for men(21).

Evaluation of diet

Dietary assessment methods need to be validated in order to be used to evaluate the relationships between diet and diseases. Common methods to assess diet in observational studies include food-frequency questionnaire (FFQ), 24-h recall, and 7-day food record. Various designs of FFQ have long been a key research tool to assessing habitual food intake in nutritional epidemiology (48). Despite limitations, the FFQ is inexpensive and easily administered tool for intake estimation(48). An FFQ should include a list of food items and dishes that are frequently consumed by the study population. FFQ validity can be assessed by comparison with a reference method, such as a food record or biomarkers(49, 50). The 7-day food record is considered a “gold standard” way of assessing food intake(51). Dietary biomarkers like FA composition in adipose tissue or blood compartments have useful features in validations of dietary assessment methods (52).

Fatty acid composition

FAs are the main building block of lipids, and are incorporated as structural components of cell membranes, precursors in eicosanoid production and regulate gene expression(53). FA is characterized by a carbon backbone with a carboxyl group at one end and a methyl group at the other end. The nomenclature is derived from the number of carbon atoms, and double bonds, and the position of the first double bond of the methyl terminus, such as n-3 (or omega 3), n-6, n-7, etc.(54). The most common FAs in the diet have 16 or 18 carbon atoms. There are three major groups of fatty acids. SFAs are straight in shape with no double bonds. MUFAs have one double bond.

PUFAs are more than one double bond. MUFAs and PUFAs are classified into families according to the location of the double bond closest to the methyl end, as described above(53). Essential FAs cannot be endogenously synthesized in the human body and must be derived from diet(55). Examples of essential FAs include LA and alpha-linolenic acid (ALA).In the human body, triacylglycerol is an ester derived from glycerol and three FAs. It is a major energy store (main constituent of body fat in human) and the major form of dietary fat. Other forms of body fat include cholesterol (1%), and PLs (5%). Also, free FAs or non-esterified fatty acids circulate in plasma bound to albumin and are released from adipose tissue by lipolysis(55).

Table 2. Fatty acids and desaturase activity investigated in this thesis

Type of fatty acids	**Chemical structures**
Saturated fatty acid (SFA)	
Myristic acid	14:0
Pentadecanoic acid	15:0
Palmitic acid (PA)	16:0
Stearic acid	18:0
Monounsaturated fatty acid (MUFA)	
Palmitoleic acid	16:1n-7
Polyunsaturated fatty acid (PUFA)	
Linoleic acid (LA)	18:2n-6
Alpha-linolenic acid (ALA)	18:3n-3
Eicosapentaenoic acid (EPA)	20:5n-3
Docosahexaenoic acid (DHA)	22:6n-3
Desaturase activity (FA ratio)	**FA ratio**
Stearoyl-CoA-desaturase (SCD)	(16:1/16:0)
Δ5-desaturase (D5D)	(20:4n6/20:3n6)
Δ6-desaturase (D6D)	(18:3n6/18:2n6)

Desaturases and elongases

In the human body, desaturase and elongase enzymes can synthesize a variety of FAs. The FAs can be converted to longer or more unsaturated FAs by elongation and desaturation. The elongation is catalyzed by elongases, which incorporate carbon atoms in the fatty acid backbone. Double bonds are formed by desaturases(54). Three desaturases are important in humans: Δ9-desaturase (also known as stearoyl CoA desaturase; SCD or SCD-1), Δ5-desaturase (D5D), and Δ6-desaturase (D6D)(55). Δ-Desaturases insert double bonds at specific positions from the carboxyl end of the FA chain. SCD catalyzes the synthesis of MUFA from SFA. D6D and D5D are required for the synthesis of highly unsaturated FAs(54). SCD-1 activity and other de-

saturase activities can be estimated by the ratio of product to precursor FA ratios. SCD-1 is considered a marker of lipogenesis and is associated with obesity (Table 3)(54).

Table 3. Desaturation and elongation of fatty acids

	n-7	***n-9***	***n-6***	***n-3***
Glucose DNL / Diet	16:0 (PA)	18:0	Diet	
Δ9-desaturase	↓	↓	↓	↓
	16:1n-7	18:1n-9	18:2n-6 (LA)	18:3n-3 (ALA)
Δ6-desaturase		↓	↓	↓
		18:2n-9	18:3n-6	18:4n-3
Elongases	↓	↓	↓	↓
	18:1n-7	20:2n-9	20:3n-6	20:4n-3
Δ5-desaturase		↓	↓	↓
		20:3n-9	20:4n-6	20:5n-3 (EPA)
Elongases		↓	↓	↓
		22:3n-9	22:4n-6→24:4n-6	22:5n-3→24:5n-3
Δ6-desaturase			↓	↓
B-oxidation			22:5n-6←24:5n-6	22:6n-3←24:6n-3 (DHA)

De novo lipogenesis (DNL)

The importance of the DNL pathway in human physiology is debatable. DNL is a metabolic pathway that synthesizes FAs from excess carbohydrates(56). These FAs can be incorporated into triglycerides for energy storage(56). DNL mainly takes place in the liver and adipose tissue in the human body(56). However, DNL significantly contributes to the serum lipid content of individuals with a high carbohydrate intake(57). DNL is highly responsive to changes in dietary intake. For instance, high carbohydrate intake activates a lipogenic response in liver tissue that leads to the increased synthesis of very low-density lipoprotein (VLDL)(58). Simple sugars are more effective than complex carbohydrates in stimulating hepatic DNL. Fructose is a monosaccharide with an especially potent stimulatory effect on DNL(58). In humans hepatic DNL is more responsive than adipose tissue of lipogenesis to carbohydrate overfeeding(56).

Fatty acids as dietary biomarkers

Intervention and cross-sectional studies have provided evidence of the value of the FA composition of adipose tissue and blood as biomarkers of fat intake(55). Assessment of dietary FA intake from different food sources is difficult. Therefore, serum FAs as a measure of dietary fat quality is probably the best way to evaluate dietary FAs(59). Quality of fat intake is also difficult to measure. The relative FA composition measured in blood or tissues can be used as biomarkers of dietary fat intake(55). A biomarker of the absolute amount of fat intake remains elusive. FA composition in serum does not perfectly reflect the dietary FA intake. Processes in the liver, such as utilization, absorption, and endogenous metabolism, also affect FA composition(60). Generally, FAs are located in different serum fractions, such as cholesterol esters (CE) and PLs. These fractions in serum reflect dietary intakes during the previous days or weeks, whereas adipose tissue reflects the intake over months(55). Some fatty acids are considered better dietary biomarkers than others. In general, the best biomarkers are FAs that cannot be endogenously synthesized. These include some PUFAs, trans FAs, and odd-numbered SFAs (15:0, 17:0)(61). For example, the essential FAs LA and ALA, as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered good biomarkers of dietary intake. The common dietary FAs 16:0 and 18:0 are not equally good biomarkers since they can be synthesized endogenously(61). The 16:0 FA is a main end-product of DNL, so changes in the proportion of 16:0 may reflect newly synthesized fat from carbohydrates as well as dietary intake(62). Furthermore, 16:0 FA seems to be relatively tightly regulated. Increases are counteracted by desaturation into palmitoleic acid (16:1n-7), and/or elongation into 18:0 FA(62). Therefore, 16:1n-7 may be considered a better marker for dietary intake of 16:0(62). In this thesis, we used the FA composition (e.g., PA, LA, EPA, DHA, etc.) of serum cholesteryl esters and PL as useful biomarkers of fat quality in dietary intake. Also, we used FA composition of adipose tissue as the best biomarker of long-term dietary intake.

Abdominal obesity and fatty acids

AO is a strong predictor of cardiometabolic disease(4, 63) and metabolic risk. The risk is correlated more closely with abdominal fat than with obesity in general(5). These complications have been attributed to increases in visceral adipose tissue (VAT) (5). Visceral obesity is an accumulation of excess intra-abdominal adipose tissue is a part of a phenotype that includes dysfunctional subcutaneous adipose tissue expansion and ectopic triglyceride storage which are closely related to a cluster of cardiometabolic risk factors(8). Abdominal adiposity is an important component of the metabolic syndrome(64,

65). WC is highly correlated with VAT as measured by computed tomography in both men and women(66). The quality of fat intake may be important in modulating fat deposition and distribution, and promoting AO(37, 67). High intake of n-3 and n-6 PUFA may reduce body fat accumulation(37, 68), whereas SFA may promote the development of AO and metabolic syndrome(37, 69). In particular LA and PA are reportedly associated with the degree of fat accumulation in both VAT and SAT(37), but in different directions.

The potential role of dietary FA in AO and FA metabolizing enzymes may influence the status of body fat storage, body weight(70, 71), and WC(72). SCD, D5D, and D6D along with elongases the main enzymes responsible for the endogenous synthesis of MUFAs and PUFAs(73).

The underlying mechanisms explaining different effects of FAs and carbohydrates on body fat composition and distribution have not been fully established. Mechanisms including lipogenesis and fat oxidation are most likely involved. Few human interventional studies have been conducted. Especially, overfeeding studies are not easy to do.

Aims

The overall aim of this thesis is to investigate the association of FA biomarkers and dietary intake with AO and related anthropometric measures among a population-based cohort of women and men residing in Stockholm County, Sweden.

Specific aims:

Paper I

To investigate cross-sectional associations between dietary fat quality assessed by serum FA composition and anthropometric measures of AO in a population-based cohort of 4232 60-year-old (60YO) Swedish men and wome

Paper II

To compare intakes estimated from a simple FFQ used in the full 60YO cohort, a 7-day food record in a provided by a sub-sample (n=301) of the men in 60YO cohort (i.e., the KOMET study), and serum FA composition measured in the 60YO cohort

Paper III

To evaluate associations between carbohydrate (including sugars) and alcohol intake with serum phospholipid and adipose tissue proportions of FAs in the DNL pathway

Paper IV

To examine associations between carbohydrate intake and anthropometric measures of AO in Swedish men

Subjects and Methods

Study designs

All observational data in this thesis were based on/or recruited from the cohort study of 60-year-old Swedish men and women (60YO) in Stockholm County (Figure 1).

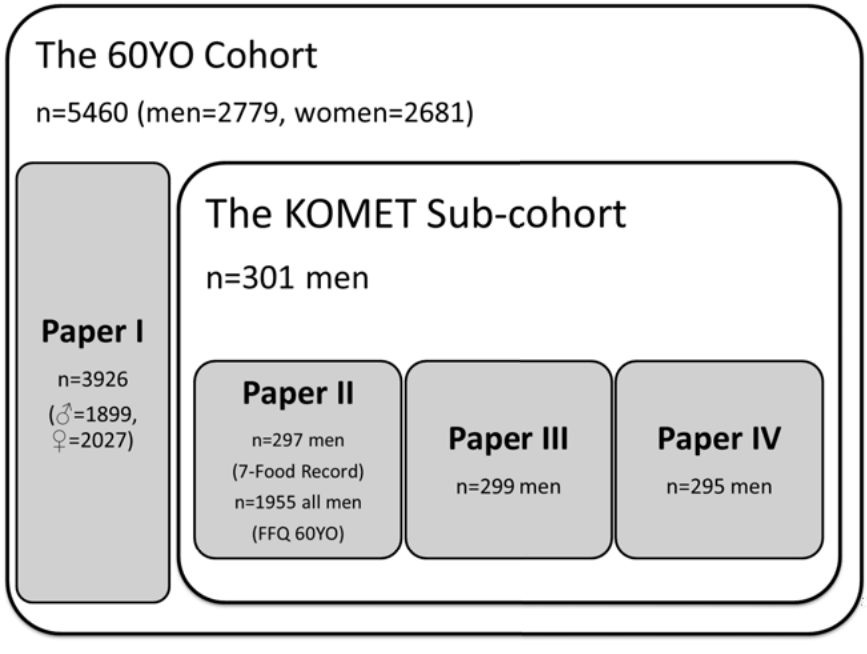

Figure 1. Illustration of the papers of this thesis based on the 60YO cohort and the KOMET sub-cohort.

Paper I – The 60YO cohort

A cross-sectional study was conducted in a large population-based cohort of 60-year-old Swedish men and women (60YO) in Stockholm County, Sweden. Data was collected between August 1997 and March 1999. This study was performed to investigate association between dietary fat quality assessed by serum FA in CE and abdominal anthropometric measures in both men

and women. The study was approved by the ethics committee at the Karolinska Institute.

Paper II – The 60YO cohort and KOMET study

All men in the 60YO cohort filled in a simple FFQ. Subsequently, a subpopulation of the men (n=301) were selected from 60YO three years later, based on their fasting insulin levels, and were required to be free from serious illness. The KOMET study was conducted between March 2000 and October 2001. Participants in KOMET completed a 7-day food record. The FFQ data from the KOMET sub-cohort was validated by two reference methods: a 7-day food record from KOMET and serum FAs in CE from 60YO. The studies were approved by the ethics committee at the Karolinska Institute.

Paper III and Paper IV – The KOMET study

Two cross-sectional studies of the 301 healthy men aged 63 years in the KOMET sub-cohort were performed. In paper III, we evaluate the associations between carbohydrates and alcohol intakes, in relation to FA composition and SCD activity estimates in serum PL and adipose tissue (AT). In paper IV, we examine the associations of carbohydrate and alcohol intake with anthropometric measures. The studies were approved by the ethics committee at the Karolinska Institute.

Participants

Paper I

Every third man and woman living in Stockholm County, Sweden, and born between July 1, 1937 and June 30, 1938, were invited to participate in the 60YO cohort study. Of the 5460 subjects (2779 men and 2681 women) randomly invited, 4232 individuals (78% response rate) comprising 2039 men and 2193 women agreed to participate.

Paper II

All participants were men in the 60YO cohort and the KOMET study. In the large 60YO cohort study, a total of 1955 men were included after excluding 62 men without data regarding dietary intake or serum FAs. A total of 299 men in the KOMET subpopulation were included after excluding two men who lacked data regarding dietary intake measured at age 60 years (60YO) or 63 years (KOMET).

Paper III

Of the 301 men of the KOMET subpopulation, two were excluded for not completing the 7-day food record, producing a final sample size of 299 men. There were no cases of CVD, pharmacological treatment of hypertension, diabetes and hypercholesterolemia, and other serious disease. The grouping into tertiles was done on all men in 60YO and equal numbers were selected from the tertiles for KOMET. Requests to participate in a study concerning diet and metabolic syndrome were randomly selected until positive responders reached approximately 100 men in each group. Classification of groups was used only to recruit subjects with a wide range of insulin concentrations and not for analyses in this study. The study was approved by the Ethical Committee at Karolinska Institutet.

Paper IV

Participants in this study were the same participants of Paper III. Of the 301 men, six were excluded due to missing data on dietary intake or anthropometric measures, leaving 295 men for this study.

Methods

Anthropometry and abdominal obesity measures

All subjects underwent examinations that included anthropometric measurements and blood sampling. AO was defined according to the definition of the National Cholesterol Education Program as WC>102 cm for men and >88 cm for women(16).

As previously described(74), an electronic scale was used to weigh the participants to the nearest 0.1 kg. Height was measured without shoes to the nearest 0.5 cm. BMI was calculated as weight (kg) divided by height (m) squared. WC was measured in underwear after a normal expiration with the subject standing up, at the midway point between the iliac crest and the lower rib margin. Hip circumference was measured horizontally at the point of largest lateral extension at the hips or over the buttocks. SAD was measured with the subject in supine position with straight legs on a firm examination table without clothes in the abdominal area after normal expiration, using a ruler and water level. These measurements were utilized to calculate WHR, waist circumference-to-height ratio (WCHR), sagittal abdominal diameter-to-height ratio (SADHR), and waist-hip-height ratio (WHHR).

Fatty acid and biochemical measurements

Blood samples were taken in the morning after an overnight fast. Serum cholesterol, insulin, glucose, and triglyceride were analyzed as previously described(74, 75). Fatty acids in serum cholesterol ester (CE) was analyzed using gas-liquid chromatography (GLC) as previously described(75). Methanol was added to serum for lipid extraction. CE was separated from other lipids by thin-layer chromatography (TLC). Proportions of individual FA were presented as percentages of all measured FA. Desaturase activities were estimated as product-to-precursor ratios and calculated as follows: SCD= 16:1/16:0, D6D= 18:3n-6/18:2n-6, and D5D= 20:4n6/20:3n-6 (76).

FFQ

FFQ in 60YO was administered as a part of a comprehensive questionnaire regarding lifestyle, medical history, and dietary habits. The questionnaire comprised 14 selected food items. Each question had three or four response categories. The categories could either be frequencies (e.g., servings/week), product preference (e.g., butter or margarine on sandwiches), or portion size (e.g., amount of butter/margarine per sandwich). For some food groups, questions were combined to provide semi-quantitative intake estimates. For example, the number of sandwiches per day was multiplied by the number of cheese slices per sandwich to provide a frequency of slices per/day. This frequency was multiplied by the standard weight of Swedish cheese slices to estimate daily cheese intake in grams(77, 78). The alcohol intake was estimated by five questions concerning the intake of beer (light, medium, and strong, with percentage of ethanol per volume of <2.5, 2.5-3.5, and >3.5%, respectively), wine, and spirits and obtained by frequency per day, per week, per month, or none using specific serving sizes such as bottles, cans, or glasses(79).

Seven-day food record

In the KOMET subpopulation, oral and written instructions were given to participants on how to complete a 7-day food record(77). The food record is an optically readable version of a questionnaire used by the Swedish National Food Administration(32). The booklet contains preprinted alternatives for commonly eaten meals and food items. The participants used household measures (e.g., servings, cups, glasses, and spoons) to estimate the amount of food intake. Photos were used to estimate portion sizes and amount of fat spread on bread(77, 80).

Assessment of dietary intake

Intake of macronutrients was calculated based on intake of energy for carbohydrates, fiber, fat, protein, and alcohol. Food composition tables were used to translate food intake into macronutrients. Total energy intake was expressed as megajoules per day (MJ). The intake of food groups was expressed as gram per 10 MJ. Intake of carbohydrates, including total carbohydrate, disaccharide, monosaccharide, and fiber were expressed as percentages of total energy intake (%). Alcohol intake was expressed as percentage of total energy intake. Intake of fat, including total, saturated, polyunsaturated, and monounsaturated fat were expressed as percentages of total energy intake.

Food groups were created based on the estimated level of carbohydrate contents. Three food groups were defined. The sugar-rich food group included sugar, syrup, honey, candy, chocolate, jam, soft drinks, lemonade, juices, ice-cream, desserts, cookies, crackers, and buns. The starch-rich food group included bread, cereals, porridge, pancakes, pizza, pasta, potatoes, and rice. The fruit and vegetables food group included fruit, berries, vegetables, and root vegetables.

SCD gene expression

For gene expression analysis, a subsample of 87 individuals was selected from all men who had fat biopsies collected. The individuals were equally distributed throughout the tertiles of fasting insulin concentration. Laboratory procedures to measure SCD expression have been previously described(81, 82). Briefly, SCD mRNA was quantified by real-time polymerase chain reaction and normalized for expression of the housekeeping gene RPLP0(81).

Ethical approval

All subjects provided written informed consent prior to inclusion. The studies were approved by the Ethics Committee at Karolinska Institutet for the 60YO cohort [Reference number/diarienummer: 96-938 (original application); 2006/157-31 (complementary application)].

Statistical analyses

Log transformation was used to make variables with highly skewed distribution less skewed. Statistical analyses were carried out with STATA version 11.0 (STATA Corporation, TX, USA). $P < 0.05$ was considered significant. The analyses conducted for each paper are described in more detail subsequently.

Paper I

Shapiro-Wilk's test was performed to examine the normality of distribution for continuous variables. Student's t-test for normally distributed variable or Wilcoxon-Mann-Whitney test for non-normally distributed variables was performed to assess sex differences of continuous variables in FA proportion and estimated desaturase activities. χ^2-test was performed in binary and ordinal variables for sex differences in prevalence of AO.

Spearman's rank correlation coefficients were calculated between FA, desaturases, and anthropometric measurements. Crude and multivariable-adjusted logistic regression models were used to calculate odds ratio (OR) and 95% confidence interval (CI) for prevalence of AO. Linear regression analysis was performed to investigate associations of mean-centered standardized FAs and estimated desaturase activities with abdominal anthropometric measures. Serum FAs were also investigated as categorized (quartiles) variables and overall trends were evaluated with quartile medians as exposure. Potential nonlinear associations were evaluated by restricted cubic splines. Associations were assessed in crude and in adjusted models where physical activity, alcohol intake, education, and smoking were used as covariates. Sex was included as a covariate and sex-differences in overall trends were evaluated in models by including interaction term of sex and exposure (sex-specific quartile median) in the analyses of the total population study.

Paper II

Differences between investigations among men in the subpopulation with normally distributed variables were assessed by two-tail unpaired t-test or paired t-test, and Wilcoxon signed rank tests or Mann-Whitney tests for variables not being normally distributed by log-transformation. Differences for categorical variables were assessed by χ^2 test.
Absolute agreement of FFQ and food records was assessed by determining the proportion of participants assigned to the same intake category by the two assessment methods. Weighted Kappa statistics (Kw) was calculated for agreement of categorization. Spearman's rank correlation coefficients were calculated between intake estimated by FFQ and food records. Differences in the proportion of FA between categories of food intake were analyzed by ANOVA and Bonferroni post-hoc tests.

Paper III

Spearman rank correlation coefficients and their 95% CIs were calculated to evaluate the relationship between carbohydrate intake and FAs, sum of SFA, and SCD activity. Associations of nutrient intakes with FAs, SCD activity, and gene expression were assessed in linear regression models, with FAs, SCD activity, or SCD gene expression as dependent variables and tertile median intake as independent variable. Crude associations were adjusted for BMI. Non-linear trends were assessed using restricted cubic splines with three knots located at the 25^{th}, 50^{th}, and 75^{th} percentiles of nutrient intake to explore the association between FAs, and SCD activity.

Paper IV

Student's t-test was conducted to assess differences in food and macronutrient intake between men with and without AO. Spearman rank correlation was used between dietary intakes expressed as energy percentage and anthropometric measures. Intakes of total carbohydrates, disaccharides, monosaccharides, fiber, and alcohol were energy-adjusted according to the residual method (83, 84). Participants were divided into tertiles based on their carbohydrate to fiber ratio (CFR) and energy-adjusted intakes of total carbohydrates, disaccharides, monosaccharides, fiber, and alcohol. Logistic regression models were used to calculate odds ratios (OR) and 95% CI for AO per intake tertiles. Associations of dietary intake and anthropometric measures were evaluated in linear regression models. Linear trends were evaluated using regression models with tertile median as exposure. Nonlinear trends were assessed using restricted cubic splines with three knots located at the 25^{th}, 50^{th}, and 75^{th} percentiles of nutrient intake. All models included energy intake as a covariate. Multivariable adjusted models additionally included smoking habits, physical activity, and energy-adjusted alcohol intake as covariates. In sensitivity analysis, all analyses were repeated after exclusion of under-reporters (n=87). The under-reporters were defined according to the Goldberg cutoff that based on energy intake should be equal to energy expenditure with assuming weight stability(85, 86).

Results

Paper I

Of the 4232 investigated subjects, 3926 (1899 men and 2027 women) were included in the study. We excluded 306 individuals because there was no data regarding serum CE-FA, anthropometric measures, physical activity, education, alcohol intake, or smoking habits. Women displayed a higher proportion of AO than men (39% vs. 29%; P<0.0001). Proportions of ALA and DHA in serum CE as well as estimated activities of SCD and D6D were higher in women compared to men. Men had greater proportion of serum PA, which was the most abundant SFA.

Serum PA was positively correlated with all abdominal anthropometric measures in men. In women, serum PA was correlated only with WHR. There was no significant difference between men and women regarding association between serum PA and AO (P=0.11). Even after adjustments for potential confounders (physical activity, alcohol intake, and smoking), PA was associated with anthropometric measures.

Prevalence of AO was significantly lower with higher LA levels with no difference between men and women. In multivariable-adjusted models, the odds of having AO was 60% lower in the highest compared to lowest LA quartile. There was a negative correlation between LA and all anthropometric measures in both sexes. After adjustment for potential confounders, inverse associations remained between LA and anthropometric measures in both sexes.

The prevalence of AO was lower in higher levels of ALA. In men, ALA was consistently inversely associated with all measures of AO, with a significant sex-difference (P = 0.0017). Similarly, ALA was negatively correlated in men with all anthropometric measures. ALA was inversely associated with WC and SAD, but not with WHR.

Serum EPA (20:5n3) was not associated with AO. Borderline significant sex-difference association was evident between EPA and AO (P = 0.05). When evaluated using multivariable-adjusted models in women, EPA and AO were associated, especially WC and SAD (P=0.03, and 0.02, respectively). Serum DHA (22:6n3) was inversely associated with AO prevalence with no significant sex-differences. In women, DHA was inversely correlated with all anthropometric measures, but only with WHR after adjustment for

potential confounders. Overall, the association between n-3 PUFA and AO and anthropometric measures appeared to be linear.

Estimated desaturase activities of SCD and D6D were associated with AO, with no significant sex-differences. SCD and D6D were correlated and associated with all the anthropometric measures. D5D activity was inversely associated with AO, with no significant sex-differences. Similarly, D5D was negatively correlated and inversely associated with all anthropometric measures. D5D and WHR were inversely associated in women, but not in men. Associations of desaturase activities were with AO and anthropometric measures were generally non-linear.

Paper II

Four men were excluded in the KOMET subpopulation (n=301) because of the lack of data regarding dietary intake measured at age 60 years (60YO) or 63 years (KOMET). Thus, a total of 297 men were available for validation of the FFQ using 7-day food records from KOMET.

At age 60 years, men in KOMET had lower BMI, WC, triglycerides, insulin, and glucose in fasting plasma, and were less likely to be smokers, compared to all men of 60YO. Comparing measurements at 60 and 63 years of age in KOMET, revealed decreased weight and increased WC in older men. Total and HDL cholesterol were higher at 63 years of age, while low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, and insulin were lower compared to the 60YO measurements.

Absolute agreement between intakes of the simple FFQ (60YO) and 7-day food records (KOMET) were highest for alcohol, egg, and total fish, but lowest for vegetables, oily fish, and bread. According to weighted Kappa statistics, the strongest agreement was for alcohol, margarine, and fruit (K_w >0.3), intermediate for fish and egg ($0.2<K_w<0.3$), but lower or absent for the remaining food groups ($K_w<0.2$).

At age 60 years, serum proportions of the long-chain n-3 PUFAs EPA and DHA were higher among men with higher total fish intake. The sum of EPA and DHA in serum differed significantly ($P<0.0001$) between all categories of total fish intake (<1, 1-2, or >2 servings/week). Serum proportions of pentadecanoic acid (15:0) were significantly higher ($P<0.01$) in men consuming ≥ 40 g/day of cheese compared to men consuming <40 g/day, but the last two highest intake groups did not differ significantly. Margarine consumers had higher serum LA and ALA compared to non-consumers, but did not differ between intake categories among margarine consumers who have >5 g/day.

Paper III

Of the 301 men, two were excluded for not completing the 7-day food record, leaving 299 men as the sample cohort. A majority (84%) of men were non-smokers. The median energy intake was 9.3 MJ/day and carbohydrates provided the highest energy intake (44%E), before fats (33%E) and proteins (16%E). The median energy percentage from disaccharides was 11%E of which sucrose was the major contributor (7%E). Alcohol contributed to a median of 4%E.

After adjusting for BMI, carbohydrate intake was inversely associated with 16:0 in PL (P=0.005). There was little evidence of linear associations of disaccharide intake with FA or SCD activity (BMI-adjusted P≥0.15). However, disaccharide intake was non-linearly associated with 16:1 and SCD activity in PL (P for non-linearity≤0.02), with apparently higher 16:1 and SCD activity between high and low disaccharide intake. Monosaccharide intake was not associated with any FA or with SCD activity after adjusting for BMI. Alcohol consumption was linearly associated with higher levels of 16:0 in PL (BMI-adjusted P<0.001) and with 16:1 and SCD activity (BMI-adjusted P<0.001 and P≤0.001, respectively) in both PL and AT. Non-linear associations of alcohol intake and 16:1 and SCD activity in PL were evident (P for non-linearity ≤ 0.02), with apparently stable levels at low and medium alcohol intake that rapidly increased at higher intakes.

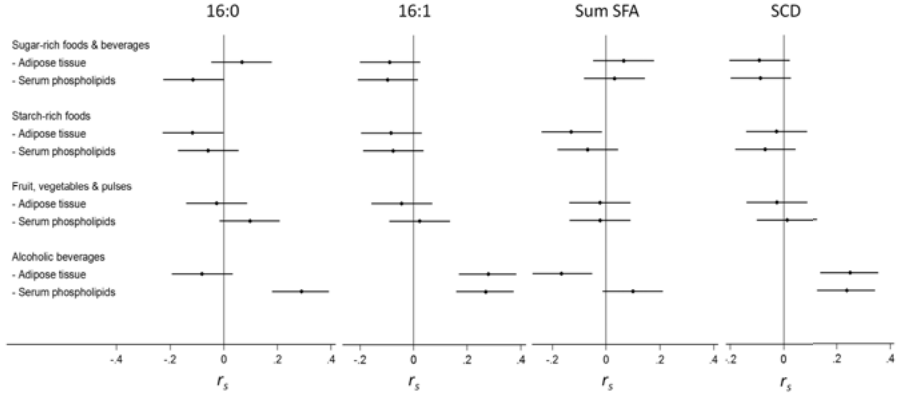

Figure 2: Correlation coefficient and 95% CIs for correlations between FAs, sum even-chain SFAs, SCD activity in PL and AT, and food groups.

Among the 81 men with dietary and gene expression data, no association of dietary carbohydrates, disaccharides, monosaccharides, alcohol, and the

carbohydrate-to-fiber ratio with SCD gene expression was evident (P for linear trend≥0.25; P for non-linearity≥0.08).

Paper IV

After excluding 6 subjects with missing data regarding dietary intake and anthropometric measures, 295 men were included for analyses. Twenty percent of the men were classified to have AO and 16% were current smokers. Carbohydrate was the major source of energy and about one-quarter of all carbohydrates were disaccharides. Men with AO had significantly lower proportions of calories from carbohydrates (P<0.001) and monosaccharides (P=0.04) compared to leaner men, but alcohol was a greater contributor of energy intake (P=0.03) in men with AO. Intake of starch-rich foods was lower in men with AO (P=0.05).

Intakes of carbohydrate, monosaccharide, fibers, and fruits and vegetables correlated negatively with anthropometric measures of overall obesity (BMI) and AO (WC, WHR, and SAD), while weaker correlations were observed for the other dietary factors evaluated.

Carbohydrate intake was significantly inversely associated with WHR and SAD. Likewise, monosaccharide intake was significantly associated with lower BMI, WC, WHR, and SAD. In contrast, alcohol intake was significantly associated with weight, BMI, WC, and SAD. Non-linear associations of alcohol intake with weight and WHR were observed (P nonlinear trend =0.03).

Intakes of carbohydrates, mono- and disaccharides, fiber, and carbohydrate-to-fiber were not associated with AO in multivariable-adjusted models (P for linear trend≥0.23). However, alcohol intake was associated with AO (P for linear trend 0.002). Men in the third highest alcohol intake group were almost three times as likely to have AO compared to the third with the lowest intake (OR 2.93, 95% CI 1.40-6.16). There was no evidence of non-linear association between the macronutrients and AO. In sensitivity analyses, exclusion of under-reporters did not substantially affect the results.

Discussion

This thesis is based on observational studies investigating the relationships between FA biomarkers, dietary intake, and AO. In line with the current dietary guidelines, our results suggest that higher intake of PUFAs, especially n-6, may help to prevent AO. A higher proportion of serum 16:0 was associated with higher prevalence of AO, whereas the opposite was true for serum LA. We found no clear evidence that higher intake of carbohydrates or sugars influences serum or adipose FA of the DNL pathway. Total carbohydrate intake was inversely associated with 16:0 in PL, but not in AT. Alcohol consumption was associated with higher 16:0, 16:1, and SCD activity in both PL and AT. Men with higher alcohol intake were more likely to have AO, and intakes of carbohydrates, monosaccharides, and starch-rich foods were associated with lower anthropometric measures of overall and AO.

Paper I

AO was associated with serum proportions of FAs, which in turn partly reflected dietary fat intake. Serum LA was strongly and inversely associated with AO in both men and women, whereas a higher serum 16:0 was linked with higher OR of AO and greater levels of all anthropometrics measures. DHA and ALA were inversely associated with AO, in a partly sex-specific manner.

Previous observational studies and clinical trials support our findings that serum 16:0 is positively related to AO(72, 87). In addition, 16:0 in other compartments (i.e., plasma, erythrocytes, and skeletal muscle phospholipids) has been associated with increased liver fat(88), body fat percentage(89), and BMI(89). Previous studies have also shown that higher proportion of serum LA is associated with lower WC(90), WHR(87), SAD(90), and BMI(87, 90). A previous publication within the present study population showed serum LA to be inversely associated with all-cause mortality, but not with CVD risk(91). A recent meta-analysis found that the LA concentration in LDL phosphatidylcholine was associated with lower WC and BMI(92). Another study reported lower LA proportions in plasma PL among overweight compared to normal weight participants(93).

A greater accumulation of liver fat(67, 94), visceral fat(94), and total body fat(94)were reported after consumption of SFA (high in 16:0) com-

pared to PUFA (high in LA). This is also supported by randomized controlled trials(37). 16:0 can be synthesized by sugars and other refined carbohydrates through DNL. 16:0 may undergo elongation or desaturation(37). Serum 16:0 proportion is therefore not exclusively determined by the intake, but also by endogenous FA metabolism. Moreover, participants in the current study likely consumed a rather non-lipogenic diet with limited consumption of sugar-sweetened beverages and high fat intake(95). Potential mechanisms behind the current associations may include greater oxidation of dietary PUFA versus SFA(96) or a potential obesogenic effect of SFA per se by up-regulation of 11β-hydroxysteroid-dehydrogenase type 1, promoting cortisol induced visceral fat accumulation(97).

Serum ALA was inversely associated with AO measures only in men. This may be due to a weaker relationship between ALA intake and serum levels in women, as previously indicated(31, 87, 91).

A recent meta-analysis reported lower plasma DHA in overweight compared to normal weight participants(93). Serum DHA was inversely associated with AO in our study population, but especially so in women. Sex-specific associations of serum n3-PUFA (DHA) and AO could partly reflect differences between men and women in many factors such as dietary and lifestyle patterns and fat accumulation(98), and hormone-dependent sex differences in lipid metabolism(99, 100). However, the associations between DHA and AO remained after adjustments for lifestyle factors.

In line with previous studies(90, 101), SCD and D6D activities were associated with AO. Higher SCD activity has also been detected in participants with high liver fat content(102). On the contrary, measures of D5D activity in plasma have been inversely associated with AO and subcutaneous adipose tissue(90, 101). In a recent meta-analysis, higher D6D and lower D5D activities were found among overweight individuals compared to those with normal weight(93). Considering the indication of elevated risk of AO in subjects with high estimated SCD activity, these finding are important for further investigation targeting their effects on individuals with AO.

Paper II

The current FFQ was not previously validated. An evaluation study to test the validity of a dietary assessment tool is important. K_w values >0.4 and correlation coefficients >0.5 in at least 50% of participants was suggested to indicate adequate validity of the dietary assessment tool, given imperfect instrument ranks of individual dietary intake(103). Intakes of margarine and fish were reasonably well captured by simple FFQ that may partly enhance better indication of fat biomarkers. There were clear differences in the serum proportion of long-chain EPA and DHA between categories of reported fish intake as determined by FFQ. Several previous studies described that serum

proportion of long-chain n-3 fatty acids EPA and DHA reflect the relative intake of these fatty acids(104, 105). LA is an essential FA and major dietary PUFA in most populations. Serum LA is considered a good biomarker of food rich in LA, such as margarine and vegetable oils(106). Also, ALA is an essential FA present in margarine and vegetable oils, but its utility as biomarker is unclear, possibly due to high metabolic turn-over(107). We found that serum proportion of LA, ALA, and pentadecanoic acid were all related to the FFQ reported intake of main food sources; margarine for AL and ALA, and cheese for pentadecanoic acid. Pentadecanoic acid is an odd-chain SFA produced in the rumen of dairy producing animals and utilized as biomarkers of dairy fat intake(108).

Paper III

We found no clear evidence that higher intake of total carbohydrate or sugar-rich foods or beverages is associated with higher proportions of DNL-derived SFA in serum PL. However, alcohol intake was strongly associated with both higher SFAs and MUFAs. Further randomized controlled trials are needed to evaluate the effect on circulating SFAs and MUFAs from the quantity and quality of carbohydrates.

In the pan European Prospective Investigation into Cancer (EPIC) InterAct study, there were indications that some carbohydrate-rich foods may be associated with even-chain SFAs in plasma, which possibly reflects SFAs synthesized from refined carbohydrates through the DNL pathway(109, 110). However, these associations were not consistent and therefore difficult to interpret. In the current Swedish population, we could not clearly observe a link between higher intake of carbohydrates or simple sugars, and 16:0 or total SFA in serum PL. Some controlled interventions studies have, however, indicated that a diet very high in carbohydrate and low in fat fed in the short-term results in significantly increased 16:0 and decreased 18:2 proportions of plasma, respectively (56, 111). In contrast to a previous study(112), we found that total carbohydrate intake was inversely associated with 16:0 in PL. Therefore, our observational study may not reflect intake of carbohydrates on FAs that have shown a clear influence on the rate of DNL in some experimental studies.

Our study population of Swedish men, as many other Western populations, had relatively high fat intake and a rather low intake of total and simple carbohydrate. DNL is stimulated mainly by high carbohydrate intake (short-chain glucose polymers (75% E)) and low fat diet, although clearly lower stimulation of DNL occurs when the carbohydrates mainly consist of starch(56, 58, 109). Another study showed that overfeeding overweight subjects with simple carbohydrates markedly increased liver fat and stimulated

DNL(113). This is in contrast to our findings that sugar intake was not associated with serum SFA.

Presently, alcohol consumption was associated with FAs (16:0, 16:1) and SCD activity. Previous studies have reported alcohol consumption to be positively associated with 16:1 in plasma PL and erythrocyte membranes(114, 115). Alcohol consumption may increase the activity of acetyl-CoA carboxylase, a key lipogenic enzyme, and therefore the synthesis of 16:0(114).

Paper IV

We found high intakes of carbohydrates and monosaccharides to be associated with lower WHR and SAD in Swedish 63-year old men. In addition, men with high alcohol consumption were more likely to have AO compared to those with low alcohol consumption. Further studies should evaluate the relationship between specific sugars intake (e.g., glucose, fructose, sucrose, and lactose) and AO.

Several studies have reported the beneficial effects on WC among participants with higher intake of carbohydrates(116-119). However, a recent cross-sectional study found no associations between carbohydrate intake and BMI, WC, and SAD among Swedish men(120). Differences in study design, such as population age, dietary assessment methods, and other factors may partly explain the disparate outcomes. Furthermore, a Finish meta-analysis of three cross-sectional studies found that total carbohydrate intake was inversely associated with obesity(39).

We found no clear evidence that disaccharides (sucrose and lactose) are associated with AO or anthropometric measures in our population. Sucrose is found in soft drinks and sweets of manufactured foods, and lactose is present in milk and milk products(21). A meta-analysis of three Finnish cross-sectional studies concluded that sucrose and lactose intakes are associated with higher and lower OR of obesity, respectively(39). Therefore, opposite associations of sucrose and lactose may partly explain our null findings for disaccharide intake.

Monosaccharide intake was inversely associated with BMI and AO-related anthropometric measurements. In the literature, various terms are used for sugar, but the most common term of “sugars” are monosaccharides and disaccharides(121). Glucose and fructose are the main glycaemic carbohydrates of monosaccharide as defined by the Food and Agriculture Organization and WHO(122, 123). Sugar consumption has increased in all Nordic countries, especially from the consumption of sugar-sweetened beverages(45, 124). In the 2010-2011 Swedish national survey of dietary habits, 44% of all monosaccharides came from, fruits, vegetables, and berries, with less contribution from juices (10%) and sweet beverages (5%).

Thus, it is likely that the large monosaccharide intake in the present study reflects a diet rich in fiber rather than rich in sugar-sweetened beverages, which in turn have been positively associated with general and AO(46, 125-128). Finally, alcohol intake was associated with AO, BMI, and SAD in this paper. Likewise, other studies have shown an association of alcohol intake with AO-related anthropometric measures(116, 129, 130).

Strengths and limitations

The results from the current studies should be interpreted cautiously in terms of causality, due to the cross-sectional study design. The 60YO cohort is restricted to 60-year-olds in Stockholm County and the current results may not be representative for other populations. Anthropometric measures used in this thesis, they cannot distinguish between different type of adipose tissue, e.g., subcutaneous and visceral fat.

In Paper I, a major strength is the use of a large population-based cohort representing Stockholm County with high participation rate, in whom serum FA composition has been determined. Both men and women were included, allowing investigation of sex-specific relationships. One limitation of this study is that measures of serum FA and AO were only performed once, which may lead to misclassifications due to intra-individual variation.

One strength of Paper II is that this evaluation study was performed in approximately 300 individuals, which could be considered a high number in this context. Another strength is the use of FA biomarkers, which can provide a more objective estimate of dietary compositions compared to traditional assessment methods based on self-reports. The simplicity of the FFQ limits the capacity of explaining a large proportion of variability in the true intake and also the possibilities of capturing dietary habits. Limitations include that the evaluation was performed only in men and that there was a time-lag between assessment methods of the FFQ and the 7-day food record.

A strength of Paper III is, to our knowledge, that it represents the first investigation on association between carbohydrate intake and circulating and adipose tissue fatty acids in a Swedish population. All men were of similar age, thereby reducing the potential bias due to age differences.

A major strength of Paper IV is the use of a 7-day food record, which is considered the gold standard for dietary assessment. To our knowledge, this is the first study to investigate relationships of total carbohydrate and subtype (mono- and disaccharide) carbohydrate intakes with anthropometric measures of AO in Swedish men. Limitations of this study include the fact that we only investigated men. Moreover, we were unable to relate specific disaccharides such as sucrose and lactose or monosaccharides liker fructose and glucose with AO. Therefore, we cannot specify whether a certain saccharide may be responsible for the observed association in our study.

Clinical implications

Our understanding of the relationship between FAs, dietary intake and AO has increased considerably over the past 20- years. Still there is more knowledge to be gained concerning the role of dietary components (e.g. fat and carbohydrate quality) on abdominal fat accumulation. In addition, inconsistencies of AO definitions and clinical screening parameters (e.g. anthropometric measures) have complicated to study this topic clinically. For example, various organizations (NCEP-ATP III, WHO, etc.) have proposed somewhat different cut-offs and methods to identify individuals with abdominal obesity.

The results from this thesis suggest that FA biomarkers may reflect markers of abdominal fat accumulation in Swedish population that could be useful in clinical assessment tools. The current abdominal anthropometric measure could be cheaper, relatively accurate and feasible to measures AO. Our results support current dietary guidelines that dietary fat should be mainly unsaturated fat. Saturated fat (16:0) was positively associated with AO whereas PUFA (LA) was inversely associated, which is in line with our previous randomized controlled trial(37). Further, the results suggest that higher consumption of alcohol could increase AO in men. Therefore, reduction of alcohol consumption may decrease the risk of developing abdominal obesity. In general, replacing SFA by PUFA, as supported by some randomized studies(37) that could be an individual advice to improve the dietary intake and lipid profile and reduce the risk of NCDs.

Conclusions

AO was more common among participants with relatively high serum proportions of 16:0, while the opposite was true for LA/18:2n6. Alcohol, fish, and margarine intakes as evaluated by a short FFQ against 7-day food records and serum biomarkers of fat intake reflected their intake reasonably well. There was no clear evidence indicating that higher carbohydrate intake or sugar-rich foods or beverages is associated with higher SFA in PL and AT, whereas alcohol intake was consistently associated with higher SAF and MUFA. Individuals with higher intake of alcohol were more likely to have AO compared to those with lower intake.

Future perspectives research

Recent results have demonstrated that AO is critical in the clinical assessment of subjects to improve health status, particularly if they are obese and have unhealthy dietary intake. Overall AO is associated with higher plasma glucose and insulin concentrations, hyperlipidemia, and together constitutes a cluster of risk factors for CVD, type-2 diabetes, and cancer as shown in several prospective studies. In fact, current abdominal anthropometric measures describe a whole fat mass of abdominal area without distinguish between excess fat in the central (visceral abdominal fat) vs. peripheral obesity (gluteofemoral and/or subcutaneous fat).

Several gaps of knowledge exist that challenge dietitians and clinicians in the field of AO and relation to fatty acid biomarkers and dietary intakes. This thesis provides interesting findings from a Swedish population that opens the door for some investigating in further studies. These are considered below.

The impact of dietary fat quality in 60YO was investigated by using dietary biomarkers that in many ways are preferable to a self-report. But we still need more confirmation by randomized controlled trials. All participants of 60YO were 60 years of age. It would be interesting to perform an observational study with primary aim to investigate the relation between AO and FA compositions at different ages.

The various structures of simple carbohydrates such as glucose, sucrose, and fructose have different effects and works via different mechanisms on

AO. Therefore, it would be interesting to investigate their specific effects on AO, especially targeting a Swedish population.

In paper III and IV only men were investigated. Thus, it would be important to perform a clinical trial in a Swedish population consisting of both men and women to evaluate the effect of different carbohydrate sources in foods, as well as of alcohol intakes on circulating levels of fatty acids in the DNL pathway.

Finally, different effects and mechanisms of the dietary intake are apparent on different adipose tissue depots, mainly visceral adipose tissue tissue(131). Further research, including prospective studies and randomized clinical trials, is needed to examine the association between macronutrients intake and adipose tissue deposition. A focus may be on visceral adipose tissue in the abdominal cavity as it associates more strongly with NCDs(131).

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