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Impact of Dietary Fat on Insulin Sensitivity, Glucose Metabolism, Serum Lipids and Coagulation Factors

Doctoral dissertation

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ABSTRACT

The incidence of type 2 diabetes is rapidly increasing on a global scale. This is mainly due to the increase in the prevalence of obesity and a sedentary lifestyle that are strong risk factors for type 2 diabetes. Many dietary factors may contribute to the increased risk. These factors include high (especially saturated) fat content in diet, low fibre intake and high glycaemic load in the diet. The association of dietary fat with glucose and insulin metabolism has been reported in several animal experiments and epidemiological studies. However, there are few human intervention studies where the effects of dietary fat modification on measures of glucose and insulin metabolism would have been carefully evaluated.

The aim of the present study was to examine the effects of stearic acid (Study I) and trans fatty acids (Study II) on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women and the effects of two fat-modified diets (monounsaturated fat enriched diet vs reduced-fat diet) on the same outcome variables in hyperlipidaemic middle-aged subjects (Study III). In addition, the relation between changes in the serum lipid fatty acid profile during the two fat-modified diets (high-fat monounsaturated fat enriched diet vs reduced-fat polyunsaturated fat enriched diet) and changes in glucose and insulin metabolism was evaluated in subjects with impaired glucose tolerance (IGT) in Study IV. There were 15 female subjects in Studies I and II, 18 subjects (13 males/5 females) in Study III and 31 subjects (18 males/13 females) in Study IV. In all four studies, glucose and insulin metabolism were evaluated by a frequently sampled intravenous glucose tolerance test. In studies I-III also serum lipids and lipoproteins and plasma fibrinogen, factor VII coagulant activity (studies I-III) and plasminogen activator inhibitor-1 activity (study III) were measured. In study IV, serum triglyceride, cholesterol, cholesteryl ester and phospholipid fatty acid composition were analysed.

In young healthy women, the effects of stearic acid (5 E%) did not differ from the effects of oleic acid on glucose and insulin metabolism, serum lipids and lipoproteins or coagulation factors (study I). There were no differences between the effects of trans fatty acids (5 E%) and the effects of oleic acid with respect to glucose and insulin metabolism or coagulation factors. Serum total to HDL cholesterol ratio and serum total, HDL and LDL triglyceride and apo-B concentrations were significantly higher after the consumption of the trans fatty acid enriched diet (study II). The insulin sensitivity in hyperlipidaemic subjects was significantly higher after the reduced-fat diet compared with the high-monounsaturated fat diet. The change in insulin sensitivity had a strong negative association with the change in the serum triglyceride concentration. There were no significant differences between the diets in the mean values of serum lipids and lipoproteins or coagulation factors (study III). In subjects with IGT, a higher insulin sensitivity index at baseline was associated with a lower proportion of saturated fatty acids in serum lipid fractions. Indicators of glucose metabolism (a higher glucose effectiveness index and lower fasting plasma glucose) were associated with higher proportions of oleic and α-linolenic acids at baseline. After the consumption of the reduced-fat diet and the high-monounsaturated fat diet the increases in proportions of oleic and α-linolenic acids were associated with a decrease in fasting plasma glucose and an increase in oleic acid also with an increase in glucose effectiveness index (study IV).

In conclusion, moderate enrichment of stearic or trans fatty acids in diet had no effect on insulin sensitivity in young healthy subjects. However, subjects with hyperlipidaemia/impaired glucose metabolism may be more sensitive to changes in dietary fat content or quality. Moderate fat content and enrichment of diet with unsaturated fat seem beneficial for glucose metabolism in these groups.

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Anne Louheranta
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles which are referred to in the text by their Roman numerals I-IV.

I  Louheranta AM, Turpeinen AK, Schwab US, Vidgren HM, Parviainen MT and Uusitupa MIJ. A high stearic acid diet does not impair glucose tolerance and insulin sensitivity in healthy women. Metabolism 1998;47:529-534


IV Louheranta AM, Sarkkinen ES, Vidgren HM, Schwab US, Uusitupa MIJ. Association of the fatty acid profile of serum lipids with glucose and insulin metabolism during 2 fat-modified diets in subjects with impaired glucose tolerance. Am J Clin Nutr 2002;76:331-337
ABBREVIATIONS

AIR               Acute insulin response
Apo(a)            Apolipoprotein(a)
ApoA-1            Apolipoprotein A-1
ApoB              Apolipoprotein B
BMI               Body mass index
CV%               Coefficient of variation
DM                Diabetes mellitus
E%                Percent of energy intake
FFQ               Food frequency questionnaire
FSIGT             Frequently sampled intravenous glucose tolerance test
FVII              Factor VII
FVIIc             Factor VII coagulant activity
GDM               Gestational diabetes mellitus
HDL               High-density lipoprotein
IFG               Impaired fasting glucose
IGT               Impaired glucose tolerance
LDL               Low-density lipoprotein
Lp(a)             Lipoprotein(a)
MCT               Medium chain triglycerides
MUFA              Monounsaturated fatty acids
PAI-1             Plasminogen activator inhibitor-1
PAI-1ag           Plasminogen activator inhibitor-1 antigen
PL                Phospholipids
PUFA              Polyunsaturated fatty acids
SAFA              Saturated fatty acids
TG                Triglycerides
S_0              Glucose effectiveness index
S_1              Insulin sensitivity index
VLDL             Very-low-density lipoprotein
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1. INTRODUCTION

The incidence and prevalence of type 2 diabetes is rapidly increasing on a global scale. There were approximately 150 million individuals with diabetes in the year 2000 and the number will double by the year 2025 (1). This increase relates particularly to type 2 diabetes. There is also a trend for the age of onset shifting to younger age groups (2). The macro- and microvascular complications of the disease result in high premature morbidity and mortality. The mortality rates among persons with type 2 diabetes are 2-4 times higher than in the general population (3, 4). In Caucasian populations, most of the excess mortality is attributable to cardiovascular disease (3-5). Given the increasing prevalence, the shift in the age of onset to younger age groups and the increased morbidity and mortality associated with type 2 diabetes, the disease will place a huge burden on healthcare systems in the future both in the developing as well as in affluent countries.

In conjunction to genetic susceptibility, this increase in the number of people with type 2 diabetes is largely due to an increase in the prevalence of obesity and a sedentary lifestyle that are strong risk factors for type 2 diabetes. Many dietary factors may contribute to the increased risk (2). Dramatic changes in the prevalence and incidence of type 2 diabetes have been observed in communities where people have changed from a traditional lifestyle to a ‘Western’ lifestyle, with corresponding changes in their diet and physical activity (6). There is also evidence from recent lifestyle intervention studies that targeting these risk factors significantly reduces the risk of type 2 diabetes (7, 8). Diet related risk factors include high fat content, especially high saturated fat content in diet, low fibre intake and high glycaemic load in the diet (9, 10). In some studies, also low magnesium and vitamin E intakes have been suggested to increase the risk (11-15). The association of dietary fat with glucose and insulin metabolism and the risk of type 2 diabetes has been reported in several animal experiments and epidemiological studies (9), (10, 16). Associations have been reported also between serum and skeletal muscle fatty acids that are to some extent modified by dietary intake and glucose and insulin metabolism this being the case both in animals and in humans (16). However, there are very few human intervention studies where the effects of single dietary fatty acids or dietary fat modification on measures of glucose and insulin metabolism would have been carefully evaluated.

To explore the effects of different dietary fatty acids on glucose tolerance, we set up a series of studies. Specifically, in randomized controlled crossover trials we examined the effects of stearic acid, a single saturated fatty acid, and trans fatty acids on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women and the effects of two fat-modified diets (monounsaturated fat enriched diet vs reduced-fat diet), on glucose and insulin metabolism, serum lipids and lipoproteins as well as coagulation factors in hyperlipidaemic middle-aged subjects. In addition, the relations between changes in serum lipid fatty acid profile during the two fat-modified diets (high-
fat monounsaturated fat enriched diet vs reduced-fat polyunsaturated fat enriched diet) and changes in glucose and insulin metabolism were evaluated in subjects with impaired glucose tolerance. Ultimately, the goals were to determine the optimal fatty acid composition of the diet in terms of glucose metabolism.
2. REVIEW OF THE LITERATURE

2.1 Type 2 diabetes and impaired glucose metabolism

The term diabetes mellitus describes a heterogeneous group of metabolic disorders characterized by chronic hyperglycaemia. There are two main forms of diabetes, type 1 and type 2 diabetes (17). Type 1 diabetes is due to an autoimmune-mediated destruction of pancreatic β-cells resulting in absolute insulin deficiency. Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion either of which can predominate.

The prevalence of type 1 diabetes is low relative to type 2 diabetes which accounts for over 90% of cases of diabetes mellitus globally (2). Based on prevalence figures and projections from national surveys, it has been estimated that worldwide there were approximately 150 million individuals with diabetes in the year 2000 and the number will double by the year 2025 (1), this being attributable mostly to type 2 diabetes. In Finland, it has been estimated that there will be a 70% increase in the number of patients with type 2 diabetes by the year 2010 (18). The onset of type 2 diabetes typically occurs after 50 years of age (19). However, in Pacific islanders and in other high-risk populations, the onset in the 20-30-year age group is now increasingly common (20). Although type 1 diabetes remains the main form of diabetes in children and adolescents, recently type 2 diabetes has been found also in these age groups, especially in high-risk populations (21, 22) The trend of increasing prevalence of type 2 diabetes and shift in the age of onset to younger age groups will place a huge burden on health-care systems in the future both in the developing as well as in affluent countries.

Type 2 diabetes is frequently asymptomatic and at this stage is detected mainly by screening. It is diagnosed when fasting plasma glucose is equal or higher than 7.0 mmol/l and/or the 2-hour value in an oral glucose tolerance test is higher than 11.0 mmol/l (17). For a person presenting with the symptoms of hyperglycaemia, one measurement in the diabetic range fulfills the criteria for diagnosis. For an asymptomatic person, two measurements in the diabetic range are required. The onset of clinical type 2 diabetes is preceded typically for years by milder forms of impaired glucose metabolism, impaired glucose tolerance (IGT) or impaired fasting glycaemia (IFG). IGT is defined by an oral glucose tolerance test when fasting plasma glucose is below 7 mmol/l and a 2-hour value from 7.8 to 11.0 mmol/l. IFG is defined when fasting plasma glucose is between 6.1-6.9 mmol/l and, if measured, 2-hour value in the oral glucose tolerance test is below 7.8 mmol/l (17). A special form of impaired glucose metabolism, gestational hyperglycaemia/diabetes (GDM) is defined by onset or first recognition during pregnancy (17). Following delivery, glucose tolerance is likely to become normal but women with gestational hyperglycaemia are at a higher risk of subsequently developing diabetes (23, 24).
Type 2 diabetes is a multifactorial disease with a clear genetic background, but there is a strong influence of environmental factors such as obesity, sedentary lifestyle and diet. The macro- and microvascular complications of the disease result in premature morbidity and mortality. In Caucasian populations, much of the excess mortality is attributable to cardiovascular disease (3-5), but in other populations, such as Asians and American Indians renal disease can be a major debilitating factor (25, 26). In some developing nations, an important component of the excess mortality is attributable to infections (27). With the diagnosis of type 2 diabetes or IGT/IFG a broader underlying disorder - the metabolic syndrome - is frequently detected. The metabolic syndrome is a cluster of risk factors for cardiovascular diseases. In addition to glucose intolerance, the features of metabolic syndrome include hyperinsulinaemia, dyslipidaemia, hypertension, visceral obesity, hypercoagulability and microalbuminuria (17). In a Finnish cohort of 3606 women and men (age 35-70 years), respectively, the metabolic syndrome (17) was recorded in 10 and 15% of subjects with normal glucose tolerance, 42 and 64% of those with IGT/IFG, and 78 and 84% of those with type 2 diabetes. The presence of the metabolic syndrome increased the risk of cardiovascular disease threefold in subjects with the syndrome compared to subjects without this syndrome (28). Recognition of the heterogeneity of type 2 diabetes has changed the approach to the therapy of the disease. According to current recommendations, instead of just treating hyperglycaemia, also other risk factors for cardiovascular disease, hypertension and hyperlipidaemia should be effectively treated (29).

2.1.1 Risk factors

Genetic factors

Type 2 diabetes has a strong genetic component. The concordance rate of type 2 diabetes has been reported to be higher in monozygotic twins (50-96%) than in dizygotic twins (10-37%) (30-33). Furthermore, type 2 diabetes and IGT show a familial aggregation (34). The lifetime risk for type 2 diabetes is about 40% in offspring with one diabetic parent and 70% if both parents have diabetes (35, 36). The risk of type 2 diabetes seems to be greater in offspring whose mothers had type 2 diabetes than in offspring whose fathers had type 2 diabetes (37). There is also a large variation in the prevalence of type 2 diabetes in different ethnic populations, the highest prevalence figures have been found in Pima Indians living in Arizona (~50%) and Micronesian Nauruans (~40%) (38). However, the genetic basis of type 2 diabetes is still poorly understood. Only in some uncommon subtypes of type 2 diabetes (maturity-onset diabetes of the young and maternally transmitted form of diabetes, often associated with deafness) have the genes involved in the development of the disease been characterized (39).
Age, gender and impaired glucose metabolism

The prevalence of type 2 diabetes increases with age, although the patterns of incidence vary considerably. In Caucasian populations in the United States and Europe, the prevalence starts to increase from about the age of 50 years and continues to increase with age at least into the seventies (40). In high-risk populations, the prevalence may increase markedly from the younger adult years, 20-35 years of age (20). Recently, type 2 diabetes has been found also in children and adolescents, especially in high-risk populations (21, 22) Gender seems to have little effect on the prevalence of type 2 diabetes. In most populations, type 2 diabetes is equally prevalent among men and women, though there is some evidence of a male preponderance in early middle age (41).

All types of impaired glucose metabolism (IGT, IFG, GDM) are risk factors for subsequent development of type 2 diabetes. Approximately 40% of subjects with IGT progress to diabetes over 5-10 years, some revert to back to normality or remain IGT. Also IFG is associated with increased risk of future diabetes (2). However, of these two categories of glucose intolerance, IGT seems to be a better predictor of future diabetes and of mortality (42-44). Women with GDM during pregnancy carry a high risk for developing diabetes subsequently, the cumulative incidence of type 2 diabetes is about 50% at 5 years (23, 24, 45).

Obesity

Obesity is a strong risk factor for type 2 diabetes. The prevalence of obesity in newly diagnosed type 2 diabetes patients is high. In one patient group in Denmark the prevalence of obesity was 80% compared to 40% in the background population of the same age (46). Data from a large American cohort of women suggest that the lowest risk of diabetes occurs in individuals who have a body mass index (BMI) less than 22 kg/m², the risk increases with increasing BMI. Furthermore, the weight gain since early adulthood significantly contributes to the risk (47). Similarly, a strong association between current BMI, BMI at age 21 and weight gain throughout adulthood and the risk of type 2 diabetes has been reported for American men (48). In Pima Indians, also obesity in childhood and adolescence (49) and duration of obesity (50) defined as the time since BMI was first known to be at least 30 kg/m² have been reported to increase the risk of subsequent type 2 diabetes. The distribution of fat seems to be an equally important risk factor for type 2 diabetes as is BMI. Large waist circumference or waist-to-hip ratio, reflecting abdominal obesity, have been identified as strong risk factors for type 2 diabetes (48, 51, 52).
Physical inactivity

Several prospective cohort studies have indicated the importance of physical inactivity in the development of type 2 diabetes (53-55). American women who reported exercising vigorously had a 30% lower risk of diabetes compared to women who exercised less frequently (53). In American men who exercised at least once per week, the risk of type 2 diabetes was 36% lower compared to men who exercised less frequently. The inverse relation of exercise to the risk of type 2 diabetes was particularly pronounced among overweight men (54). In data from British middle-aged men, moderate physical activity reduced the risk of type 2 diabetes by 50% compared to the risk for inactive men. Even occasional and light physical activity decreased the risk (56).

Diet

With respect to the diet-related factors, there are most data about dietary fat and the risk of type 2 diabetes. A high fat content and especially a high saturated fat content in diet have been identified as risk factors (9, 10). The associations of dietary fat and single fatty acids with glucose and insulin metabolism are reviewed in detail in the following chapters (2.2 and 2.3) in this thesis. Other dietary factors which have been identified as risk factors for type 2 diabetes in prospective cohort studies are low fibre (especially cereal fibre) content (11-13) and high glycaemic load in diet (11, 12). The association with high glycaemic load has not been observed in all studies (13). There are also some studies, where magnesium intake and vitamin E level in blood have been associated with the risk of type 2 diabetes. In three large prospective cohort studies, high intake of magnesium has been associated with lower risk of type 2 diabetes (11-13). However, this association has not been reported in all studies (57). There are two studies, one prospective cohort study and one case-control study, where the association of blood level of vitamin E and the risk of type 2 diabetes has been evaluated (14, 15). Both studies reported a higher risk of type 2 diabetes with low levels of vitamin E in blood. However, in the nested case-control study by Reunanen et al., (15) the association disappeared when the risk ratio was adjusted for various coronary heart disease risk factors. Also a low serum vitamin D concentration has been reported to be associated with a deterioration in glucose tolerance in a small prospective cohort study (58, 59). In some prospective cohort studies, a high intake of meat or processed meat has been proposed as a risk factor (60-62).

Other factors

Some drugs e.g. glucocorticoids and thiazides are known to increase the risk of type 2 diabetes (17). Smoking has been identified as an independent risk factor for type 2 diabetes in several prospective cohort studies (56, 63-68). In a few studies, smoking has
been a predictor of type 2 diabetes only in men (67, 68). In a large cohort of American women (69) alcohol consumption was associated with a lower risk for type 2 diabetes. However, there was a strong inverse relation between alcohol consumption and body weight which could explain much of the protective effect. In a large cohort of American males (65, 70) those consuming more than 2-4 drinks per week had a lower incidence of type 2 diabetes. On the other hand, an increased risk of type 2 diabetes was reported in another prospective cohort study (71) for men consuming more than 21 drinks per week. In that study there was no association between alcohol intake and the risk of diabetes in women. There are two prospective cohort studies in males from UK (72) and Sweden (73) where the lowest risk for type 2 diabetes was found in moderate drinkers but the highest risk was in heavy drinkers.

The intrauterine environment also influences the risk of developing type 2 diabetes. Offspring of diabetic pregnancies tend to develop obesity in childhood and are at high risk of developing type 2 diabetes at an early age (74). Among offspring born to mothers before and after the development of type 2 diabetes, those born after the mother developed diabetes have a three-fold risk of developing diabetes compared to those born before the illness appeared (37, 75). An association between low birth weight and higher risk of type 2 diabetes and abnormal glucose/insulin metabolism in later life has been shown in numerous studies (76-79). In Pima Indians, this relationship was found to be U-shaped with the highest prevalence both in high and low birth weight infants (80). Moreover, the highest prevalence of type 2 diabetes has been found in those with low birth weight and who became obese as adults (76, 81). Early feeding may also play a role; the prevalence of type 2 diabetes was 50% lower in Pima Indians exclusively breastfed for the first two months of life (6).

2.2 Dietary fat and markers of glucose and insulin metabolism

Both the amount and type of dietary fat may modify glucose tolerance and insulin sensitivity (9, 10, 16). In animal experiments, several mechanisms have been proposed to account for this relationship. A high fat content in the diet may result in a deterioration of glucose tolerance by decreased binding of insulin to its receptors, impaired glucose transport, reduced proportion of glycogen synthase and accumulation of stored triglycerides in skeletal muscle (82-86). The fatty acid composition, in turn, affects tissue phospholipid composition, which may impact on insulin action by altering membrane fluidity and insulin signalling (16). There are data from animal experiments that fatty acids may also affect insulin secretion (87). The inhibitory effects are coupled to the fatty acid oxidation in \( \beta \)-cells (87), there is also recent evidence that chronic exposure to fatty acids may alter insulin receptor and insulin receptor substrate gene expressions in insulin producing cells (88).
2.2.1 Amount of fat

In experimental animals all high-fat diets (with the exception of n-3 fatty acids) have been shown to result in insulin resistance relative to high carbohydrate diets (16, 85, 89, 90). Furthermore, exposure of the β-cells to high levels of fatty acids for long periods has altered insulin secretion in animal experiments. Increased insulin secretion has been observed in the presence of low glucose concentration and a blunted insulin response in response to high glucose concentration (91). The evidence from epidemiological studies is less consistent (Tables 1 and 2). A higher total fat intake has been associated with higher fasting insulin concentrations in two cross-sectional studies (92, 93) and in a prospective cohort study where the high fat intake was associated with lower insulin sensitivity index (94). However, in many cross-sectional studies or prospective cohort studies, no association has been found (Tables 1 and 2.) The number of intervention studies comparing high fat, low carbohydrate diets is small and the results have been inconsistent (95-102). A recent study in healthy subjects reported lower insulin sensitivity (101) after consumption of a high-fat diet (50 % of energy (E%) as fat, 35 E% carbohydrates) relative to a high-carbohydrate diet (20 E% as fat, 55 E% as carbohydrates). With a very high fat content (83%, liquid diet) a reduction in ability of insulin to suppress endogenous glucose production (102) relative to the high-carbohydrate diet (85 %, liquid diet) was reported in healthy subjects.

2.2.2 Saturated fat

In animal experiments, saturated fat has caused insulin resistance when fed as high-fat diets (59 E% as fat) (16, 85, 89). In cultured rodent β-cells, long-chain saturated fatty acids appear to stimulate insulin release more potently than unsaturated ones, however, the number of studies is small (87). Mainly in cross-sectional studies, high saturated fat intake has been associated with higher fasting glucose (103-105) and insulin concentrations (92, 106, 107). In a cross-sectional study of over 4500 Italian men and women, a higher consumption of butter was associated with significantly higher blood glucose in both sexes (105). In a group of 652 men aged 43-85 years, saturated fatty acid intake was independently associated with fasting and postprandial insulin concentrations in a cross-sectional study (92) even after adjusting for age, smoking and physical activity. In that group, a decrease in saturated fat intake from 14 to 8 E% would predict a 18% decrease in fasting and a 25% decrease in postprandial insulin. Saturated fat intake had a significant positive correlation with fasting insulin also in a group of men with coronary artery disease (106). The association between saturated fat and fasting insulin has been shown in women as well. In the cross-sectional study of Mayer et al, (93) high saturated and total fat intake were associated with high fasting insulin. Furthermore, a higher proportion of saturated fatty acids in serum phospholipids was associated with higher insulin
concentration in a study population of nearly 4000 American men and women, this association being sustained also after adjustment for BMI (108). In a Swedish population, insulin sensitivity was inversely correlated with the proportion of palmitic and palmitoleic acids in the serum cholesterol esters of 70-year old men (109). The correlation of palmitic acid and insulin sensitivity was even stronger when fatty acids were measured in skeletal muscle phospholipids. There are only a few intervention studies in which the effects of single saturated fatty acids on glucose and insulin metabolism have been examined (Table 3.). In three short-term studies, no differences between the effects of lauric and palmitic (110) or palmitic and stearic acids (111, 112) on glucose and insulin metabolism were found. In a recent study comparing the effects of palmitic, oleic and elaidic acid enriched diets on insulin sensitivity in normal and overweight subjects no difference was found (113).

2.2.3 Unsaturated fat

With respect to saturated fat, both monounsaturated and polyunsaturated fat (with the exception of n-3 fatty acids) have caused insulin resistance in animal experiments when fed as high-fat diets (16, 85, 89, 90). In observational studies in humans, higher vegetable fat (unsaturated fat) and PUFA intakes have been associated with lower fasting glucose and insulin concentrations (92, 93, 104, 105). Furthermore, a higher proportion of long-chain polyunsaturated fatty acids in skeletal muscle phospholipids has been linked to better insulin sensitivity in humans (114, 115). With respect to monounsaturated fatty acids, the epidemiological data produce a less clear picture, some cross-sectional studies and prospective cohort studies indicate that a high intake of monounsaturated fat may be harmful for glucose metabolism (106, 116). However, it should be noted that in the Western diet the monounsaturated fatty acids are not derived from vegetable oils but to a large extent from other sources such as meat and milk products. Thus the inverse associations reported (106, 116, 117) are likely to be due to the strong intercorrelation between saturated and monounsaturated fat intake (116). In two earlier intervention studies, replacement of a considerable portion of saturated fat by unsaturated fat improved glucose tolerance in young healthy women (118) and middle-aged glucose-intolerant hyperlipidaemic subjects (119). A recent intervention study by Vessby et al. (120) confirmed that the substitution of monounsaturated fat for saturated fat significantly improved insulin sensitivity in healthy middle-aged subjects after a 3-month diet period. An interesting interaction between the intake and fatty acid composition of dietary fat was reported: the favourable effect of substituting monounsaturated fat for saturated fat was lost in individuals consuming more than 37 E% fat (120). In most of the reported intervention studies (Table 3) in healthy subjects or in subjects with type 2 diabetes substituting either monounsaturated or polyunsaturated fat for saturated fat has had a
beneficial effect on various measures of glucose and insulin metabolism. However, there are few intervention studies in this field.

**Trans fatty acids**

There are little data on the effects of trans fatty acids on glucose metabolism. In mouse islet cells, trans fatty acids potentiated insulin secretion relative to cis isomers (121). In patients with type 2 diabetes, a 6-week experimental diet high in trans fatty acids (20% of energy, E%) increased postprandial C-peptide and insulin responses compared with a diet high in cis-monounsaturated fatty acids (122). In the recent study of Lovejoy et al (113), the effects of 9 E% enrichment of elaidic acid in diet on glucose and insulin metabolism did not differ from the effects of a 9 E% enrichment of either oleic or palmitic acids in diet in healthy adults.

**N-3 fatty acids**

In rodent studies, long-chain n-3 fatty acids ameliorate insulin resistance induced by high-fat feeding (10, 85, 90). Most intervention studies aimed at investigating the effect of fish oil on insulin sensitivity have been done in patients with type 2 diabetes but no effect has been seen (123-127). Also in a recent study in healthy subjects, there was no effect of fish oil on insulin sensitivity (120).

**2.3 Dietary fat and the risk of type 2 diabetes**

A high intake of fat has been associated with an increased risk of type 2 diabetes and impaired glucose tolerance (Tables 1 and 2). In the cross-sectional study by Marshall et al (128), the risk ratio for diabetes (with fasting glucose > 10 mmol/l (140/dl)) was 3.0 and for impaired glucose tolerance (IGT) 2.7 for every increase of 40 g/day in fat intake. Based on the prospective data from the same study population (129), an increase in fat intake of 40 g/day was associated with a 3.4-fold risk of developing type 2 diabetes during the 1-3 year follow-up in a group of subjects with impaired glucose tolerance. The association remained significant after controlling for obesity and markers of glucose metabolism. In a cross-sectional study, women with recurrent GDM had a significantly higher fat intake than women with no recurrence (38.4 vs 34.1 E%, respectively) (130). The groups did not differ with respect to family history of diabetes, age, parity or last recorded BMI. In the 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study, the intake of total fat was higher 20 years before diagnosis in men with newly diagnosed diabetes (116).

With respect to the different subtypes of fat, in several prospective cohort studies high consumptions of vegetable fat and PUFA have been associated with a reduction (61, 103,
and a high SAFA (116) and trans fat (131) consumption with an increase in the risk of type 2 diabetes. In an earlier report from the prospective Nurses’ Health Study, an inverse association of vegetable fat intake with the risk of type 2 diabetes was seen only in non-obese individuals (61). In recent reports from the same study (131) as well as from another large cohort of women from USA (103) with more cases and a longer follow-up, an inverse association between vegetable fat intake and the risk of type 2 diabetes was seen independent of BMI. However, in the Health Professionals Follow-up Study with a large cohort of men and a 12-year follow-up, the linoleic acid intake was associated with a lower risk of diabetes only in men <65 years of age and BMI < 25 kg/m² (62). A higher saturated fat intake was associated with glucose intolerance (IGT/DM) during pregnancy in a cross-sectional study (132) and in the earlier mentioned prospective Seven Countries Study (116). However, there are several prospective cohort studies where no association has been observed between intake of total fat or different subtypes of fat and the risk of type 2 diabetes (11, 12, 63, 94, 133).

In line with the associations found for diet and the risk of type 2 diabetes in the epidemiological data, the proportion of palmitic acid has been reported to be higher in serum cholesteryl esters (134, 135) and triglycerides and phospholipids (135) in diabetic compared to normoglycaemic subjects. Furthermore, a higher proportion of saturated fatty acids and a lower proportion of polyunsaturated fatty acids in serum cholesteryl esters were associated with a higher risk of type 2 diabetes in Swedish men during a 10 year follow-up (136).

There are two recent intervention studies where both a reduction in fat intake and saturated fat intake and an increase in unsaturated fat intake have been a part of a lifestyle intervention aiming to prevent type 2 diabetes in overweight, middle-aged subjects with impaired glucose tolerance (7, 8). Together with increase in exercise, weight loss and increase in dietary fibre intake, the dietary fat modification was efficient in reducing the incidence of type 2 diabetes. In both studies, the estimate of risk reduction was 58% with an average follow-up time from 2.8 to 3.2 years.
<table>
<thead>
<tr>
<th>Study, country, year (ref)</th>
<th>No of subjects</th>
<th>Sex, age</th>
<th>Diet assessment</th>
<th>Outcome</th>
<th>Adjustment</th>
<th>Reported associations with dietary fat (direction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zutphen Study, Netherlands, 1990 (104)</td>
<td>394</td>
<td>M, 50-70</td>
<td>Dietary history</td>
<td>Fasting glucose</td>
<td>Age, subscapular skinfold thickness, energy intake</td>
<td>SAFA (+), dietary cholesterol (+)</td>
</tr>
<tr>
<td>Italian Nine Communities Study, Italy, 1990, (105)</td>
<td>4903</td>
<td>M/F, 20-59</td>
<td>FFQ⁴</td>
<td>Fasting glucose</td>
<td>Age, BMI³, other fats</td>
<td>Positive associations with butter or margarine (+), vegetable oils (-)</td>
</tr>
<tr>
<td>San Luis Valley Diabetes Study, USA, 1991 (128)</td>
<td>211</td>
<td>M/F, 30-74</td>
<td>24-h recall</td>
<td>DM2 or IGT</td>
<td>Age, sex, ethnicity, BMI, energy intake</td>
<td>total fat (+)</td>
</tr>
<tr>
<td>Stanford Coronary Risk Intervention Project, USA, 1991 (106)</td>
<td>215</td>
<td>M, 32-74</td>
<td>Food records</td>
<td>Fasting insulin</td>
<td>Age, BMI</td>
<td>SAFA (+)</td>
</tr>
<tr>
<td>Normative Aging Study, USA, 1993 (92)</td>
<td>652</td>
<td>M, 43-85</td>
<td>FFQ</td>
<td>Fasting or postpr. insulin</td>
<td>Age, smoking, physical activity</td>
<td>SAFA (+)</td>
</tr>
<tr>
<td>Kaiser Permanente Women Twins Study, 1993 (93)</td>
<td>544</td>
<td>F, 30-84</td>
<td>FFQ</td>
<td>Fasting or postpr. insulin</td>
<td>Energy intake, age, body fat, waist-hip ratio</td>
<td>total fat (+), SAFA, oleic and linoleic acids (+)</td>
</tr>
<tr>
<td>Study, country, year (ref)</td>
<td>No of subjects</td>
<td>Sex, age</td>
<td>Diet assessment</td>
<td>Outcome</td>
<td>Adjustment</td>
<td>Reported associations with dietary fat (direction)</td>
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</tr>
<tr>
<td>Zutphen Elderly Study, Netherlands, 1994 (137)</td>
<td>389</td>
<td>M, 70-89</td>
<td>Dietary history</td>
<td>Fasting insulin</td>
<td>Age, BMI, physical activity, CHD, energy intake</td>
<td>SAFA (+), inverse association with PUFA (-)</td>
</tr>
<tr>
<td>Hoorn Study, Netherlands, 1995 (138)</td>
<td>2484</td>
<td>M/F, 50-74</td>
<td>FFQ</td>
<td>2h-glucose</td>
<td>Other risk factors, energy intake</td>
<td>polyunsaturated fat (+) in men</td>
</tr>
<tr>
<td>San Luis Valley Diabetes Study, 1997 (107)</td>
<td>1069</td>
<td>M/F, 20-74</td>
<td>24-h recall</td>
<td>Fasting insulin</td>
<td>Age, sex, ethnicity, BMI, waist circumference, physical activity, energy intake</td>
<td>total and saturated fat intake (+)</td>
</tr>
<tr>
<td>Wollongong, Australia, 1997 (130)</td>
<td>35</td>
<td>F, 34 (mean)</td>
<td>Diet history, food records</td>
<td>Recurrence of GDM</td>
<td>None</td>
<td>total fat intake (+)</td>
</tr>
<tr>
<td>Torino, Italy, 2001 (132)</td>
<td>504</td>
<td>F, 33 (mean)</td>
<td>Diet history</td>
<td>IGT or GDM during pregnancy</td>
<td>Gestational age</td>
<td>SAFA (+)</td>
</tr>
</tbody>
</table>

1 IGT = impaired glucose tolerance, GDM = gestational diabetes mellitus, DM2 = type 2 diabetes; 2 M=males, F=females; 3 saturated fatty acids; 4 food frequency questionnaire; 5 body mass index; 6 coronary heart disease; 7 polyunsaturated fatty acids
<table>
<thead>
<tr>
<th>Study, country, year (ref)</th>
<th>No of subjects</th>
<th>Sex¹, age at baseline</th>
<th>Follow-up (y)</th>
<th>Diet assessment</th>
<th>Outcome</th>
<th>Adjustment</th>
<th>Reported associations with dietary fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pima Indians, USA, 1984 (94)</td>
<td>277</td>
<td>F, 25-44</td>
<td>NA³</td>
<td>Dietary history</td>
<td>DM2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Gothenburg, Sweden, 1989 (133)</td>
<td>1462</td>
<td>F, 38-60</td>
<td>12</td>
<td>24-h recall, diet records</td>
<td>DM2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Zuthpen study, Netherlands, 1989 (63)</td>
<td>841</td>
<td>M, 40-59</td>
<td>25</td>
<td>Dietary history</td>
<td>DM2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Netherlands, 1991 (139)</td>
<td>175</td>
<td>M/F, 64-87</td>
<td>4</td>
<td>Dietary history</td>
<td>IGT or GDM</td>
<td>Other risk factors, energy intake</td>
<td>fish intake (-)</td>
</tr>
<tr>
<td>Nurses’ Health Study, USA, 1992 (61)</td>
<td>84360</td>
<td>F, 34-59</td>
<td>6</td>
<td>FFQ¹</td>
<td>DM2</td>
<td>Other risk factors, energy intake</td>
<td>vegetable fat intake in the non-obese (-)</td>
</tr>
<tr>
<td>San Luis Valley Diabetes Study, 1994 (129)</td>
<td>134</td>
<td>M/F, 30-74</td>
<td>1-3</td>
<td>24h-recall</td>
<td>DM2</td>
<td>Age, sex, ethnicity, obesity, blood glucose/insulin</td>
<td>fat intake (+)</td>
</tr>
<tr>
<td>Seven Countries Study, Netherlands/Finland 1995 (116)</td>
<td>338</td>
<td>M, 50-70</td>
<td>20</td>
<td>Dietary history</td>
<td>2-h glucose</td>
<td>Age, BMI², energy intake</td>
<td>SAFA³ (+) and total fat (+), fish intake (-)</td>
</tr>
<tr>
<td>Study, country, year (ref)</td>
<td>No of subjects</td>
<td>Sex, age at baseline</td>
<td>Follow-up (y)</td>
<td>Diet assessment</td>
<td>Outcome</td>
<td>Adjustment</td>
<td>Reported associations with dietary fat</td>
</tr>
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<tr>
<td>Nurses' Health Study, USA, 1997 (11)</td>
<td>65173</td>
<td>F, 40-65</td>
<td>6</td>
<td>FFQ</td>
<td>DM2</td>
<td>Other risk factors and energy intake</td>
<td>None</td>
</tr>
<tr>
<td>Health Professionals Follow-up Study, USA, 1997 (12)</td>
<td>42759</td>
<td>M, 40-75</td>
<td>6</td>
<td>FFQ</td>
<td>DM2</td>
<td>Other risk factors and energy intake</td>
<td>None</td>
</tr>
<tr>
<td>Nurses' Health Study, USA, 2001 (131)</td>
<td>84204</td>
<td>F, 34-59</td>
<td>14</td>
<td>FFQ</td>
<td>DM2</td>
<td>Other risk factors, energy intake, other fats</td>
<td>vegetable fat (-) and PUFA(^\text{1}) (-), trans fat (+)</td>
</tr>
<tr>
<td>Iowa Women's Health Study, USA, 2001 (103)</td>
<td>35988</td>
<td>F, 55-69</td>
<td>11</td>
<td>FFQ</td>
<td>DM2</td>
<td>Other risk factors, dietary magnesium, cereal fiber, other fats</td>
<td>vegetable fat (-)</td>
</tr>
<tr>
<td>Health Professionals Follow-up Study, USA, 2002 (62)</td>
<td>42504</td>
<td>M, 40-75</td>
<td>12</td>
<td>FFQ, repeated 3 times</td>
<td>DM2</td>
<td>Other risk factors, energy intake, cereal fiber and magnesium intake, BMI</td>
<td>linoleic acid (-) in men &lt; 65 years and BMI &lt; 25 kg/m(^2)</td>
</tr>
</tbody>
</table>

\(^1\)IGT = impaired glucose tolerance, GDM = gestational diabetes mellitus, DM2 = type 2 diabetes; \(^2\)M=males, F=females; \(^3\)not available; \(^4\)food frequency questionnaire; \(^5\)body mass index; \(^6\)saturated fatty acids; \(^7\)polyunsaturated fatty acids
Table 3. Human intervention studies on saturated fatty acids, trans fatty acids and monounsaturated fatty acids and indicators of glucose and insulin metabolism

<table>
<thead>
<tr>
<th>First author year (ref)</th>
<th>No of subjects</th>
<th>Sex, age, glucose tolerance</th>
<th>Design</th>
<th>Intervention</th>
<th>Duration (wk)</th>
<th>Outcome measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saturated fatty acids and trans fatty acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessby, 1980 (119)</td>
<td>30</td>
<td>12F/18M, 23-78y, NA</td>
<td>crossover</td>
<td>SAFA(^2) (P/S 0.2) vs PUFA(^3) (P/S 2.0)</td>
<td>2</td>
<td>IVGTT(^3)</td>
<td>elimination rate constant for glucose (K-value) higher after PUFA diet in subjects with type IV hyperlipoproteinaemia (n= 6)</td>
</tr>
<tr>
<td>Uusitupa, 1994 (118)</td>
<td>10</td>
<td>F, 23 y (mean), NGT(^8)</td>
<td>crossover</td>
<td>SAFA vs MUFA(^6) (20 E%)</td>
<td>3</td>
<td>IVGTT</td>
<td>AUC(^7) for glucose lower after MUFA diet</td>
</tr>
<tr>
<td>Schwab, 1995 (110)</td>
<td>15</td>
<td>F, 19-34y, NGT</td>
<td>crossover</td>
<td>palmitic vs lauric acid (4 E%)</td>
<td>4</td>
<td>FSIGT(^9)</td>
<td>no difference</td>
</tr>
<tr>
<td>Christianse n, 1997 (122)</td>
<td>16</td>
<td>7F/9M, 55 y (mean), DM2(^10)</td>
<td>crossover</td>
<td>SAFA vs MUFA vs trans-MUFA (20 E%)</td>
<td>6</td>
<td>AUC for insulin and C-peptide after a meal test</td>
<td>AUC for serum insulin and C-peptide higher after SAFA and trans-MUFA diets</td>
</tr>
<tr>
<td>Schwab, 1997 (140)</td>
<td>8</td>
<td>F, 21-25y, NGT</td>
<td>crossover</td>
<td>SAFA (21 E%) vs PUFA(^11) (18 E%)</td>
<td>3</td>
<td>IVGTT</td>
<td>no difference</td>
</tr>
<tr>
<td>Schwab, 1997 (141)</td>
<td>8</td>
<td>F, 20-31y, NGT</td>
<td>crossover</td>
<td>palmitic vs stearic acid (5 E%)</td>
<td>4</td>
<td>FSIGT</td>
<td>no difference</td>
</tr>
<tr>
<td>First author year (ref)</td>
<td>No of subjects</td>
<td>Sex¹, age, glucose tolerance</td>
<td>Design</td>
<td>Intervention</td>
<td>Duration (wk)</td>
<td>Outcome measure</td>
<td>Results</td>
</tr>
<tr>
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</tr>
<tr>
<td>Storm, 1997 (112)</td>
<td>15</td>
<td>7F/8M, 53 y (mean), DM2</td>
<td>crossover</td>
<td>palmitic (16 E%) vs stearic acid (13 E%)</td>
<td>3</td>
<td>HbA₁c, fasting glucose</td>
<td>no difference</td>
</tr>
<tr>
<td>Perez-Jimenez, 2001 (142)</td>
<td>59</td>
<td>29F/30M, 23 y (mean), NGT</td>
<td>crossover</td>
<td>SAFA (20 E%) vs Mediterranean (22 E% MUFA) vs high-carbohydrate (57 E%)</td>
<td>4</td>
<td>glucose suppression test, isolated monocytes</td>
<td>lower steady state plasma glucose and increased glucose uptake in monocytes after MUFA and high-carbohydrate diets</td>
</tr>
<tr>
<td>Vessby, 2001 (120)</td>
<td>162</td>
<td>76F/86M, 30-65y, NGT/IGT⁻¹³</td>
<td>parallel</td>
<td>SAFA (17 E%) vs MUFA (23 E%)</td>
<td>12</td>
<td>FSIGT</td>
<td>S₁⁰² reduced by 10% in the SAFA group, no change in the MUFA group</td>
</tr>
<tr>
<td>Lovejoy, 2002, (113)</td>
<td>25</td>
<td>13F/12M, non-diabetic</td>
<td>crossover</td>
<td>palmitic vs oleic vs elaidic acid (9 E%)</td>
<td>4</td>
<td>FSIGT, insulin pulsatile secretion</td>
<td>no difference, Nonsignificant reduction in S₁ by 24% after the palmitic acid enriched diet in overweight subjects compared to oleic acid enriched diet</td>
</tr>
<tr>
<td>Summers, 2002 (143)</td>
<td>17</td>
<td>9F/8M, 50-56 y (mean), DM2/non-diabetic</td>
<td>crossover</td>
<td>SAFA (21 E%, P/S 0.19) vs PUFA (9 E%, P/S 1.19)</td>
<td>5</td>
<td>hyperinsulinaemic euglycaemic clamp</td>
<td>Improvement in insulin sensitivity after PUFA diet</td>
</tr>
<tr>
<td>First author year (ref)</td>
<td>No of subjects</td>
<td>Sex¹, age, glucose tolerance</td>
<td>Design</td>
<td>Intervention</td>
<td>Duration (wk)</td>
<td>Outcome measure</td>
<td>Results</td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
<tr>
<td>Nielsen, 1995 (144)</td>
<td>10</td>
<td>3F/7M, 50-75y, DM2</td>
<td>crossover</td>
<td>MUFA (30 E%) vs high-carbohydrate (50 E%)</td>
<td>3</td>
<td>fasting glucose, fructosamine, HbA₁c</td>
<td>no difference</td>
</tr>
<tr>
<td>Espino-Montoro, 1996 (145)</td>
<td>20</td>
<td>M, 19-26y, NGT</td>
<td>crossover</td>
<td>MUFA (22 E%, olive oil) vs MUFA (22 E%, high-oleic-acid sunflower oil vs high-carbohydrate (55 E%))</td>
<td>3-4</td>
<td>fasting insulin and glucose</td>
<td>lower fasting insulin and glucose after the two MUFA diets</td>
</tr>
<tr>
<td>Garg, 1998 (146)</td>
<td>133</td>
<td>104M/29F, DM2</td>
<td>meta-analysis</td>
<td>MUFA (37-50 E%) vs high-carbohydrate (49-60 E%)</td>
<td>2-6</td>
<td>fasting glucose and insulin</td>
<td>lower fasting glucose by 0.23 mmol/l in the MUFA group</td>
</tr>
<tr>
<td>Thomsen, 1999 (147)</td>
<td>16</td>
<td>10F/6M, 35 y (mean), NGT</td>
<td>crossover</td>
<td>MUFA (25 E%) vs high-carbohydrate (55 E%)</td>
<td>4</td>
<td>FSIGT</td>
<td>no difference</td>
</tr>
</tbody>
</table>

¹ M=males, F=females; ² saturated fatty acids; ³ intravenous glucose tolerance test; ⁴ not available; ⁵ polyunsaturated fatty acids; ⁶ monounsaturated fatty acids; ⁷ area under the curve; ⁸ normal glucose tolerance; ⁹ frequently sampled intravenous glucose tolerance test; ¹⁰ type 2 diabetes mellitus; ¹¹ polyunsaturated fatty acids; ¹² insulin sensitivity index; ¹³ impaired glucose tolerance
2.4 Dietary fat and serum lipids

2.4.1 Cholesterol

The earliest estimates of the serum cholesterol response to different types of dietary fat come from the 1960s from Keys et al (148) and Hegsted et al (149). Using data from numerous feeding studies where the intake of dietary fat was compared to that of carbohydrates, these investigators reported that saturated fatty acids raised blood total cholesterol concentrations about twice as much as n-6 polyunsaturated fatty acids lowered them. Monounsaturated fatty acids were considered as neutral. More recent reports based on randomized controlled trials have shown that monounsaturated fatty acids are mildly hypocholesterolemic and about half as potent as n-6 polyunsaturated fatty acids in lowering serum total cholesterol (150, 151).

Saturated fat, when substituted for carbohydrates, is known to raise also low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)- cholesterol concentrations in intervention studies. Monounsaturated fatty acids have been reported to increase HDL-cholesterol concentrations when substituted for carbohydrate, the effect on total and LDL-cholesterol being similar to a high-carbohydrate diet. However, there are some studies where the cholesterol-lowering effect has been greater in the high-monounsaturated fat diet (152, 153) compared to the high-carbohydrate diet. Several studies have suggested that monounsaturates have similar effects on plasma lipids and lipoproteins when 4-14 E% was substituted for polyunsaturates (154-158). When n-6 polyunsaturated fatty acids have been compared to carbohydrates, the n-6 PUFAs have been shown to effectively reduce total and LDL-cholesterol. When the n-6 PUFAs have been compared to saturated fatty acids, there have been significant decreases in total, LDL- and HDL-cholesterol concentrations (159). One special subgroup of polyunsaturated fatty acids are long-chain n-3 fatty acids of marine origin. They have been reported to be neutral with respect to total cholesterol, but they raise LDL-cholesterol by 5-10% and HDL-cholesterol by 1-3 %, especially in hypertriglyceridaemic subjects (160).

More specific data on the effect of single fatty acids on total, LDL and HDL cholesterol levels were summarized by Kris-Etherton and Yu in 1997 (159). The most prevalent saturated fatty acid in the Western diet is palmitic acid (C16:0), followed in order of abundance by stearic (C18:0), myristic (C14:0) and lauric (C12:0) acids (161). Of these single saturated fatty acids, myristic acid has been found to be most potent with respect to the cholesterol-raising effect followed by lauric and palmitic acids. There are little data on medium-chain saturated fatty acids, caprylic (C8:0) and capric (C10:0) acids, but estimates vary from their having a neutral to similar cholesterol-raising effect as palmitic acid (159). However, the intake of medium-chain fatty acids is low and therefore their effect on serum cholesterol concentration is likely to be small. Stearic acid is unique among the saturated
fatty acids, it has been estimated that compared to the other saturated fatty acids, it has a cholesterol-lowering effect similar to that of oleic acid (C18:1) and slightly weaker than that of linoleic acid (C18:2). However, stearic acid has also been reported to reduce the concentration of HDL-cholesterol when compared to the other saturated fatty acids (162-164).

Oleic acid is by far the most abundant of the monounsaturated fatty acids, it accounts for more than 95% of the total amount. When substituted for saturated fatty acids, the hypcholesterolemic effect of oleic acid has been estimated as being similar to that of linoleic acid or a little weaker (159).

Trans fatty acids are unsaturated fatty acids with one or more double bonds in the trans configuration. They are formed when vegetable oils or fish oils are hardened by partial hydrogenation. Trans fatty acids are also naturally present in the butterfat and meat of ruminants. In the Finnish diet, the estimated intake of trans fatty acids is low, 0.9 E% (165) e.g. in the U.S. the estimated intake in the diet is several times higher, 2-4 E% (166). The effect of the trans fatty acids, the most common of which is the trans isomer of oleic acid (elaidic acid, C18:1t), on serum lipids is intermediate between the saturated and unsaturated fatty acids. They lower serum total and LDL cholesterol concentrations as compared to saturated fatty acids but raise the concentrations compared to monounsaturated fatty acids. However, they reduce the HDL-cholesterol concentration compared to both saturated and monounsaturated fatty acids (159).

2.4.2 Triglycerides

High-fat diets differ from high-carbohydrate diets in that they generally do not raise serum triglyceride levels (167-170). Only medium-chain fatty acids (C8-10) of the saturated fatty acids increase triglyceride concentrations to about the same extent as carbohydrates, other saturated fatty acids do not elevate triglyceride concentrations (171). Diets high in monounsaturated fatty acids have been reported to lower triglyceride concentrations relative to high-carbohydrate diets (172-176). There are a few experimental studies (156, 177, 178) and a meta-analysis (179) indicating that relative to n-6 polyunsaturated fatty acids, monounsaturated fatty acids may increase the triglyceride concentration. Marine n-3 polyunsaturated fatty acids of the single fatty acids are by far the most effective in lowering triglyceride concentrations at small intakes. A considerable number of studies have produced a similar estimate of the effect: an intake of 3-4 g n-3 fatty acids/day can decrease serum triglyceride concentrations by 25-30% in both normotriglyceridaemic and hypertriglyceridaemic subjects (160).
2.4.3 Apolipoproteins A-1 and B, lipoprotein (a)

In general, the changes in concentrations of apolipoprotein A-1 (apo A-1) which is a part of the HDL-particle and apolipoprotein B (apo B) a component of the LDL and very-low-density lipoprotein (VLDL) particles mirror closely the changes in the respective HDL, LDL and VLDL cholesterol concentrations (180). There is one apolipoprotein molecule per lipoprotein particle and if the size of the lipoprotein particle changes, the concentrations of apolipoprotein and cholesterol in the lipoprotein particle may change differently. As an example, there is some evidence that a low-fat diet as compared to high-saturated fat diet can lower the LDL cholesterol more than the apo B concentration, which indicates that there has been a decrease in the LDL particle size and an increase in particle density, i.e. an unfavorable change in the serum lipid profile (181). In contrast, substitution of monounsaturated fatty acids for saturated fatty acids has been reported to induce an equal lowering of LDL cholesterol and LDL apoB (182). In contrast, there are also recent data indicating that enriching the diet with different types of unsaturated fat (olive, rapeseed or sunflower oil) compared to high saturated fat diet causes a small but significant decrease in LDL particle size. However, the small magnitude of this reduction suggests that the composition of fat is not a major factor affecting LDL size (183). Lipoprotein (a) (Lp(a)), which is a complex of apoB containing lipoprotein, usually LDL, and apolipoprotein(a) seems to be very little influenced by the amount or type of dietary fat (184) with the exception of trans fatty acids. In several intervention studies, trans fatty acids have elevated Lp(a) (185-187), though this effect has not been seen in all studies (188, 189).

2.5 Dietary fat and haemostatic factors

2.5.1 Fibrinogen

Fibrinogen is a procoagulant factor in the haemostatic system acting as substrate for fibrin formation. Epidemiological studies indicate that elevated plasma levels of fibrinogen are predictive for both nonfatal and fatal coronary events (190, 191). The fibrinogen concentration is uninfluenced by dietary fat content or composition except for inconsistent reports of a reduction after dietary enrichment with n-3 fatty acids (192).

2.5.2 Factor VII (FVII)

FVII is a key protein in the initial phases of coagulation process (193). In prospective cohort studies, elevated plasma levels of FVII have been associated with an increased risk of arterial thrombosis (190, 191). In intervention studies low-fat, high-fiber diets have
lowered fasting FVII coagulant activity (FVIIc) in several subject groups: subjects with type 2 diabetes (194) or hypertriglyceridaemia (195) and in healthy subjects (196). However, later reports have indicated that the importance of total fat intake may be limited and that the type of carbohydrates (simple vs. complex) may be at least of equal importance (197, 198). The observed reduction in FVIIc in response to a low-fat diet is due to a reduction in both the FVII total protein concentration and in the proportion of active FVII (199). It has been estimated that the recommended low-fat high-fiber diet could reduce fasting FVIIc by 5-15% compared to the average diet in most Western countries (198, 200, 201). The variation in the estimated reduction might be partly dependent on the genetic factors: Niskanen et al (202) reported a reduction in FVIIc level after a low-fat diet as compared to a monounsaturated fat diet only in subjects without the MspI polymorphism in their FVII gene.

The fatty acid composition of diet seems to have little if any effect on fasting FVIIc levels. There was no difference in fasting FVII measurements between diets rich in monounsaturated or polyunsaturated fat (199, 203). In agreement, Rankinen et al (194) reported no effect on FVIIc by dietary counselling intended to decrease the saturated and increasing polyunsaturated fat intake (204). Furthermore, a study from New-Zealand comparing high-SFA, high-MUFA and high-PUFA diets resulted in similar FVIIc levels, despite expected the occurrence of the expected changes in the serum lipid concentrations (205). Also marine n-3 fatty acids seem to have no effect on fasting FVIIc levels (206). In the study of Mitropoulos et al. (207) two high-fat (62 E% as fat) diets, one high in saturated fat and the other high in unsaturated fat were compared to a low-fat diet (<20 E% as fat). Factor FVIIc was 6.5% higher with the unsaturated and 13.1% higher with the saturated fat diets compared to the low-fat diet. In that study, the plasma free stearic acid concentration had a strong positive association with FVIIc. The total FVII protein concentration also increased with the high saturated fat diet as compared to the two other diets. In contrast to the previous study (207), Tholstrup et al. (162) reported that a high stearic acid diet reduced FVIIc by 15-20% compared with fats high in palmitic acid or a combination of lauric and myristic acid. In line with the study of Tholstrup et al., a high stearic acid diet reduced FVIIc in healthy men compared to a high palmitic acid diet (208). There are also studies where no specific effect of stearic acid has been observed: in a strictly controlled Finnish trial, there were no differences in FVIIc between a high stearic acid diet and a high saturated fat diet (mainly dairy fat) (209). It is possible that the non-lipid constituents of shea fat accounted for the observed FVIIc decreasing effect in the study of Tholstrup et al. (162) as discussed by the author herself. However, in the study of Kelly et al. (208) an interesterified baking margarine was used as the source of stearic acid and thus an independent effect of stearic acid cannot be ruled out. The study of Mitropoulos et al. (207) had a very small number of subjects (n=5) and therefore the results from that study should be interpreted with caution. In another study by Tholstrup and coworkers, small fasting FVIIc differences between fats high in myristic or palmitic acids
were found (163) but, as previously stated, these are single observations against a large body of literature indicating no major effects of single fatty acids on fasting FVII levels.

2.5.3 Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is a major fibrinolysis inhibiting enzyme in the haemostatic system and an elevated plasma PAI-1 concentration has been found to predict the occurrence of a first acute myocardial infarction (210) and it is also an independent risk factor for reinfarction (211). There is little known about the influence of dietary fat on PAI-1. A change in fat intake from 39 E% to 31 E% with a similar fatty acid and fiber content in the diets did not affect PAI-1 antigen (PAI-lag) or PAI activity levels in a group of healthy subjects (197). Similarly, 8 month consumption of a low-fat high-fiber diet did not affect the PAI-lag level in another study from the same research group (200). However, the type of carbohydrate in the diet may play a role. There is recent data from Sweden where a low-glycaemic index diet reduced PAI-1 activity by 50% compared to a high-glycaemic index diet (212).

In two different studies in healthy young males, a high-monounsaturated fat diet (38 E% fat, 22-24 E% monounsaturated fat) resulted in lower PAI-1 plasma activity than a low-fat, high-fiber diet (213, 214) or high-saturated fat diet (38 E% fat, 20 E% saturated fat) (214). However, in a group of subjects with impaired glucose tolerance, no change in PAI-1 activity was observed either during a low-fat, high-fiber diet or a high-monounsaturated fat diet (202).

With respect to single fatty acids, an oleic acid enriched diet was reported to result in lower PAI-1 activity than a palmitic acid enriched diet (215) in a group of healthy males and females. No difference in PAI-1 was reported in a similar group of subjects between oleic acid and linoleic acid enriched diets (216). Almedingen et al (217) reported higher PAI-lag and PAI activity levels after consumption of a partially hydrogenated soybean oil diet than after consumption of a partially hydrogenated fish oil diet or butter diet. However, the effect of trans fatty acids was not confirmed by Mutanen and Aro (209) who reported no difference between a dairy fat diet and a trans fatty acid enriched diet in PAI-1 activity. The associations of serum cholesterol ester fatty acids with PAI-1 activity were evaluated in elderly Swedish men (218). After adjustment for features of the insulin resistance syndrome, γ-linolenic acid and arachidonic acid were positively associated with PAI-1 activity. This indicates that the dietary unsaturated fat intake may be associated with PAI-1 activity (218).
3. AIMS OF THE STUDY

The present study is one facet of a series of studies evaluating the effects of single fatty acids and fat-modified diets on glucose, insulin and lipoprotein metabolism carried out in the Department of Clinical Nutrition, University of Kuopio. The main aim of this study was to examine the effects of stearic acid, a single saturated fatty acid, and trans fatty acids on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women and the effects of two fat-modified diets on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in hyperlipidaemic middle-aged subjects. In addition, the relation between the changes in serum lipid fatty acid profile during two fat-modified diets to changes in glucose and insulin metabolism was evaluated in subjects with impaired glucose tolerance.

The specific study questions were as follows:

1. Does the effect of stearic acid on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women differ from the effect of oleic acid?

2. Does the effect of trans fatty acids on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women differ from the effect of oleic acid?

3. Is there a difference between the effects of a reduced-fat diet and a medium-fat, monoene-enriched diet on serum lipids and lipoproteins, glucose and insulin metabolism and coagulation factors in a group of hypercholesterolaemic and hypertriglyceridaemic subjects?

4. How are the changes in glucose and insulin metabolism during two fat-modified diets, monounsaturated-fat enriched diet and reduced-fat polyunsaturated-fat enriched diet related to changes in the serum lipid fatty acid profile in subjects with IGT?
4. SUBJECTS AND METHODS

4.1 Subjects

All studies were carried out in the Department of Clinical Nutrition, University of Kuopio. In *Study I* and *II* the subjects were healthy females and were recruited from the students and the staff of University of Kuopio by advertisements and internal mail. All subjects had normal liver, kidney and thyroid function before the study as evaluated by serum gammaglutamyltransferase, creatinine and thyroid stimulating hormone levels. Five of the subjects in *Study I* and 10 of the subjects in *Study II* were using low-estrogen oral contraceptives or a hormone-releasing intrauterine device. No medication affecting lipid or glucose metabolism was in use. In *Study III* the subjects were hypercholesterolaemic and hypertriglyceridaemic (inclusion criteria: serum total cholesterol concentration > 5 mmol/l and serum total triglyceride concentration > 1.5 mmol/l). They were recruited from the Lipid Clinic of the Kuopio University Hospital and from previous diet studies performed in the Department of Clinical Nutrition of the University of Kuopio. Four of the women were postmenopausal and one was premenopausal. Six of the subjects were patients of the Lipid Clinic of the Kuopio University Hospital and used regular hypolipidemic medication (3 subjects used lovastatin, 1 simvastatin and 2 bezafibrate). In *Study IV*, subjects were recruited from the 1992 Finnish FinMonica Survey (National Public Health Institute, Helsinki, Finland) from the Kuopio area. The primary criterion for inclusion was IGT in two consecutive oral glucose tolerance tests according to the World Health Organization criteria in 1985 (219). On entry, six of the subjects were using β-blockers and three were taking diuretics for hypertension. Their medication was kept unchanged during the study and there were no differences in the use of medication between the groups. In all four studies, subjects with diabetes (219) were excluded. All subjects had regular exercise habits and they were asked to maintain these habits unchanged during the study. The smokers (*n*=2, *Study I; n*=4, *Study III*) were asked to retain their regular smoking habits throughout the study. Subjects provided informed consent to participate in the studies. The study plans were approved by the Ethics Committee of the Kuopio University Hospital and University of Kuopio. The baseline characteristics of subjects are presented in Table 4.
Table 4. Subject characteristics, mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>subjects randomized N(M/F) (^1)</td>
<td>16 F</td>
<td>16 F</td>
<td>28 (19M/9F)</td>
<td>31 (18M/13F)</td>
</tr>
<tr>
<td>subjects that completed the study N (M/F)</td>
<td>15 F</td>
<td>14 F</td>
<td>18 (13M/5F)</td>
<td>31 (18M/13F)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>23 (4)</td>
<td>23 (3)</td>
<td>53 (10)</td>
<td>56 (5)</td>
</tr>
<tr>
<td>BMI(^2) (kg/m(^2))</td>
<td>22.1 (2.2)</td>
<td>20.8 (2.1)</td>
<td>29.6 (3.5)</td>
<td>30.0 (2.6)</td>
</tr>
<tr>
<td>Glucose tolerance status</td>
<td>normal(^3)</td>
<td>normal(^3)</td>
<td>normal (n=17) to IFG(^2) (n=1)(^3)</td>
<td>IGT(^5)</td>
</tr>
</tbody>
</table>

\(^1\) M=males, F=females; \(^2\) body mass index; \(^3\) based on fasting plasma glucose concentration; \(^4\) impaired fasting glucose; \(^5\) impaired glucose tolerance

4.2 Study designs

Studies I, II and III were carried out as randomized cross-over studies (Figure 1).

![Cross-over study design used in Studies I-III](image)

- blood samples
- blood samples, frequently sampled intravenous glucose tolerance test
- FR food record

Figure 1. Cross-over study design used in Studies I-III
In *Study I*, the effects of stearic acid and in *Study II*, the effects of trans fatty acids were compared to those of oleic acid. In *Study III*, the effects of monoene enriched (MUFA) diet and reduced-fat diet were compared. The two experimental diet periods in *Studies I-III* were preceded by consumption of a baseline diet for 2 weeks. After this run-in period, all of the subjects consumed the experimental diets for 4 weeks. Body weight and blood pressure were measured at 2-week intervals. Laboratory samples (serum lipid, lipoprotein, and apolipoprotein concentrations and coagulation factors) were collected after a 12-hour fast at the beginning and the end of the experimental diet periods (four times altogether). In *Study III*, the blood samples for measurements of serum lipid and lipoprotein concentrations were collected on two consecutive days and the mean of the two consecutive values was used in the results. A frequently sampled intravenous glucose tolerance test (FSIGT) was performed at the end of the experimental diet periods (two times altogether). In *Study I*, FSIGT was performed also at the end of the run-in period (three times altogether).

*Study IV* had a parallel design (Figure 2) where the effects of a monounsaturated-fat enriched (Mono) diet were compared to a polyunsaturated-fat enriched (Poly) diet. After consuming the Run-in diet for 3 weeks, the subjects were randomized into one of two diet groups for 8 weeks. Weight, blood pressure and concentrations of serum lipids and lipoproteins were measured at baseline and 2, 4, and 8 weeks after randomization. The FSIGT was performed and fatty acid composition of serum lipids was determined at baseline and 8 weeks.

![Diagram](image)

- **blood samples, frequently sampled intravenous glucose tolerance test**
- **blood samples**
- **FR** food record

Figure 2. Parallel study design used in *Study IV.*
4.3 Methods

4.3.1 Diets

The baseline diets in all four studies were planned to resemble the average Finnish diet and were medium-fat high-saturated fat diets. The goal for the fat intake of the baseline diets was 36 E%, the saturated fat content varied from 16 (Study II) to 18 E% (Studies I, III, IV). The carbohydrate (48-50 E%) and protein (15 E%) contents were identical in the baseline diet and experimental diets in Studies I and II. In Studies III and IV, the goals for carbohydrate (48-50 E%) and protein content (14-16 E%) were identical in the baseline diets and the monoene-enriched diets. In the reduced-fat diets (Studies III and IV), the goal for the fat content was lower (30 E%) and the content of carbohydrates 50-55 E% therefore higher than in the baseline and monoene-enriched diets. The goal for protein intake in Studies III and IV was similar in all three diets (14-16 E%). The goals for the fatty acid composition for the experimental diets are presented in Table 5. In Studies I-III, the energy requirement of the subjects was estimated by a 3-day food record kept before the study, in Study IV a 4-day food record was used. The subjects received detailed written instructions about the diets, specifying the number of servings of individual foodstuffs classified according to the main food groups (dairy products, cereals, vegetables and roots, fruit and berries, meat and meat products and sugar and sweets) or number of grams (fats). The fatty acid modification of the diets was carried out using different kinds of spreads and oils. The main fat sources in experimental diets are presented in Table 5. The subjects received the fat products and vegetable oils free of charge, in Studies I and II also the dairy products were provided free of charge. To control compliance to the diets in Studies I-III, the subjects kept 7-day food records during 5 weekdays and 2 weekend days. During the baseline and washout periods 4-day food records (3 weekdays and 1 weekend day) were kept. In Study IV, a 4-day food record was kept before each visit (altogether 12 days from the 8-week diet period). To obtain another estimate of the composition of the diets the subjects collected double portions of everything they ate (except coffee, tea and tap water) on 1 day during both experimental periods for analysis of the fatty acid composition of the diets in Studies II and III. The fatty acid composition of serum lipids (triglycerides in Studies I-IV and cholesteryl esters in Studies I-III) was used as an objective marker of compliance in all studies.

The diets were planned and the nutrients in food records were calculated using the Micro-Nutrica® dietary analysis program based on the database of the Finnish Social Insurance Institute. The food composition tables are based on Finnish food analyses and values taken from international food composition tables (220). Information about new food products (with the emphasis on products important for fat intake) was collected from manufacturers and the database was regularly updated by the study personnel. In addition
to food records and double portions, determination of the fatty acid composition of serum triglycerides and cholesterol esters was used as an indicator of compliance to the experimental diets during the study.
Table 5. The goals for the fatty acid composition and the main fat sources for the experimental diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main fat source</td>
<td>Stearic</td>
<td>Oleic</td>
<td>Trans</td>
<td>Oleic</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>36</td>
<td>36</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Olive oil</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>TFA enriched margarine</td>
<td>11</td>
<td>11</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Sunflower rapeseed oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SF oil + SF based spread</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

1 trans fatty acid enriched margarine; 2 high-oleic-acid sunflower oil; 3 low-erucic acid rapeseed oil; 4 sunflower oil; 5 % of energy intake; 6 saturated fatty acids; 7 monounsaturated fatty acids; 8 polyunsaturated fatty acids
4.3.2 Laboratory methods

Frequently sampled intravenous glucose tolerance test (FSIGT)

FSIGT was performed as previously described (221). First, two intravenous catheters were inserted in the antecubital veins of both arms and the fasting samples were drawn. A glucose dose of 300 mg/kg of body weight was given intravenously as a 50 % solution in 1.5 min followed by 10 ml of 0.9 % NaCl solution. Thereafter, a 0.9 % NaCl solution was slowly infused until a bolus of 0.03 U/kg of insulin was rapidly injected 20 minutes after the glucose dose. The NaCl infusion was continued at full speed for 1.5 min after the insulin dose. To determine plasma glucose and insulin levels, venous blood samples were collected before the glucose dose (-5 and 0 min) and 23 times after the glucose dose (at 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 24, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160 and 180 min) via the catheter in the contralateral arm. To arterialize venous blood, the arm was kept in a 50 °C electric pad during the test. The plasma glucose concentration was analyzed by the glucose oxidase method (Glucose Auto & Stat, Model GA-110, Daiichi, Kyoto, Japan) and plasma insulin by a radioimmunoassay method (Phadeseph Insulin RIA 100, Pharmacia Diagnostica, Uppsala, Sweden). The data were analyzed by calculating with the Minmod program (222) glucose effectiveness (S_e) which represents the effect of glucose itself to enhance glucose uptake and suppress endogenous glucose production at basal insulin and insulin sensitivity index (S_i) which represents the effect of insulin secreted above basal to enhance glucose effectiveness. In addition, the acute insulin response (AIR) was determined by calculating the area under the insulin curve above the baseline level from 0 to 10 minutes.

Serum lipids and lipoproteins

To remove the very low density lipoprotein (VLDL) fraction, lipoproteins were separated by ultracentrifugation for 18 h at a density of 1.006 kg/l. VLDL cholesterol was calculated as the difference between the mass of cholesterol in the serum and in the infranatant. High density lipoprotein (HDL) in the infranatant was separated from low density lipoprotein (LDL) by precipitation of LDL with dextran sulfate and magnesium chloride (223). LDL cholesterol was calculated as the difference between the mass of cholesterol in the infranatant and HDL. Enzymatic colorimetric methods were used for the determination of cholesterol and triglycerides from the whole serum and lipoprotein lipids with commercial kits (Monotest® Cholesterol and Triglyceride GPO-PAP, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) with an automated instrument (Kone Specific Clinical Analyzer, Kone Ltd, Espoo, Finland). The coefficient of variation (CV%) between measurements for
serum total cholesterol was 1.6-1.7% (2 standards) and for total triglycerides 2.2-2.3 % (2 standards). The CV% for HDL-cholesterol was 1.1-1.6 % (2 standards) and for HDL-triglycerides 4.4 % (1 standard).

Serum samples for apolipoprotein A-I and B were stored at -78 °C until analyzed at the end of the study. Analyses were based on the measurement of immunoprecipitation enhanced by polyethylene glycol at 340 nm. An automated Kone Specific Clinical Analyzer and apolipoprotein A-1 and apolipoprotein B reagents from Kone Instruments (Espoo, Finland) were used in the analyses. The CV% for apolipoprotein A-1 within the measurement was 2.0-3.7 % (2 standards) and for the apolipoprotein B 2.1-2.6 % (2 standards).

Lipoprotein (a)

Apolipoprotein(a) [Apo(a)] in lipoprotein(a) [Lp(a)] was measured using the solid-phase two-site immunoradiometric assay from Merckia AB (Uppsala, Sweden). This assay is based on a direct sandwich principle in which two monoclonal antibodies are directed against separate antigenic determinants on the Apo(a) molecule. The assay is calibrated against a highly purified Lp(a) preparation. To transform the concentration of Apo(a) to Lp(a), apo(a) concentrations were multiplied by 0.7 and the results are expressed in units per liter (U/L). The analytical parameters were validated to meet the criteria as defined by the manufacturer. The method sensitivity is 8.4 U/L (routine standardization at 16.8 - 840 U/L), and the levels above 840 U/L were reanalyzed after further dilution (measuring range up to 3360 U/L). Reasonably low assay imprecision was found: between assay CV% were 2.5, 7.1, 3.5 and 6.0% at the Lp(a) levels of 93.5, 123, 311 and 372 U/L, respectively. The assay is specific for Apo(a), and apolipoprotein B exhibits no measurable crossreaction. Serum plasminogen up to 5 g/L gives no measurable crossreaction in the assay.

Free fatty acids

Concentration of serum free fatty acids were determined with a turbidimetric method and analyzed with the Specific-analyzer (Kone Ltd, Espoo, Finland).

Fatty acid composition of serum lipid fractions

The serum samples were stored at -78 °C prior to the analysis of the fatty acid composition of serum triglycerides (TG), cholesteryl esters and phospholipids (PL). Lipids were extracted from 100 μl serum with chloroform-methanol (2:1), and the lipid fractions were separated by solid phase extraction with an aminopropyl column
(224). The fatty acids of triglycerides and cholesteryl esters were transmethylated with 14% boron trifluoride in methanol. Fatty acid methyl esters were analyzed with a gas chromatograph (HP 5890 Series II, Hewlett-Packard Company, Waldbronn, Germany) equipped with a HP-FFAP capillary column (25-m long, diameter 0.2 mm, phase layer 0.3 μm) with helium as the carrier gas.

**Fatty acid composition of double portions**

Double portions of foods were homogenized and two samples of 100 g from each portion were frozen after addition of butyl-hydroxy-toluene extracted with ethanol (1 g/1000 g food). Samples were stored (-18 °C) until all portions were collected. Then the samples were thawed (+10 °C) and all samples from the same diet period were pooled. The pooled mass was carefully homogenized and six samples of the pooled mass were taken, frozen and stored (-70 °C) until freeze-dried. From the freeze-dried sample, 50 mg was taken for the determination of fatty acid composition of double portions. The lipids were extracted with dichloromethane-methanol (1:1). The extract was evaporated in a nitrogen stream and the lipids remaining in the tube were dissolved in toluene. The fatty acids were transmethylated and analyzed in the same way as the fatty acid composition from the serum sample.

**Haemostatic factors**

Blood samples for measurements of haemostatic factors were taken without compression. If the first puncture was not successful, the samples were taken from the contralateral arm. All exceptions from the normal procedure such as difficulties in blood collection, in preparation of the sample, nausea or fainting and use of analgetics by the subject were recorded.

Fibrinogen was measured with an ACL 300 R coagulometer (Instrumentation Laboratory, Milan, Italy) from the light scattered by the clot during the prothrombin time assay (PT-fibrinogen, Instrumentation Laboratory, Milan, Italy). A single lot of IL Calibration plasma was used as a standard throughout the study. The intra-assay precision of the method is 3.6 % and inter-assay precision is 2.3%. The samples were measured in duplicate; the difference of the duplicates had to be within 10% of the mean or the analysis was repeated using a split sample (225). Factor VII coagulant activity (FVIIc) was measured with the one-stage method using rabbit brain thromboplastin (Thromboplastin IS, Baxter Dade, Miami, Florida, USA) and human immunodepleted FVII deficient plasma (Behring, Marburg, Germany). The assays were carried out with an ACL 300 R coagulometer. A frozen plasma pool was used as the standard. The intra-assay precision of this method is 2.4% and the interassay precision 3.9% (225). PAI-1 activity was measured with a chromogenic
method (Coatest PAI; Chromogenix, Mölndal, Sweden). The detection limit is 5 AU/ml and interassay precision is 5.5%.

4.3.3. Statistical analysis

The data were analyzed by using SPSS 4.0 (Study I) (226) and 6.0.1 (Studies II-IV) statistical programs (227). Before further analysis, the normal distribution of the variables was checked with the Shapire-Wilks test. To control the overall α-level, the Bonferroni adjustment was used. All data were expressed as mean ± SEM (Studies I-III) or mean ± SD (Study IV). The 2-tailed p-value < 0.05 was considered to be statistically significant.

In Studies I-III, the results from the run-in and wash-out periods were pooled and are presented as baseline values in the results section. The values after both experimental diets were used as the main outcome measures. An ANOVA for repeated measurements was carried out to assess whether there was any carry over effect. In Study III, an order effect was found in insulin sensitivity and these results are presented in the whole group and according to the order of the experimental diets. Paired t-tests were used for two-tailed comparisons except for analysis of the FSIGT and for all variables not normally distributed after mathematical transformations. For those variables, the Wilcoxon matched-pairs signed-ranks tests were used. In Study III, Spearman correlation coefficients were calculated between selected variables. The Mann-Whitney test was used to compare the responses of the subjects with or without hypolipidemic medication. Since no differences in the responses according to the medication status were found, the data are presented for the whole group.

In Study IV, differences in the means and in the absolute and percentage changes (0 vs 8 weeks) between the two diet groups were analyzed with the Mann-Whitney test. Since the majority of variables were not normally distributed and did not become so after mathematical transformations, non-parametric tests were selected for analyses. The Wilcoxon matched-pairs signed-ranks test was used for analysis of the within group changes in glucose metabolism and fatty acid profile of serum cholesterol esters, triacylglycerols and phospholipids. Spearman correlation coefficients were calculated between selected variables.
5. RESULTS

5.1 Body weight, blood pressure and diets

**Study I (Stearic vs Oleic diet)**

Body weight (BMI after the baseline diet 22.1±0.6 kg/m², after the Stearic diet 22.0±0.6 kg/m², after the Oleic diet 22.0±0.6 kg/m²) and blood pressure remained stable during the study. The difference in the stearic acid content between the experimental diets was 4.5 E%, for the monounsaturated fatty acids the difference was 6.5 E%. The amount of other energy yielding nutrients as well as dietary cholesterol and fiber did not differ between the diets. Furthermore, the significant changes in the fatty acid composition of serum triglycerides and cholesteryl esters after consumption of the study diets indicated good compliance (Article I, p. 531, tables 2 and 3).

**Study II (Trans vs Oleic diet)**

Weight remained stable during the study (BMI after the baseline diet 20.8±0.5 kg/m², after trans diet 20.6±0.5 kg/m², after oleic diet 20.4±0.5 kg/m²). There was no change in blood pressure values during the study. Between the Trans diet and the Oleic diet, the difference in the trans fatty acid content was 5.1 E% and for the monounsaturated acids, the difference was 6.0 E%. The analyzed fatty acid composition of double portions was comparable with what would be expected from the food record data. Total fat, cholesterol and fiber intakes did not differ among the different diet periods during the study. The increase in the intake of trans fatty acids and oleic acid during the respective diet periods was reflected in the fatty acid composition of triglycerides confirming that the dietary compliance was good (Article II, p. 873, table 3).

**Study III (Mufa vs Reduced-fat diet)**

The average body weight was similar after the diet periods (after the baseline diet 84.5 ± 2.8 kg, after the Reduced-fat diet 84.1 ± 2.8 kg and after the Mufa diet 84.2 ± 2.9 kg, mean ± SEM), although during the Mufa diet, the subjects' average energy intake was slightly higher than during the other diets. Compared to the diet plan, the fat content (30 E% vs 33.8 E%, planned vs calculated from food records) and the content of monounsaturated fat (12 E% vs 16.0 E%) of the Reduced-fat diet were somewhat higher than planned. The difference between the Reduced-fat diet and the Mufa diet was 5.2 E% for total fat and 5.3 E% for monounsaturated fat. The
analyzed fatty acid composition of the diets was comparable with the data from diet records. The significant changes in fatty acid composition of serum cholesterol esters further confirmed that the compliance was good (Article III, p. 181, table 3).

**Study IV (Mono vs Poly diet)**

BMI decreased marginally by 1% during the study in both groups. The compliance with the fatty acid modification of both diets was good. The Mono diet contained 40 E% fat of which 11 E% was saturated, 19 E% monounsaturated and 8 E% polyunsaturated fatty acids, the Poly diet contained 34 E% fat of which of which 11 E% was saturated, 10 E% monounsaturated and 10 E% polyunsaturated fatty acids. Results on the fatty acid composition of serum lipids (triglycerides, cholesteryl esters) were in accordance with nutrient intake analysis from food records and indicated that the goals of the fatty acid composition of the experimental diets were well achieved (228).

**5.2 Glucose and insulin metabolism**

**Studies I-III**

In *Studies I* (Stearic vs Oleic diet) and *II* (Trans vs Oleic diet) no differences were found in fasting plasma glucose or insulin concentrations or in $S_i$, $S_o$ or AIR at the end of the experimental diet periods (Figure 3).

![Graph showing insulin sensitivity indices](image)

*Figure 3. Insulin sensitivity indices (mean±SEM) after consumption of the study diets.*
In Study III, $S_i$ was 40% greater after the Reduced-fat diet than after the Mufa diet ($2.42 \pm 0.42$ vs. $1.73 \pm 0.24 \cdot 10^4 \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}^{-1}$, $p = 0.02$, Figure 3). Due to the MANOVA analysis indicating an order effect in insulin sensitivity the results in Study III were also analyzed according to the order of the test diets. In the group starting with the Mufa diet the insulin sensitivity index after the Mufa diet vs the Reduced-fat diet was $1.84 \pm 0.42$ vs. $3.04 \pm 0.74 \cdot 10^4 \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}^{-1}$, $p < 0.02$ and in the group starting with the Reduced-fat diet $1.63 \pm 0.26$ vs. $1.79 \pm 0.33 \cdot 10^4 \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}^{-1}$, $p < 0.44$, respectively. In both groups, the average insulin sensitivity index was higher after the Reduced-fat diet but in the group beginning with the Reduced-fat diet the difference was not statistically significant. Glucose effectiveness ($S_e$) did not differ between the diets ($0.0161 \pm 0.001$ vs. $0.0158 \pm 0.001 \text{min}^{-1}$, NS). There were no statistically significant differences in fasting plasma insulin or glucose concentrations nor did the acute insulin response differ after the experimental diet periods. The change in insulin sensitivity had a strong inverse correlation with the change in the serum triglyceride concentration ($r = -0.75$, $p < 0.0001$, Figure 4).

![Figure 4. Association of the change in serum triglyceride concentration with the change in insulin sensitivity ($S_i$) between the Reduced-fat diet and the Mufa diet in Study III ($r = -0.75$, $p < 0.01$)](image)

**Study IV**

In Study IV, the fasting glucose concentration was lower after the Mono diet than at baseline whereas after the Poly diet it remained unchanged. $S_i$ and AIR did not
change significantly with either of the diets. The $S_G$ was higher in the Mono group than in the Poly group after the consumption of the diets. At baseline, a higher $S_G$ was associated with higher proportions of oleic ($r=0.57$, $p=0.04$) and $\alpha$-linolenic acids ($r=0.64$, $p=0.01$) in PL. A higher $S_I$ at baseline was associated with a lower proportion of saturated fatty acids in TG ($r=-0.55$, $p=0.04$). Associations between the changes in the proportions of fatty acids in serum lipid fractions and indices of glucose and insulin metabolism during the study were analyzed with both groups combined. The increase in $S_G$ correlated with the increase in the proportion of oleic acid ($r=0.55$, $p=0.004$) and with the decrease in the proportion of arachidonic acid ($r=-0.40$, $p=0.04$) in PL (Figure 5).

Figure 5. Association of changes in the proportions of oleic acid (panel A, $r=0.55$, $p=0.004$) and arachidonic acid (panel B, $r=-0.40$, $p=0.04$) in phospholipids (PL) with glucose effectiveness, diet groups combined.
The increase in the proportions of oleic acid and α-linolenic acid in PL correlated with the decrease in the fasting plasma glucose concentration ($r = -0.53$, $p=0.002$ and $r = -0.47$, $p=0.009$, respectively, Figure 6).

Figure 6. Association of changes in the proportions of oleic acid (panel A, $r = -0.53$, $p=0.002$) and α-linolenic acid (panel B, $r = -0.47$, $p=0.009$) in phospholipids (PL) with fasting plasma glucose, diet groups combined.

5.3 Serum lipids and lipoproteins

Study I

There were no significant differences in serum lipid, lipoprotein, apo A-I and B or Lp(a) concentrations between the Stearic and Oleic diets. Compared with the high-saturated-fat baseline diet, the consumption of both experimental diets resulted in
lower total (5.9-7.7%) and LDL-cholesterol (6.4-9.9%) concentrations. HDL-cholesterol was significantly lower after the Stearic diet (5.3%) and tended to be lower after the Oleic diet (4.7%) when compared to baseline diet. There were no differences in VLDL cholesterol or total, LDL, HDL or VLDL triglyceride concentrations compared to the baseline diet after consumption of either the Stearic diet or the Oleic diet.

**Study II**

The 5 E% enrichment of trans fatty acids in the Trans diet did not affect serum total, HDL or LDL cholesterol concentrations differently from that of 5 E% oleic acid enrichment in the Oleic diet after the consumption of otherwise identical diets. However, the ratio of total cholesterol to HDL cholesterol was 7% higher after the consumption of the Trans diet than the Oleic diet. Furthermore, serum total, HDL and LDL triglyceride and apo-B concentrations were 8-27% higher after the Trans diet compared to the Oleic diet (p<0.05). There were no differences in ApoA-I or Lp(a) concentrations between the experimental diets. When Trans and Oleic diets were compared to the baseline diet, the serum total and HDL cholesterol were 8-12% lower after the consumption of the experimental diets. A significant decrease of 9-38% in LDL and VLDL cholesterol and total, HDL and LDL triglycerides and apoB concentrations was observed only after the Oleic diet. ApoA-I was 8-10% lower after both experimental diet periods compared with the baseline concentration.

**Study III**

There were no significant differences in concentrations of serum lipids, lipoproteins, apo A-I and B or Lp(a) between the MuFA and Reduced-fat diets. Compared with the baseline diet, both diets lowered serum total and LDL cholesterol concentrations by 7-8%. Only the Reduced-fat diet decreased the apoA-I (4%) and B concentrations (6%) compared with the baseline diet.

5.4 Haemostatic factors

The dietary fat modifications used in Studies I-III did not affect the level of fibrinogen or FVIIc or PAI-1 activities.
6. DISCUSSION

6.1 Subjects and study designs

In Study I (Stearic vs Oleic diet) and Study II (Trans vs Oleic diet) the subjects were young healthy women, 15 subjects in both studies. They were volunteers from the students and staff of University of Kuopio. In Study III (Mufa diet vs Reduced-fat diet) the subjects were 18 middle-aged hypercholesterolaemic and hypertriglyceridaemic subjects recruited from the Lipid Clinic of Kuopio University Hospital and from previous diet studies performed in the Department of Clinical Nutrition. Overt diabetes was an exclusion criteria in all three studies. However, as would be expected, the middle-aged, hyperlipidaemic subjects in Study III were insulin resistant (S, 1.7-2.4 × 10^9 min^-1·μU^-1·ml^-1) compared to the two groups of healthy women in Studies I and II (S, 4.8-5.4 × 10^9 min^-1·μU^-1·ml^-1) whose insulin sensitivity index was in the range previously seen in other groups of healthy subjects (110, 141). In Study IV, the subjects were 31 middle-aged persons who had IGT carefully documented by two consecutive oral glucose tolerance tests using the WHO 1985 criteria (219, 228).

In Studies I-III, a cross-over design was used. In that kind of trial the results of one diet period are compared to the results from the same person from another diet period. This reduces significantly the variation in measurements and fewer subjects are needed than in the parallel design. On the other hand, the study is longer since all of the subjects have to go through all diet periods. In Studies I-III, the duration of the study including the run-in and wash-out and 2 experimental diet periods in each study was 3 months. However, there were very few drop-outs in Studies I and II and they were unrelated to the duration of the study (1 subject in Study I quit the study because she felt participating in the FSIGT uncomfortable and 1 subject was excluded because of pregnancy in Study II). In Study III, the number of drop-outs was higher, 10 subjects quit during the early phase of the study mainly because of the high frequency of study visits. In Study IV, all randomized subjects completed the study. It similarly had a duration of 3 months but it was carried out using a parallel design with two diet groups (Mono vs Poly diet). The use of a cross-over design would have been impractical because the total duration of the study with that design would have been extended to 6 months which might have seriously risked the compliance of the study subjects.

The number of subjects in Studies I-III enabled us to detect a 10% difference in serum lipids between the dietary periods. For evaluating the indices of glucose metabolism where the variation is larger, the difference should have been much more marked. In Study IV, a post-hoc analysis was made on the changes in serum lipid fatty acid profile and changes in glucose and insulin metabolism during the
study. For that analysis, the two diet groups in the study were combined and the total number of subjects in this analysis was 31. There might arise problems in post-hoc analysis because the study was not primarily designed to answer the study question addressed later and the interpretation of the results should be conservative. In the present study for each significant association the distribution of single observations was carefully checked and the role of possible outliers evaluated.

6.2 Diets and compliance of the subjects

In Studies I-IV, all subjects received detailed oral and written instructions about the diet. The body weight was kept constant during the studies and the energy level of the diet was adjusted according to the body weight measurements of each individual subject. In Studies I-III, there were no significant differences in body weight between the diet periods. In Study IV there was a marginal decrease of 1% in body weight in both groups and there was no difference between the groups. The study subjects received the study fats in Studies I-IV and in Studies I and II also some dairy products (cheese and yoghurt) free of charge. This together with frequent counselling promoted good compliance. The compliance was evaluated during the experimental diet periods with 7-day food records (Studies I-III) and with a 4-day food record repeated three times (Study IV). The number of days in the food records, seven days in Studies I-III and altogether 12 days in Study IV during experimental diet periods are considered sufficient to obtain a reliable estimate of energy yielding nutrient intakes at a group level (229). Underreporting is a common phenomenon associated with food records possibly confounding the interpretation of diet data. Age, gender and weight are known to affect the degree of underreporting (230). However, in Studies I-III, where a cross-sectional design was used, the results were compared within the same subject and it is highly unlikely that there would have been any differences in the degree of underreporting of an individual subject between the diet periods. In Study IV, where a parallel design was used, the groups were similar with respect to age, the number of males and females and BMI and it is unlikely that the degree of underreporting would have been significantly different between the groups. Furthermore, the complianc was indirectly evaluated by measuring the fatty acid composition of serum lipids in all four studies and in Studies II and III also double portions from one day during experimental diet period were collected and their fatty acid composition analyzed.

In Studies I and II, the dietary goals were well met as indicated by food record data, the changes in serum lipid fatty acid composition and the analysis of double portions in Study II. In Studies III and IV, the fat content in both the reduced-fat and monounsaturated-fat enriched diets exceeded the goals. However, the food record data as well as the fatty acid composition of serum lipids and the analysis of the
double portion data (Study III) confirmed that the excess fat consumption was due to
the consumption of study fats and the difference in fat content between the diets was
similar to that intended: 5 E% in Study III and 6 E% in Study IV. However, with a fat
content of 34 E% one can no longer consider this to be a low-fat diet and this has
been taken into account in the interpretation of the data, starting from renaming the
diets as reduced-fat diets instead of low-fat diets.

6.3 Methods

Frequently sampled intravenous glucose tolerance test (FSIGT)

In Studies I-IV FSIGT was performed as previously described (221). In non-diabetic
subjects, the insulin sensitivity index determined by FSIGT correlates well with the
insulin sensitivity measured with a clamp-study which is considered as the gold
standard for this measurement (231). The method has also been shown to be
reproducible with acceptable intraindividual variation (232). The benefit of the
FSIGT method is that it gives an estimate of insulin secretion (acute insulin
response, AIR) in addition to glucose effectiveness and insulin sensitivity indices
which is not possible during a clamp-study. The method was proven to be also
technically feasible, there was only one subject in Study I and three subjects in Study
IV for whom the data were not available due to technical difficulties. However, as
for other measurements of glucose and insulin metabolism, the day-to-day variation
is rather high, for S, in healthy males under conditions of fixed diet and limited
physical activity the mean CV% was 20%, for S, 25% and for AIR 20% (233). The
high CV% of the method reduces its statistical power to detect differences in a study.
In Studies I-IV with their relatively small number of subjects, the differences in
indices from FSIGT should have been marked to reach statistical significance.
Gender, BMI, waist circumference, maximal aerobic capacity and use of oral
contraceptives in women were associated with variation in S, from FSIGT in 380
young healthy Caucasians (234). The mean S, was found to be higher in women
than men. Gender, age and waist circumference were associated with variation in
AIR (234). In women also the menstrual cycle can affect S; it is highest during the
follicular phase and lowest during the luteal phase, S, did not change as a function
of menstrual cycle (235). In a cross-over design in Studies I-III, the background
factors of subjects do not confound the comparison between diet periods as the
results from different periods are compared within the same subject. However, in
Studies I and II where subjects were premenopausal women, the 2-week washout
period between the diet periods has caused some difference in the phase of menstrual
cycle at the time of the measurements and as a consequence increased variation
thereby lowering the statistical power to detect differences in $S_i$. In *Study IV*, the background factors were similar between the groups.

**Serum lipid and lipoprotein concentrations**

The serum lipid and lipoprotein concentrations were measured using the standard methods described previously and with a reasonable low CV%, 1.1 - 4.4% between measurements. There is substantial intraindividual variation reported in serum cholesterol concentrations with a mean CV% between 5 and 10% (236) and it has been estimated that two to three samples are required to estimate the true cholesterol measure with 95% confidence (237). In women, the cholesterol concentration fluctuates also during the different phases of menstrual cycle (238) and the levels of cholesterol and apolipoproteins have been reported to be higher in women using oral contraceptive therapy (239). With respect to the responses to dietary changes, differences between men and women cannot be excluded (240) and clearly some people are hyperresponders whereas some others are hyporesponders to dietary changes (241, 242). Also the cholesterol content of the diet may have an effect (243) as well as the individual’s baseline level of serum cholesterol - those with higher serum cholesterol concentrations appear to respond best to dietary changes (244). Part of the variation in the response may be attributable to genetic factors (245).

In *Studies I and II*, participants were young premenopausal women and part of them used oral contraceptives. In a cross-over design, however, the results from one diet period are compared to the results of another diet period from the same person. Therefore, differences in oral contraceptive use do not affect the differences observed between diet periods as long as the contraceptive use is similar during both diet periods as it was during these studies. The same applies to the individual differences in responses to dietary changes and baseline cholesterol level of individuals. The cholesterol content of the different diets was kept constant during the *Studies I and II* so that would not contribute to the variation to measurements. In both studies, the length of the experimental diet period was four weeks and the length of run-in and wash-out periods 2 weeks which means that the subjects have been in a different phase of the menstrual cycle at the end of the diet periods. That together with a single measurement of serum lipids has produced more variation to the measurements reducing the power to detect the differences between the diets. If all diet periods had been 4 weeks in length and contained more than one serum lipid measurement, this variation could have been reduced. However, these studies were one part of a continuum of studies evaluating the effects of single fatty acids on glucose and insulin metabolism which have been carried out in the Department of Clinical Nutrition and the similar study design in all studies enabled comparability between studies. *Study III* included both men and women as study subjects. Due to
the cross-over design, a different response to the diets or individual differences in baseline levels of serum lipids does not contribute any variation to the measurements because all subjects go through all diet periods and the results from different diet periods are compared within the same person. From the five women that participated in the study, only one was premenopausal so the effect of fluctuation during menstrual cycle has had only a marginal effect on the evaluation of the results. The cholesterol content of the different diets was kept constant during the study. Due to the larger day to day variation of serum lipids in hypertriglyceridaemic subjects, the mean of two consecutive serum lipid measurements was used, thus reducing the variation in measurements and increasing the statistical power of the study to detect differences in serum lipids between the diet periods.

**Serum lipid fatty acid composition**

The serum lipid fatty acid composition was determined as previously described (224). Moilanen et al have demonstrated that the fatty acid composition of serum remained unchanged for at least one year at -60 °C (246). In Studies I-IV, samples were stored at an even lower storage temperature -78 °C and for a shorter period of time (up to 6 months) so it is unlikely that the storage would have caused any changes in the fatty acid composition of the samples. Lipids were extracted with chloroform-methanol (2:1), this solvent allows good recovery for lipids in serum and plasma. The lipid fractions were separated by solid phase extraction with aminopropyl columns. This method is more rapid than the thin-layer chromatography and it offers higher recovery and purity (224). Analysis of the fatty acid composition of serum lipids can be used as a biochemical indicator for assessment for adherence to experimental diets during the intervention studies (247). The fatty acid composition of serum triglyceride fraction reflects the fatty acid intake from the last few days and the fatty acid composition of serum cholesteryl esters reflects the dietary intake of the preceding 3-4 weeks and whereas the composition of phospholipids reflects diet over several months (247-249). The fatty acid composition of serum triglycerides and cholesteryl esters were used as indicators of dietary compliance in Studies I-III. In Study IV, in addition to these two lipid fractions also the composition of serum phospholipids were reported. Phospholipids are the main component of cell membranes thought to mediate the effects of fatty acids and therefore could be most relevant in evaluating the associations with glucose and insulin metabolism.
**Haemostatic factors**

The fibrinogen concentration, FVIIc and PAI-1 activity were determined as previously described (225). In all three measurements there were acceptable CV\%, varying from 2.3 to 5.5\%. Obesity, insulin resistance, type 2 diabetes and hypertriglyceridaemia are all associated with higher levels of fibrinogen, FVIIc, and PAI-1 (250-254). Also age, oral contraceptive use and menopause can modify fibrinogen and FVII levels. With respect to fibrinogen, also smoking has been identified as a background factor contributing to the variation (255). Gender variability was not observed for fibrinogen and FVII, however, intraindividual variation was 7.0-7.6\% for fibrinogen and 13.5-13.9\% for FVII (256). Diurnal variation has been observed in PAI-1 especially in obese subjects (257). Differences in background factors (age, obesity, glucose tolerance etc.) do not cause confounding in a cross-over design (Studies I-III). All samples were taken in the morning in standard conditions so the diurnal variation in PAI-1 did not interfere with the results. The rather large intraindividual variation reported for fibrinogen and FVII will reduce the statistical power to detect differences with a small number of subjects as was the case in Studies I-III.

**6.4 Results**

**6.4.1 Glucose and insulin metabolism**

There was no difference between the Stearic and the Oleic diet in any of the measures of glucose and insulin metabolism. There is another short-term study where the effects of stearic acid have been compared to palmitic acid in a group of healthy women and no effect on glucose or insulin metabolism was seen (141). On the other hand, there are several epidemiological studies where a high saturated fat intake has been associated with higher fasting insulin and glucose concentrations (92, 103-107, 120) and increased risk of type 2 diabetes (116, 132). There is also a recent intervention study where substitution of monounsaturated fat for saturated fat for a 3-month period improved insulin sensitivity in middle-aged subjects (120). Stearic acid is unique compared to the other saturated fatty acids with respect to serum lipids, it is considered neutral (159) and it is possible that its effect on glucose and insulin metabolism could be different from that of other saturated fatty acids.

The effect of Trans diet on glucose and insulin metabolism did not differ from the effect of Oleic diet in healthy normal-weight women. These results are supported by a recent study by Lovejoy et al (113). A 9% enrichment of diet by elaidic acid, oleic acid or palmitic acid did not have significant impact on insulin sensitivity or secretion in lean or overweight individuals. There are little data on trans fatty acids
and glucose and in insulin metabolism. However, some studies indicate a detrimental effect. In the Nurses Health Study, high intake of trans fatty acids had an association with increased risk of type 2 diabetes (131). There are also data that a very high intake of trans fatty acids (20 E%) in patients with type 2 diabetes could alter their postprandial insulin and C-peptide response (122). In mouse islet cells, trans fatty acids potentiated insulin secretion relative to cis isomers (121).

In the present studies where the effects of stearic acid and trans fatty acids on glucose and insulin metabolism were evaluated, the subjects were young, healthy and lean women. The length of the dietary periods was 4 weeks. The results might have been more pronounced if the length of the study had been longer or the intake of fatty acids higher. It is also possible that insulin resistant or glucose intolerant subjects might have been more susceptible to this dietary change. This is supported to some extent by recent data (113) where a reduction in insulin sensitivity after palmitic acid enrichment compared to oleic acid enrichment was seen in the overweight but not in lean subjects after 4-week diet periods. However, the reduction was not statistically significant probably due to the small number of overweight subjects. Obesity is known to be associated with insulin resistance. Insulin sensitive healthy subjects as in the present studies might be able to compensate for the possibly deteriorating short-term changes in insulin sensitivity induced by the diet. Furthermore, the intra-individual variation in insulin sensitivity is high and the number of subjects in both studies was rather small which means that only a marked difference (similar to the difference observed in Study III) in insulin sensitivity between the diets would have reached statistical significance. Therefore, a difference of a smaller magnitude between the diets cannot be excluded on the basis of present data.

In epidemiological studies, a high intake of fat has been associated with increased risk of type 2 diabetes and impaired glucose tolerance (116, 128-130). On the other hand, high intake of unsaturated fat has been associated with a beneficial effect on the risk of type 2 diabetes (61, 103, 131). In the present study in hyperlipidaemic subjects, the insulin sensitivity index was 40% higher after the Reduced-fat diet than after the Mufa diet. In the previously cited intervention study by Vessby et al (120), the beneficial effects of the monounsaturated fat enriched diet compared to saturated fat enriched died on insulin sensitivity were lost in individuals consuming more than 37 E% fat. In the present study, the median fat intake during the monoene enriched diet was 38.3 E%. Assuming that there is a threshold effect as suggested by the study of Vessby et al (120), the high fat content in this diet could have contributed to the fact that it had no beneficial effect on insulin sensitivity.

The observed improvement in insulin sensitivity in the present study after the Reduced-fat diet compared to the Mufa diet was associated with a decrease in serum triglyceride concentration. There were no significant differences in group means of serum triglyceride concentrations between the diets but there was extensive variation
between individuals, especially in their responses to the monoene enriched diet. Nonetheless, the observed association indicates that within a single subject, a change in insulin sensitivity was accompanied by a change in serum triglyceride concentration. The inverse association between serum triglycerides and insulin sensitivity has been previously established in several subject groups: in healthy subjects and in subjects with IGT or type 2 diabetes (258-261). Moreover, it has been reported that hypertriglyceridaemic subjects with type 2 diabetes are more insulin resistant than matched non-hypertriglyceridaemic patients (262, 263). It has been suggested that this association could be due to the increased lipid availability to muscle which could interfere with glucose metabolism and contribute to insulin resistance in muscle tissue (264, 265). Piatti et al reported a decrease of peripheral glucose uptake when plasma triglyceride concentration in healthy subjects was experimentally increased (266). However, in two studies with hypolipidemic medication, insulin sensitivity was improved only in severely (267) but not in mildly hypertriglyceridaemic (268) subjects. This discrepancy in the available data could be interpreted to indicate that triglycerides may play a role in glucose metabolism only when they exceed a threshold concentration, 5 mmol/l as suggested by Piatti et al (266). With respect to the present findings, it can be suggested that in a group of individuals already with a mean serum triglyceride concentration of 3.2 mmol/l a high fat diet could result in an impairment in insulin sensitivity. However, simply on the basis of a correlation, no causal relationship can be determined. Even in the case where the variables are related to each other, it is still impossible to deduce which of the variables is the primary one followed by a change in the other variable. Insulin resistance as such is known to affect VLDL metabolism (265) and the observed correlation could also reflect a reduction in serum triglyceride concentration as a consequence of improved insulin sensitivity in this group of hyperlipidemic individuals.

In subjects with IGT, the Mono diet resulted in beneficial changes in the fasting plasma glucose concentration and \( S_0 \). At baseline, a high \( S_1 \) was related to a lower proportion of saturated fatty acids in triglycerides. There are previous data suggesting that a greater proportion of saturated fatty acids in cholesteryl esters is associated with a greater risk of type 2 diabetes (136). In the present study, the higher proportions of oleic and \( \alpha \)-linolenic acids were associated with better \( S_0 \) and lower fasting plasma glucose at baseline. After the consumption of the study diets, increases in proportions of oleic and \( \alpha \)-linolenic acids were associated with a decrease in fasting plasma glucose and an increase in oleic acid also with an increase in the glucose effectiveness index. To our knowledge there are no previous data linking the proportions of oleic or \( \alpha \)-linolenic acids in serum lipids to glucose tolerance. However, beneficial effects of monounsaturated-fat enriched diet on fasting (145) and postprandial plasma glucose (269) in type 2 diabetic subjects have been reported. In epidemiological studies, unsaturated fatty acids in general have
been associated with a lower risk of type 2 diabetes (61, 103, 131). One of the mechanisms mediating the effect of dietary fat on glucose and insulin metabolism is hypothesized to be diet induced changes in the fatty acid composition of cell membranes (16). This study indicates that also the beneficial effects of oleic and ω-linolenic acids could be mediated by this mechanism.

6.4.2 Serum lipids and lipoproteins

In line with previous studies, the effect of stearic acid on serum lipids was similar to that of oleic acid (159). The effect on lipid metabolism of trans fatty acids was similarly in line with previous findings (188, 270). The total/HDL cholesterol ratio, total, HDL and LDL triglyceride concentrations and apoB concentration were higher after the Trans diet as compared to the Oleic diet. The elevated apo B concentration in the presence of similar LDL cholesterol and the only marginal difference in VLDL cholesterol could indicate a reduced lipoprotein particle size after the consumption of the trans fatty acid enriched diet. The tendency to a smaller LDL particle size after the use of a hydrogenated margarine enriched diet has been previously reported (271). In Lp(a) concentration there was no difference between the diets, this being in line with the data from Clevé et al (189) and Lichteneck et al (188), however, there are several studies where a significant increase in Lp(a) concentration after consumption of a trans fatty acid enriched diet has been reported (185-187). The role of genetic factors in determining the Lp(a) concentration is thought to be strong (161), and this could provide one explanation for the divergent results.

In the study comparing the Mufa diet and the Reduced-fat diet in hyperlipidaemic subjects, the concentrations of serum lipids and lipoproteins were similar after the two diets which is in line with previous studies (228, 269, 272). A lower triglyceride concentration could have been assumed in these hypertriglyceridaemic subjects after a high monoene diet (159). The result in the present study is probably explained by the fact that the Reduced-fat diet had a moderate content of fat instead of a very low content of fat, which may have a triglyceride elevating effect (273).

6.4.3 Haemostatic factors

In healthy young women, the effects on coagulation factors were similar between the Stearic and the Oleic diets as well as Trans and Oleic diets. Of the measured variables, fibrinogen is known to be uninfluenced by dietary fat composition, with the potential exception of long-chain n-3 fatty acids (192). With respect to fasting levels of FVIIc or PAI-1, there are no previous data on the effect of stearic acid compared to oleic acid but compared to other saturated fatty acids, stearic acid has
resulted in lower FVIIc activity (162). One study comparing the effects of oleic, stearic and linoleic acid rich meals found no differences between the effects of these fatty acids on postprandial activities of either FVIIc or PAI-I (274). Although these results are not directly comparable to fasting levels, similar to present results no difference between the effects of oleic and stearic acids was observed. In agreement with the present study, no effect on fibrinogen or FVIIc by trans fatty acids was observed by Almedingen et al (217). The data on trans fatty acids and PAI-1 are controversial (209, 217), in the present study no effect was seen. In hyperlipidaemic middle-aged subjects, there were no differences in coagulation factors between the Mufa and the Reduced-fat diets. However, as previously emphasized, the Reduced-fat diet was not a low-fat diet, which in some studies has been reported to induce positive changes in haemostatic factors (198, 200).

6.5 Practical implications

The present study was one part of a series of studies carried out in the Department of Clinical Nutrition, University of Kuopio aiming to determine the optimal type of fat in the diet for glucose, insulin and lipid metabolism. None of the investigated individual fatty acids seem to have such a large impact on glucose metabolism that these fatty acids or their major sources should be emphasized in the dietary advice given to subjects with abnormalities in glucose metabolism. Instead, the attention should be directed to total proportions of different fat types i.e. saturated and unsaturated fat. A summary of the accumulated data is that the optimal diet for glucose metabolism should include a low content of saturated fat and a moderate content of unsaturated fat. Translated to the food level this means that the individual should choose low-fat dairy and meat products and combine this with moderate use of vegetable oils, vegetable-oil based margarines, and salad dressing. A high fat intake as such is likely to be detrimental for glucose and insulin metabolism. The exact upper limit is not known but on the basis of recent evidence it should not exceed 37 E%, but the optimal limit might be lower. In the current Finnish and European recommendations for diabetic patients the upper limit for fat intake is 35 E%. However, as dietary fat is an important determinant of body weight, in those individuals aiming at weight loss or weight maintenance the fat content of diet should not exceed 30 E%. The optimal diet should also be rich in fiber which means the daily use of whole grain products, vegetables, berries and fruits. To sum up, a diet beneficial for glucose metabolism is well in line with current recommendations for healthy and diabetic populations. Furthermore, this recommended diet would have a beneficial effect on the other risk factors for cardiovascular disease including serum lipids and haemostatic factors, and therefore it would provide a safe approach
in several fronts to reduce the risk of morbidity in those individuals with type 2 diabetes or those at risk of the disease.
7. SUMMARY AND CONCLUSIONS

The aim of the study was to examine the effects of stearic acid and trans fatty acids on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women and the effects of two fat-modified diets (monounsaturated fat enriched diet vs reduced-fat diet) on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in hyperlipidaemic middle-aged subjects. In addition, the relation between changes in serum lipid fatty acid profile during two fat-modified diets (high-fat monounsaturated fat enriched diet vs reduced-fat polyunsaturated fat enriched diet) to changes in glucose and insulin metabolism was evaluated in subjects with impaired glucose tolerance. Fasting glucose and insulin, acute insulin response, insulin sensitivity and glucose effectiveness measured by the frequently sampled intravenous glucose tolerance test were used to evaluate glucose and insulin metabolism. Lipid metabolism was evaluated by serum and lipoprotein cholesterol and triglycerides, apolipoproteins A-I and B and lipoprotein(a) and coagulation factors by fibrinogen, PAI-1 and FVII. The results of this study can be summarized as follows:

1. The effects of moderate stearic acid enrichment in the diet (5 E%) did not differ from the effects of oleic acid on glucose and insulin metabolism, serum lipids and lipoproteins and coagulation factors in young healthy women.

2. The effects of moderate trans fatty acid enrichment in the diet (5 E%) did not differ from the effects of oleic acid with respect to glucose and insulin metabolism or coagulation factors in young healthy women. Serum total to HDL cholesterol ratio and serum total, HDL and LDL triglyceride and apo-B concentrations were significantly higher after the consumption of the trans fatty acid enriched diet.

3. Insulin sensitivity in hyperlipidaemic middle-aged subjects was significantly higher after the reduced-fat diet compared with the high-monounsaturated fat diet. The change in insulin sensitivity had a strong inverse association with the change in serum triglyceride concentration. There were no significant differences between the diets with respect to serum lipids and lipoproteins or coagulation factors.

4. A higher insulin sensitivity at baseline was associated with a lower proportion of saturated fatty acids and tended to be associated with a higher proportion of long-chain unsaturated fatty acids in serum lipid fractions. Indicators of glucose metabolism (a higher glucose effectiveness index and lower fasting plasma glucose) were associated with higher proportions of oleic and α-linolenic acids at baseline.
After the consumption of the reduced-fat diet and the high-monounsaturated fat diet, the increases in proportions of oleic and α-linolenic acids were associated with a decrease in fasting plasma glucose and increase in oleic acid also with an increase in glucose effectiveness index.

In conclusion, moderate enrichment of stearic or trans fatty acids in diet had no effect on insulin sensitivity in young healthy subjects. However, subjects with hyperlipidaemia/impaired glucose metabolism may be more sensitive to changes in dietary fat content or type. Moderate fat content and enrichment of diet with unsaturated fat seem to beneficial for glucose metabolism in these groups. None of the investigated individual fatty acids seem to have such a large impact on glucose metabolism that these fatty acids or their major sources should be emphasized in dietary advice for subjects with abnormalities in glucose metabolism. Instead, the attention should be directed to total proportions of different fat types i.e. saturated and unsaturated fat. The optimal diet for glucose metabolism has a low content of saturated fat and a moderate content of unsaturated fat with a high content of fiber - this is all well in line with current recommendations for healthy and diabetic populations. The benefits with the recommended diet exceed the effect on glucose metabolism - also serum lipids and haemostatic factors are favourably affected. Thus diet provides a safe approach to reduce the risk of morbidity in those individuals with type 2 diabetes and in those at risk of the disease.
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