Dietary Fatty Acids, Body Composition and Ectopic Fat

Results from Overfeeding Studies in Humans

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Abstract

The aim of this thesis was to investigate the effects of dietary fatty acids on body composition and ectopic fat in humans, with emphasis on the role of the omega-6 polyunsaturated fatty acid (PUFA) linoleic acid (18:2n-6) and the saturated fatty acid (SFA) palmitic acid (16:0). The overall hypothesis was that linoleic acid would be beneficial compared with palmitic acid during overfeeding, as previously indicated in animals.

Papers I, II and IV were double-blinded, randomized interventions in which different dietary fats were provided to participants and Paper III was a cross-sectional study in a community-based cohort (PIVUS) in which serum fatty acid composition was assessed as a biomarker of dietary fat intake.

In Paper I, overfeeding with sunflower oil (n-6 PUFA) for 7 weeks caused less accumulation of liver fat, visceral fat and total body fat (as assessed by MRI) compared with palm oil (SFA) in young and lean subjects despite similar weight gain among groups. Instead, sunflower oil caused a larger accumulation of lean tissue.

In Paper II, plasma from Paper I was analyzed with NMR-based metabolomics, aiming to identify metabolites differentially affected by the two dietary treatments. Acetate decreased by PUFA and increased by SFA whereas lactate increased by PUFA and decreased by SFA.

In Paper III, the proportion of linoleic acid in serum was inversely associated with contents of visceral-, subcutaneous- and total body adipose tissue whereas the proportion of palmitic acid was directly associated with visceral- and total body adipose tissue in 70-year old men and women.

In Paper IV, overfeeding with sunflower oil for 8 weeks caused less accumulation of liver fat compared with palm oil also in overweight and obese subjects. SFA increased visceral fat in men only. Accumulation of lean tissue was similar between groups.

In conclusion, SFA (palmitic acid) from palm oil promotes marked liver fat accumulation in both normal-weight and overweight/obese subjects during overeating, whereas n-6 PUFA (linoleic acid) from sunflower oil prevents such liver fat accumulation. Diverging effects of SFA and PUFA on visceral adipose tissue and lean tissue may only be applicable in some groups and/or circumstances. These results imply that negative effects associated with weight gain (e.g. fatty liver) may be partly counteracted by the type fat in the diet, overall supporting a beneficial role of diets higher in unsaturated fat compared with saturated fat for preventing liver fat accumulation.

Keywords: Linoleic acid, Palmitic acid, SFA, PUFA, Fatty acids, Body composition, Liver fat, Ectopic fat, Adipose tissue

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Den mätta dagen, den är aldrig störst.
Den bästa dagen är en dag av törst.
Nog finns det mål och mening i vår färd -
men det är vägen, som är mödan värd.
Det bästa målet är en nattlång rast,
där elden tänds och brödet bryts i hast.
På ställen, där man sover blott en gång,
blir sömnen trygg och drömmen full av sång.
Bryt upp, bryt upp! Den nya dagen gryr.
Oändligt är vårt stora äventyr.

Karin Boye – I rörelse
The cover picture illustrates the main findings of this thesis, namely that overeating sunflower oil and palm oil results in different body compositions and amounts of ectopic fat, despite similar weight gains. The picture is designed by me and drawn by Marion Rosqvist.
List of Papers

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


III **Rosqvist, F., Bjermo, H., Kullberg, J., Johansson, L., Michaelsson, K., Ahlström, H., Lind, L., Risérus, U. Visceral and subcutaneous adipose tissue content is diversely associated with serum polyunsaturated and saturated fatty acids. Submitted**

IV  **Rosqvist, F., Kullberg, J., Orho-Melander, M., Cederholm, T., Ahlström, H., Risérus, U. Effects of overfeeding polyunsaturated and saturated fat on lean tissue, liver fat and visceral fat accumulation in overweight and obese humans. In manuscript**
Contribution

The contribution of Fredrik Rosqvist to the papers included in this thesis was as follows:

I. Designed and planned the study in collaboration with supervisor, participated in baking of the muffins, performed the study, compiled the data and analyzed the results, wrote the first manuscript, revised the paper in collaboration with supervisor and co-authors

II. Interpretation of the results, wrote part of the discussion, contributed to revision of the paper

III. Performed the analyzes, wrote the manuscript, contributed to revision of the paper

IV. Designed and planned the study in collaboration with supervisor, performed the study, compiled the data and analyzed the results, wrote the first manuscript

Related papers
(not included in the thesis)


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Abbreviations

ACC  Acetyl-coenzyme A carboxylase
ADP  Air-displacement plethysmography
BAT  Brown adipose tissue
BMI  Body mass index
ChREBP Carbohydrate-responsive element-binding protein
CLP  Conjugated linoleic acid
CPT  Carnitine palmitoyltransferase
D5D  Delta-5 desaturase
D6D  Delta-6 desaturase
DHA  Docosahexaenoic acid
DXA  Dual energy X-ray absorptiometry
E%  Energy %
EPA  Eicosapentaenoic acid
FAS  Fatty acid synthase
GPR  G-protein coupled receptor
LPL  Lipoprotein lipase
MRI  Magnetic resonance imaging
MRS  Magnetic resonance spectroscopy
mTOR Mechanistic target of rapamycin
MUFA Monounsaturated fatty acid
n-3 Omega-3
n-6 Omega-6
NAFLD Non-alcoholic fatty liver disease
NMR Nuclear magnetic resonance
PNPLA3 Patatin-like phospholipase domain-containing protein 3
PPAR Peroxisome proliferator-activated receptor
PUFA Polyunsaturated fatty acid
SAT Subcutaneous adipose tissue
SCD Stearoyl-coenzyme A desaturase
SFA Saturated fatty acid
SREBP Sterol responsive element-binding protein
UCP Uncoupling protein
VAT Visceral adipose tissue
Introduction

Lipids and dietary fatty acids

The term *lipids* include a broad range of molecules, and can in general be defined to be any molecule that is insoluble in water and soluble in organic solvents. The consortium LIPID MAPS classifies lipids in eight different categories (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides), with further sub-classification within each class; together comprising thousands of distinct molecules. The main biological functions of lipids include their central role in energy storage (as triacylglycerols), as structural components (as phospholipids in cell membranes) and as signaling molecules.

However, when talking about dietary fats, we are mainly concerned with one of these eight categories, namely the fatty acyls, which include the fatty acids. A fatty acid consists of a carbon chain (of variable length) with a carboxyl group at one end and a methyl group at the other. The carbon chain can be either saturated (no double-bonds) or unsaturated (one or several double-bonds). A saturated fatty acid (SFA) has a straight shape. The double-bonds in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) gives them bent shapes (see Figure 1). The nomenclature derives from the carbon chain length, number of double-bonds and the position of the first double-bond from the methyl end (designated n-x). However, in addition to this systematic nomenclature, many of the fatty acids also have common/trivial names. The unsaturated fatty acids are commonly classified into three main families; n-3, n-6 and n-9; although in the context of metabolism and this thesis, the n-7 family is also of importance.

Of the lipids in the diet, the vast majority (~95%) is in the form of triacylglycerols (triglycerides), which is glycerol with three esterified fatty acids. Other forms of common dietary lipids are phospholipids and cholesterol.

In the Swedish diet, the most common dietary SFA is by far palmitic acid (16:0). The most common (virtually the only) dietary MUFA is oleic acid (18:1n-9) and the most common dietary PUFA is linoleic acid (18:2n-6), followed by alpha-linolenic acid (18:3n-3). The approximate fat composition of various oils and fats is visualized in Figure 2 and Figure 3 presents some selected fatty acids and their classification.
**Figure 1.** Structure of fatty acids

**Figure 2.** Fat composition (% of total fat) of various oils and fats
The relative fatty acid composition measured in blood (or tissues) can be used as a biomarker of dietary fat intake (1). Some fatty acids are considered better dietary biomarkers than others. In general, the essential fatty acids 18:2n-6 and 18:3n-3, as well as the long-chain PUFAs 20:5n-3 (EPA) and 22:6n-3 (DHA) are considered good biomarkers. Additionally, the odd-chain SFAs 15:0 and 17:0, synthesized by the microbiota in ruminants, are also considered good biomarkers. Although they can be present in small amounts in certain species of fish, this source can probably be neglected within the Swedish diet. Not all fatty acids are good biomarkers of dietary intake due to endogenous fatty acid metabolism. Some fatty acids can be synthesized endogenously, and fatty acids can also be desaturated, elongated and shortened. Unfortunately, the proportions of 16:0, 18:0 (stearic acid) and 18:1n-9 are not as good biomarkers of dietary intake. Palmitic acid is the main end-product of de novo lipogenesis (DNL), i.e. changes in the proportion of 16:0 may reflect newly synthesized fat (from e.g. carbohydrates) as well as dietary intake. The rate of DNL is however considered to be very limited with a “Western” background diet relatively high in fat (>25E%). Furthermore, the proportion of 16:0 seems to be relatively tightly regulated, i.e. increases are
counteracted by desaturation into 16:1n-7 (palmitoleic acid), and/or elongation into 18:0 (which can be further desaturated into 18:1n-9). Therefore, 16:1n-7 may be a better marker for dietary intake of 16:0 (although it could also reflect DNL in some populations). In fact, the proportion of 16:1n-7 in serum cholesterol esters has been shown to increase in response to increased dietary intake of 16:0 during isocaloric, highly controlled conditions (2). The enzyme responsible for the desaturation of 16:0 into 16:1n-7 (and of 18:0 into 18:1n-9) is a delta-9 desaturase called stearoyl-CoA desaturase (SCD). Two other desaturases important for fatty acid metabolism are the delta-5 and delta-6 desaturases (D5D and D6D, respectively). An overview of selected desaturation and elongation reactions is shown in Figure 4.

Desaturase enzymes

The activities of the three desaturases are cumbersome to assess directly, but they can be estimated by using product to precursor fatty acid ratios (activity indices) in blood or tissue: 16:1n-7/16:0 for SCD, 20:4n-6/20:3n-6 for D5D and 18:3n-6/18:2n-6 for D6D. The hepatic mRNA expression of SCD has been shown to be strongly correlated with the product to precursor fatty acid ratio in blood in humans (3).

SCD is considered a lipogenic enzyme, i.e. changes in the SCD activity index can reflect changes in lipogenesis, which has been verified by isotopically labeled tracers (4). Which underlying metabolic pathways that are reflected by changes in D5D and D6D are less well known, but D6D and SCD are generally associated in the same direction whereas D5D is generally associated in the opposite direction. For example, both SCD and D6D are positively associated, whereas D5D is negatively associated, with incidence of type 2 diabetes (5-7), which may partly be explained by liver fat accumulation (7).
 Obesity, body composition and body fat distribution

Obesity is now a global problem, affecting both affluent "Westernized" populations as well as poorer populations in transition. Obesity is associated with metabolic derangements and increased risk of type 2 diabetes. However, obesity is not a uniform condition. A subgroup of obese individuals (often termed “metabolically healthy obese”) appear not to exhibit disturbed metabolic traits compared with other, equally obese, individuals. Although this concept is under debate (as the “healthy” phenotype has been shown to be transient (8, 9) and not protective for incidence of type 2 diabetes (10)), the cross-sectional metabolic differences between “healthy” and “unhealthy” obesity may be due to differences in body composition and body fat distribution (11).

Ectopic fat and NAFLD

Excess energy can be stored as fat (triacylglycerols) in white adipose tissue, either subcutaneously (SAT) or may accumulate in other places such as intra-abdominally (visceral adipose tissue, VAT) or in the liver and other organs. Fat stored outside of SAT is commonly called ectopic. The storage of ectopic fat may not always be visible from the outside or deduced from simple measures such as BMI or waist circumference, as illustrated in Figure 5.
There is also another type of adipose tissue, called brown adipose tissue (BAT), which differs significantly from the classical white adipose tissue, e.g. by having higher mitochondrial density. The primary purpose of the BAT is not to store surplus energy but rather to generate heat (by oxidizing fatty acids).

Figure 5. Amounts of ectopic fat may not be deduced from simple measures such as BMI or waist circumference. Individual A, B and C have the same BMI but different amounts of intra-abdominal adipose tissue (IAAT) whereas individual D, E and F have the same waist circumference but different amounts of IAAT. Figure from Thomas et al. (12) The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat; Obesity 2012. Reproduced with permission, © 2011 The Obesity Society

SAT seems to be relatively harmless as long as the tissue is healthy and have the ability to dynamically respond to changes in energy flux (see below), whereas the amount of ectopic fat such as VAT and liver fat has been shown to be better predictors of metabolic derangements than both SAT and obesity per se (13-15). Also, VAT is independently associated with the conversion from “metabolically healthy obese” to “metabolically unhealthy obese” (16). Furthermore, liver fat has been reported to be the best predictor of insulin sensitivity (in both liver, skeletal muscle and adipose tissue) independent of BMI and percent total body fat (17). Interestingly, a 10-unit increase in BMI did not further impair insulin sensitivity when subjects were matched for liver fat content (18). The term non-alcoholic fatty liver disease (NAFLD) is used when the liver fat content is above ~5.5% (in the absence of excess
alcohol intake and competing liver diseases). NAFLD is on the rise, and the global prevalence has been estimated to be ~25%, whereas the prevalence in individuals with type 2 diabetes or morbid obesity may reach 75-90% (19). Alarming findings since NAFLD may be a causative factor for cardiovascular disease and type 2 diabetes (20-22). Importantly, improvement of NAFLD is associated with reduced incidence of type 2 diabetes (22, 23).

Although obesity is associated with increased liver fat content at the group level, the amount of liver fat may vary considerably at the individual level and do not always follow the amount of e.g. abdominal SAT as shown in Figure 6.

![Figure 6. Magnetic resonance imaging (MRI)-images focused on the livers in three subjects from Paper IV. From left to right, the liver fat contents are 0.57%, 14.2% and 30.8%.](image)

**Skeletal muscle**

When considering body composition and metabolic traits, skeletal muscle should not be neglected. Skeletal muscle is responsible for taking up ~80% of the postprandial glucose and may also affect circulating lipids. Therefore, it comes as no surprise that skeletal muscle mass has been inversely associated with insulin resistance and prediabetes (24, 25). Additionally, skeletal muscle is more energetically costly also in the inactive state compared with adipose tissue, thereby potentially preventing weight gain. The combination of low muscle mass and excess fat accumulation (“sarcopenic obesity”) is especially deleterious and associated with increased mortality (26, 27).

Overall, from a metabolic- and public health perspective, much is gained by decreasing (ectopic) fat accumulation and increasing skeletal muscle mass.

**Overfeeding as a model**

Although there are some studies in humans comparing the effects of different dietary fats on body composition and fat distribution, Paper I and Paper IV are the first ones doing so during positive energy balance. This is relevant to study because the effects may well differ depending on if the metabolic system is at homeostasis or slightly “overloaded”; i.e. some differential effects of dietary fatty acids may only be observable when the system is chal-
lenged and may therefore go unnoticed in isocaloric studies. Hence, the results are relevant also from a public health perspective as the general adult population tends to slowly gain weight over the years.

The popularity of performing overfeeding studies has waxed and waned over time during the last three decades and there are surprisingly many studies, although with various duration, quality and primary outcomes. Relating to the “metabolically healthy obese” phenotype, not all individuals experience deleterious health effects even during the metabolically challenged setting of experimental overfeeding. This has led to a concept called “adipo- pose tissue expandability hypothesis”, its essence being that negative health effects occur first when the SAT is no longer capable of efficiently storing the surplus energy, thereby giving rise to ectopic fat storage. This theory was supported by Alligier et al. (28) who overfed (+760 kcal/day) 41 healthy males for 8 weeks and showed that accumulation of VAT was highest in subjects with impaired regulation of genes involved in lipid storage in SAT. Further, Fabbrini et al. (29) showed that subjects defined as “metabolically healthy obese” were resistant to adverse effects following weight gain compared with “metabolically unhealthy obese” subjects, and the former group had upregulated lipogenic pathways in SAT compared with the latter. However, the expandability hypothesis is not unequivocally supported (30).

Although the animal studies overviewed in the sections below seldom are explicitly labeled as overfeeding studies, many of them are as the common practice seems to be to use young and still growing animals. This is an important limitation to bear in mind when trying to extrapolate the findings to humans.

Dietary fat and body composition

Although various dietary fatty acids in general are considered to provide similar amounts of energy, differential effects on body composition have repeatedly been demonstrated. The following sections provide an overview of what has been shown, both in animal and human studies.

Animal studies

Rats
Shimomura et al. (31) fed 4-week old male Sprague-Dawley rats isoenergetic diets based on safflower oil or beef tallow (45 E% fat) for 4 months. Despite that the two groups consumed similar energy and had similar body weight gain during the experiment, rats fed safflower oil had lower total carcass fat content compared with beef tallow. However the abdominal adipose tissue weight and carcass protein content were similar between groups. Similarly, Matsuo et al. (32) fed 5-week old male Sprague-Dawley rats
isoenergetic diets based on safflower oil or beef tallow (45 E% fat) for 8 weeks. Both groups gained similar in body weight during the experiment, but both total carcass fat content and abdominal adipose tissue weight were significantly higher after beef tallow compared with safflower oil. Total carcass protein content was similar between groups. Matsuo et al. (33) also performed a similar study in 4-week old male Wistar rats, feeding them isoenergetic diets based on safflower oil, soybean oil and beef tallow for 8 weeks. All groups gained similar in weight but beef tallow resulted in significantly higher carcass fat content compared with both safflower and soybean oil. Carcass protein was similar in all groups.

Partly congruent results were reported by Su et al. (34), who fed (ad libitum) male Sprague-Dawley rats with fish oil (menhaden), safflower oil, olive oil and beef tallow (42 E% fat) for 12 weeks. Food intake and weight gain were similar among groups during the experiment. Gain of fat mass was highest after beef tallow and olive oil and lowest after fish oil. Fat mass gain by safflower oil was in between and not significantly different from any group. Gain of lean body mass was highest after fish oil and significantly different from beef tallow and olive oil, but not from safflower oil. Gain of lean body mass after safflower oil was significantly higher than after beef tallow.

Differential effects on body fat was further observed by Okuno et al. (35), who fed (ad libitum) 4-week old male Sprague-Dawley rats diets containing perilla oil, safflower oil, olive oil or beef tallow (26 E% fat) for 12 weeks. Body weight did not differ between groups but perilla oil caused significantly lower accretion of VAT compared with beef tallow and olive oil. Safflower oil was almost as effective as perilla oil but was not significantly different from any of the other groups. Further, Takeuchi et al. (36) fed 4-week old male Sprague-Dawley rats isoenergetic diets based on lard, high oleic acid safflower oil, safflower oil and linseed oil (39.4 E% fat) for 12 weeks. Carcass fat content was significantly higher after lard compared with all other groups, but abdominal adipose tissue weight and carcass protein content did not differ between any of the groups.

Finally, Dulloo et al. (37) took another approach and investigated the effect on body weight recovery and body composition in rats that initially were food restricted (50%) for 2 weeks and thereafter refed for 2 weeks with isoenergetic high-fat diets (53 E% fat) varying in fatty acid composition. Seven-week old male Sprague-Dawley rats were refed with high-fat diets based on lard, coconut oil, olive oil, safflower oil and fish oil (menhaden). One group of rats was refed with a low-fat control diet. Final body weight did not differ between groups, but rats refed with either safflower oil or coconut oil had lower body fat content compared with the other high-fat groups, and they also did not differ in body fat content from rats refed the low-fat control diet. Interestingly, accumulation of body protein also differed between groups, where rats refed safflower oil had higher body protein dep-
osition compared with rats reared with coconut oil, olive oil and fish oil. Importantly, this study indicates that different sources of SFA (lard vs coconut oil) and PUFA (safflower oil (n-6) vs fish oil (n-3)) can have differential effects on both fat and protein accretion. Using the same model, Yepuri et al. (38) tested three different safflower oils varying slightly in linoleic acid content and found that the highest accretion of lean mass and lowest accretion of fat mass were achieved with the safflower oil containing most linoleic acid. Yepuri et al. then proceeded to test other oils relatively rich in linoleic acid, namely sunflower oil, grapeseed oil and corn oil, and found that all oils resulted in higher lean mass and lower fat mass compared with an isoenergetic lard-based diet. The authors concluded from these experiments that all oils rich in linoleic acid (compared with a lard-based diet) had a favorable impact on body composition but that their effect varied as a function of their linoleic acid content. Yepuri et al. then proceeded to investigate the effects of the n-6/n-3 ratio by feeding different mixtures of safflower oil and linseed oil (1:0, 0:1, 1:1, 2:1, 1:2) and found that all five mixtures were equally effective at increasing lean tissue and reducing body fat compared with an isoenergetic lard-based diet. The authors concluded that it is the total intake of essential fatty acids rather than the specific intake of either one that affects body composition.

In contrast to the results in the abovementioned studies, Stachon et al. (39) reported that feeding male Wistar rats a diet based on sunflower oil for 6 weeks caused a higher deposition of body fat and a lower deposition of body protein compared with diets based on palm oil, lard and rapeseed oil.

Mice

Already three decades ago, Mercer and Trayhurn (40) fed lean and obese (ob/ob) male mice isoenergetic high-fat diets (20 weight%) containing corn oil or beef tallow for 2 weeks. Gains in body weight and energy were higher after beef tallow than corn oil. In lean mice fed corn oil, only 18% of the excess energy intake was retained in the carcass, compared with 77% in mice fed beef tallow. In obese mice, the same pattern was observed although the differences were not as dramatic (31% vs 56%). Further, Catta-Preta et al. (41) fed (ad libitum) 12-week old male C57BL/6 mice high-fat diets (60 E% fat) based on lard, olive oil, sunflower oil and canola oil for 10 weeks. Although food intake did not differ between groups, mice fed with lard increased significantly more in body weight and visceral fat mass compared with all other groups. Lard also increased SAT significantly more compared with both sunflower oil and canola oil. Similarly, Timmers et al. (42) fed (ad libitum) 7-week old male C57BL/6 mice high-fat diets (45 E% fat) containing palm oil, cocoa butter, olive oil and safflower oil for 8 weeks. Food intake was similar in all groups, but mice fed palm oil increased significantly more in body weight compared with all other groups. Interestingly, muscle fat content increased by all high-fat diets except for safflower oil. The differ-
ential fat-promoting effects was shown also in another model by Bell et al. (43) who fed \((ad\ libitum)\) 6-week old female ARC Swiss Albino mice high-fat diets (40.8 E% fat) based on either beef fat or canola oil for 8 weeks. Mice fed beef fat gained more in weight and total body fat compared with mice fed canola oil. Lean body mass did not differ between groups.

Adding some more nuance, Van den Berg et al. (44) fed \((ad\ libitum)\) 14-week old male C57Bl/6J mice high-fat diets (45 E%) based on either palm oil, lard or palm oil with added stearate (to the level found in lard) for 5 weeks, and found that both lard and palm oil with added stearate increased total body mass and fat mass to a greater extent than palm oil.

In contrast to the abovementioned studies, Shen et al. (45) observed that a diet rich in stearic acid compared with isoenergetic diets based on safflower oil or corn oil (20% fat) drastically reduced the amount of VAT in athymic nude mice fed \(ad\ libitum\) for 18 weeks. Furthermore, total body fat decreased and lean tissue increased more by stearic acid, despite similar weight gain.

**Chickens**

Sanz et al. (46) fed 21-day-old Hybro-G female broiler chickens diets rich in either beef tallow or sunflower oil for 32 days, and showed that abdominal fat deposition was significantly lower by sunflower oil despite no differences in weight gain or feed intake between treatments; replicating their previous study (47). Sanz et al. (48) further repeated these findings by showing that abdominal fat deposition increased linearly with increasing number of days in which birds were fed the tallow-rich diet compared with the sunflower oil-rich diet. Sanz et al. (49) also showed that protein accretion was lower in chickens fed a diet rich in tallow and lard compared with sunflower oil.

In conclusion, the general picture of the results from animal studies suggests that unsaturated fat is favorable for body composition compared with saturated fat, although some inconsistencies exist. However, extrapolations to humans must be done with caution. Many of the results have been obtained from young and still growing animals. Further, diets are often extreme compared with what is reasonable in the human setting. Finally, some aspects of metabolism may differ between these various animal models and humans, potentially making the results non-transferable to humans.

**Human studies**

The first human study showing differential effects on body fat by dietary fats was done by Summers et al. (50) who compared the effects of a SFA-rich diet with a PUFA-rich diet in a 5-week cross-over study in 17 men and women, and found that abdominal subcutaneous fat content (assessed by MRI) decreased during the PUFA-rich diet compared with the SFA-rich diet.
Changes in visceral fat content were not significantly different between groups when analyzed in the whole population, but was significantly decreased by PUFA when analyzed in the subgroup having type 2 diabetes, however this result should be interpreted cautiously considering the small sample size (n=6). Congruently, Kien et al. (51) showed a borderline higher (P=0.06) fat mass accretion during 4 weeks in healthy young adults fed a liquid formula diet high in palmitic acid (16.8 E%) compared with a diet low in palmitic acid and high in oleic acid (31.4 E%). Changes in fat-free mass (FFM) did not differ between groups. Kien et al. (52) performed a similar study, this time using solid food diets and a cross-over design, and found that FFM was higher after the oleic acid-rich diet compared with palmitic acid-rich diet. However, in this study no differences in fat mass were observed between the diets.

In a crossover study, Piers et al. (53) fed (all food provided) 8 overweight and obese men isocaloric diets high in SFA or MUFA for 4 weeks each. They showed that body weight, fat mass, percent body fat, trunk fat mass and limb fat mass decreased by MUFA compared with SFA, despite that no differences in energy intake could be detected between groups. Lean tissue mass tended to decrease by SFA but differences between groups were not statistically significant. In another cross-over study, Norris et al. (54) compared the effects of supplemental conjugated linoleic acid (CLA) or safflower oil (in capsules, 8 g/day) during 16-weeks in 35 obese postmenopausal women with type 2 diabetes. Safflower oil had no effect on body weight or total adipose mass (assessed by dual energy X-ray absorptiometry, DXA) but significantly reduced trunk adipose mass and increased lean tissue mass, results that are indeed remarkable (if not created by chance) considering the low dose of oil. CLA had no effect on lean tissue or trunk adipose mass, but decreased body weight and total adipose mass.

Smith et al. (55) evaluated the effect of supplementing high-doses of EPA and DHA (4 g Lovaza/day) on muscle mass and function in 44 old (age 60-85 years) but healthy men and women for 6 month. Supplementation with n-3 PUFA had no effect on body weight or fat mass, but significantly increased thigh muscle volume (assessed by MRI) compared with control (corn oil). Furthermore, n-3 PUFA significantly increased handgrip strength and 1-repetition maximum (composite measure of 4 exercises) muscle strength.

Contrasting results were reported by Gillingham et al. (56) who performed a randomized cross-over study in 34 overweight women, comparing diets enriched in oleic acid or alpha-linolenic acid with a ‘Western diet’ containing higher amounts of SFA. Each diet (~38 E% fat) was tested for 28 days and all food was provided. No differences in body weight, fat mass, FFM, android fat or gynoid fat (assessed by DXA) were observed between groups. Further, in the context of a weight-loss study, Tan et al. (57) compared the effect of low (5 E%) and high (10 E%) PUFA diets on weight loss.
Participants were given advice on how to change their diets but half the subjects also received walnuts. Outcomes were assessed after 12 weeks in 121 obese men and women with complete data. No differential effects on weight loss, fat loss or loss of VAT (assessed by computed tomography) were observed between groups. However, no objective compliance measure (e.g. fatty acid composition in blood) was used as dietary intake was only assessed by diet history interviews and food records.

Observational studies

Warensjö et al. (58) cross-sectionally investigated the associations between fatty acid composition in serum cholesterol esters and crude markers of obesity in 849 middle-aged healthy men and women. The proportions of 16:0 and 18:0 were weakly but highly significantly positively associated with sagittal abdominal obesity, BMI and waist girth, whereas 18:2n-6 was inversely associated with all three measures. Maybe somewhat surprising, EPA was also positively associated with all three obesity measures, whereas neither DHA nor 18:3n-3 showed significant associations. Further, Vinknes et al. (59) cross-sectionally investigated the associations between PUFA in plasma (total plasma) and body fat content assessed by DXA in 2021 ~70-year old men and women, and found significant inverse associations for both n-6 PUFA and n-3 PUFA with body fat content.

Reinders et al. (60) investigated the cross-sectional and longitudinal associations between PUFA in plasma and muscle size and function in 836 elderly (age 66-96 years) men and women. Higher concentration of total PUFA in plasma, but not individual PUFAs, were cross-sectionally associated with larger thigh muscle size and greater knee extension strength. Linoleic acid was cross-sectionally associated with less intermuscular fat depots. There were no longitudinal associations with any fatty acid and change in muscle size or strength. Furthermore, no associations between fish oil consumption and muscle parameters were found, either cross-sectionally or longitudinal. Similarly, Welch et al. (61) investigated the cross-sectional associations between dietary fat intake (assessed by food-frequency questionnaire) and indices of skeletal muscle mass (assessed by DXA) in 2689 women aged 18-79 years. FFM was significantly and inversely associated with dietary intake of SFA, but directly associated with the dietary P:S ratio. Intake of linoleic acid was positively associated with FFM. Congruently, Cardel et al. (62) examined the cross-sectional associations between self-reported dietary fat intake (assessed by two 24-h recalls) and body composition (assessed by DXA and computed tomography) in 311 children aged 7-12 years. Lean body mass was positively associated with intake of total PUFA, n-3 PUFA, n-6 PUFA and P:S ratio. Body fat and intra-abdominal fat was inversely associated with total PUFA and P:S ratio. Finally, Belury et al. (63) showed that linoleic acid in erythrocytes was positively associated with appendicular
lean mass and inversely associated with trunk adipose tissue in a cross-sectional analysis of 139 healthy but overweight men and women.

In conclusion, the amount of human studies are relatively sparse, but the results are generally in agreement with what has been shown in animal studies, although some inconsistencies exists also here. An important caveat to bear in mind is that the common animal models used derive from harmonized genetic backgrounds, further impeding the generalizability and extrapolation to humans, i.e. humans are more genetically diverse and the results may only be applicable to a subpopulation and therefore clear effects may be more difficult to show in humans.

Dietary fat and liver fat

Animal studies

Rats

Kabir et al. (64) fed male Sprague-Dawley rats diets with either palm oil, safflower oil, linseed oil or perilla oil (15E% fat) for 2-3 weeks. Although food intake and animal growth were comparable between groups, palm oil feeding resulted in significantly higher (2-fold) liver fat content compared with safflower, linseed and perilla oils. Similarly, Go et al. (65) bolus-fed 8-week old male Sprague-Dawley rats with either palm oil or sunflower oil (2.5 mL/day) for 22 days. Although rats receiving sunflower oil gained significantly more body weight, rats receiving palm oil had significantly higher hepatic lipid accumulation.

Levy et al. (66) fed Fischer 344 rats with high-fat diets (45 E% fat) based on lard or fish oil for 4 weeks, with a low-fat diet as reference. Rats fed fish oil consumed more food but still increased less in weight compared with rats fed lard. Rats fed fish oil also had considerably lower levels of liver fat compared with both lard and control. Interestingly, Ferramosca et al. (67) showed that addition of n-3 PUFA as krill oil to a high-fat diet (35 E% fat) based on lard completely prevented the accumulation of liver fat in male Sprague-Dawley rats fed for 12 weeks.

Adding some nuance regarding SFA, Janssens et al. (68) fed 12-week old male Wistar rats high-fat diets (45 E% fat) based on either palm oil or lard for 10 weeks, i.e. the difference between diets being mainly the content of palmitic and stearic acid. Energy intake and body weight gain were similar between groups, but palm oil caused significantly higher deposition of liver fat, despite that palm oil increased the fatty acid oxidative capacity in isolated liver mitochondria compared with lard.

Comparing unsaturated fats, Ronis et al. (69) showed that male Sprague-Dawley rats, subjected to total enteral overfeeding for 21 days, had lower
liver fat content if the diet contained corn oil compared with olive oil (5 E% fat).

In contrast, Shimomura et al. (31) observed no difference in liver lipid content after 4 month of isoenergetic feeding of safflower oil and beef tallow, despite that carcass fat content, serum insulin and triglycerides were higher after beef tallow. Further, Hanke et al. (70) showed that only a blend of canola oil and flaxseed oil decreased liver fat content in male Sprague-Dawley rats compared with lard after 12 weeks of treatment, whereas canola oil, safflower oil and soybean oil were not different from lard.

Mice
Timmers et al. (42) demonstrated that liver fat content increased in male C57BL/6 mice fed high-fat diets (45 E% fat) containing palm oil or olive oil for 8 weeks, but no increase in liver fat content was seen after safflower oil or cocoa butter. Similarly, Pavlisova et al. (71) fed 12-week old male C57BL/6N mice high-fat diets (32-35 weight%) based on either corn oil or lard for 8 weeks. Body weight gain was similar between groups but lard caused significantly higher hepatic lipid content, in conjunction with a more than 4-fold higher hepatic expression of SCD-1. Further, de Wit et al. (72) fed 9-week old male C57BL/6J mice high-fat diets (45 E% fat) containing either palm oil, olive oil or safflower oil for 8 weeks, with a low-fat diet as a reference. Palm oil caused significantly higher liver fat accumulation compared with safflower oil. Interestingly, safflower oil decreased liver fat content also when compared with the low-fat reference diet. Results for olive oil did not differ from palm oil, but was significantly higher compared with safflower oil.

Comparing two unsaturated fats, Ferramosca et al. (73) fed 5-week old male ICR mice diets rich in either corn oil or olive oil for 8 weeks, and found that olive oil increased hepatic triglyceride content by 2.6 fold compared with corn oil.

Bargut et al. (74) showed strong suppressive effects on hepatic steatosis by fish oil compared with lard in male C57BL/6 mice during 8 weeks of treatment, even when diets contained 50% energy from fat. In parallel, hepatic expression of the lipogenic enzyme fatty acid synthase (FAS) and transcription factor sterol regulatory element-binding protein 1c (SREBP-1c) were upregulated by lard but downregulated by fish oil. In line with this, Tandy et al. (75) reported that krill oil (rich in n-3 as phospholipids rather than triglycerides) markedly reduced liver fat compared with butter in male C57BL/6 mice fed high-fat diets for 8 weeks, in conjunction with strong reductions in hepatic expression of FAS, acetyl-CoA carboxylase (ACC), SREBP-1c and SCD. Applying the reverse approach, Pachikian et al. (76) showed that removing n-3 PUFA from the diet resulted in markedly higher liver fat content in male C57BL/6J mice fed for 3 months with otherwise similar diets.
On the contrary, and in the context of a low-fat diet (15 E%), Sealls et al. (77) found no differential effects by canola oil and lard on liver fat content in 4-week old male C57BL/6 mice fed for 8 weeks. Likewise, hepatic expression of FAS, SCD, SREBP1, peroxisome proliferator-activated receptor (PPAR) alpha and PPAR gamma were similar by canola oil and lard.

In conclusion, the available animal studies rather consistently show that PUFA (both n-3 and n-6) can have strong suppressive effects on liver fat accumulation compared with SFA. There are also indications that some SFA may be worse than others, e.g. palmitic acid appear to be more deleterious than stearic acid.

Human studies

Bjermero et al. (78) randomized 67 abdominally obese men and women to a 10-week isocaloric diet high in either SFA (mainly butter) or n-6 PUFA (mainly sunflower oil and margarine). No difference in body weight was observed between groups, and subjects remained weight-stable, but SFA increased and PUFA decreased liver fat content. Liver fat was assessed both by MRI and magnetic resonance spectroscopy (MRS), and results were consistent regardless of method. Changes in liver fat content were inversely associated with changes in linoleic acid in plasma, and directly with changes in palmitic acid in plasma. This is the first intervention study in humans showing differential effects on liver fat in an isolated comparison of n-6 PUFA and SFA. When it comes to n-3 PUFA, several supplementation studies have been performed, with fairly consistent and positive results (79). In a pilot study, Capanni et al. (80) treated 42 patients with non-alcoholic fatty liver disease (NAFLD) with 1-g capsules of EPA and DHA for 12 months, and compared the effects with 14 controls not receiving n-3 PUFA. No changes in weight occurred, but n-3 PUFA decreased liver enzymes and liver fat content as assessed by ultrasonography; 64% of patients regressed from steatosis to normal, or from higher to lower degree of steatosis, compared with 0% of the controls.

Zhu et al. (81) randomized 144 patients with NAFLD to receive 6 g n-3 PUFA or placebo per day for 24 weeks. Body weight remained stable but fatty liver (as assessed by ultrasonography) was improved to a greater extent by n-3 PUFA. Further, Spadaro et al. (82) randomized 40 patients with NAFLD to receive two 1-g capsules of n-3 PUFA per day or placebo for 6 months, in the context of a hypocaloric background diet. Both groups decreased in weight, but n-3 PUFA caused a larger decrease in liver enzymes and liver fat content as assessed by ultrasonography. In another pilot trial, Tanaka et al. (83) treated 23 patients with NASH with 2.7 grams of EPA for 12 months. Body weight remained stable but liver enzymes and liver fat content decreased as assessed by ultrasonography (and liver biopsies in a sub-group).
In a double-blinded, randomized cross-over study, Cussons et al. (84) treated 25 obese women with 4 g n-3 PUFA per day or placebo for 8 weeks. Body weight did not change but n-3 PUFA decreased liver fat content as assessed by MRI, this effect was seen primarily in women with hepatic steatosis compared with women with normal liver fat content.

Nobili et al. (85) randomized 60 children with NAFLD to receive either 250 mg DHA/day, 500 mg DHA/day or placebo for 6 months and found that DHA decreased liver fat content (as assessed by ultrasonography) compared with placebo, but there were no difference between the two doses of DHA. Nobili et al. (86) also showed that these changes persisted when the treatments were continued up to 24 months.

Scorletti et al. (87) randomized 103 subjects with NAFLD to receive 4 g of n-3 PUFA per day or placebo for 18 months. In the primary, fully-adjusted intention-to-treat analysis, liver fat decreased by 3.64% (as assessed by MRS) by n-3 PUFA compared with placebo, but the difference was not statistically significant (P=0.1). However, DHA enrichment in erythrocytes was inversely associated with changes in liver fat. Scorletti et al. (88) also showed that the effects on liver fat content by DHA were modulated by the PNPLA3 gene variant.

To conclude, the available studies are indeed of variable quality with different durations, study populations and amount of n-3 PUFA provided, and several were either not randomized, blinded or had an appropriate control group. Despite these shortcomings, there seems to be a clear positive effect of n-3 PUFA on liver fat content.

Observational studies
Tiikkainen et al. (89) observed that intake of SFA was cross-sectionally associated with liver fat content (r=0.45, P=0.05) in a small group (n=23) of obese women participating in an intervention trial. In line with this, Petit et al. (90) reported that the proportions of palmitic acid and total SFA in erythrocytes were higher in patients with steatosis (n=109) compared with patients not having steatosis (n=53). Further, the proportion of 16:1n-7 and the SCD1-index were also higher in steatosis, whereas proportion of DHA and total PUFA were lower. This is also supported by Kotronen et al. (91), showing that livers of subjects with NAFLD contain less PUFA compared with subjects not having NAFLD. Further, the degree of unsaturation of liver triglycerides was strongly inversely associated with liver fat content whereas both the SCD1-index and the lipogenic index (16:0/18:2n-6) were strongly positively associated. The positive association between the SCD1-index in human livers and liver fat content was replicated by Peter et al. (92) in a larger patient population, but surprisingly there was no correlation for SCD1 mRNA expression.
taken together, unsaturated fat (primarily PUFA) seems beneficial compared with saturated fat with regard to liver fat accumulation. However, one must also bear in mind that efficient sequestration of fatty acids into triglycerides is a protective mechanism, preventing the formation of more reactive lipid intermediates (93), i.e. the increased triglyceride deposition may not necessarily always be deleterious, at least not in the acute or short-term perspective.

Potential mechanisms

Lipids as an internal language

Lipids and fatty acids are not merely carriers of energy but can also exert profound effects on various biochemical pathways by acting as signaling molecules. The composition of dietary fat will therefore modulate the overall biochemical message received by the body after a meal. Fatty acids can bind to nuclear receptors (e.g. the PPARs) or interact with several transcription factors (e.g. carbohydrate-responsive element-binding protein [ChREBP] and the SREBPs) thereby directly affecting gene transcription (94). Further, fatty acids can also bind to certain membrane-bound receptors (e.g. the G-protein coupled receptors (GPRs), giving rise to intracellular signaling cascades modifying e.g. transcription factors. Lipid mediators able to modulate signaling events may also be produced within the cell. Figure 7 provides an illustration of the concept. Adding to the complexity of signaling, complex lipids with clear signaling effects may also be endogenously synthesized in a tissue-specific manner in response to metabolic events, broadcasting the metabolic state in one tissue to others (95).

Figure 7. Illustration of the role of fatty acids as signaling molecules
Lipogenesis
Lipogenesis refers both to the processes of synthesizing fatty acids (DNL) and to the processes of triacylglycerol synthesis and storage. The amount of fatty acids available for triacylglycerol synthesis depends both on DNL and import of fatty acids from the bloodstream. DNL may be stimulated by low-fat diets by increased substrate availability and insulin signaling, but may also be differentially regulated by dietary fatty acids as reviewed in the following sections. Figure 8 provides a simplified overview of the lipogenic machinery and some of the important regulators.

![Simplified overview of the lipogenic machinery and some of the main regulators.](image)

**Figure 8.** Simplified overview of the lipogenic machinery and some of the main regulators. When glucose has been metabolized through glycolysis to pyruvate, it can be transported into the mitochondria to generate acetyl-CoA. Acetyl-CoA is the building block of fatty acids, which are synthesized in the cytosol. Acetyl-CoA is transferred to the cytosol by conversion into citrate. Acetyl-CoA can then be converted to malonyl-CoA by the enzyme ACC, and malonyl-CoA is used by the enzyme FAS to generate palmitic acid. Palmitic acid can be directly incorporated into triacylglycerols or be further metabolized by elongases or desaturases (e.g. SCD) before being incorporated into triacylglycerols. Fatty acids may also be incorporated into triacylglycerols by uptake through various membrane-bound transporters (e.g. FATP and CD36).

Zara et al. (96) showed that male Wistar rats fed with a safflower-enriched diet (15 E% fat) for 4 weeks had significantly lower (by 50%) transport activity of the hepatic mitochondrial citrate carrier (CIC). The protein and
mRNA levels were also decreased, by 30-35%. In conjunction, the activities of acetyl-CoA carboxylase (ACC) and FAS were also decreased after safflower oil. Similar results were observed by Siculella et al. for both n-6 PUFA (97) and n-3 PUFA (98) compared with beef tallow and olive oil and by Giudetti et al. (99) for fish oil compared with coconut oil. The activity of the citrate carrier is important for lipogenesis (100), as it provides cytosolic acetyl-CoA units needed for lipogenesis. Ferramosca et al. (73) investigated the effect on the hepatic citrate carrier by feeding 5-week old male ICR mice diets rich in either corn oil or olive oil for 8 weeks. Mice fed corn oil had significantly lower activity of the hepatic citrate carrier and lower protein levels compared with mice fed olive oil, where the activity and protein level were unchanged. Also, the hepatic activity of ACC and FAS were significantly reduced by corn oil but unchanged by olive oil. Further, Ferramosca et al. (67) showed that n-3 PUFA (krill oil) strongly suppressed the mitochondrial citrate carrier also within a background high-fat diet based on lard in male Sprague-Dawley rats. Again, this was accompanied by reductions in both ACC and FAS. Taken together, these results imply that n-3 and n-6 PUFA are more potent in suppressing hepatic lipogenesis compared with MUFA and SFA.

Shillabeer et al. (101) showed that male Sprague-Dawley rats fed a high-fat diet (45 E% fat) based on beef tallow had significantly higher hepatic mRNA levels of FAS, compared with rats fed an isoenergetic diet based on safflower oil. Similarly, Kabir et al. (64) showed that the hepatic activity of FAS, glucose-6-phosphate dehydrogenase and pyruvate kinase were significantly higher in rats fed palm oil compared with safflower oil, linseed oil and perilla oil.

An early study by Clarke et al. (102) showed that addition of 3% methyl linoleate to a fat-free diet for 7 days suppressed hepatic fatty acid synthesis, in conjunction with reduced levels of FAS and ACC in Sprague-Dawley rats. In contrast, addition of 8% methyl stearate had no effect on any of these parameters. Clarke et al. (103) repeated these findings in a similar study, and also reported that methyl palmitate had no suppressing effect on hepatic fatty acid synthesis. These results by Clarke et al. were soon replicated by Triscari et al. (104), showing that hepatic lipogenesis was dose-dependently suppressed in female CD rats by diets containing corn oil, but not by equivalent diets containing SFA (hydrogenated soybean oil). Similarly, Herzberg et al. (105) demonstrated that hepatic lipogenesis, and activities of FAS and other lipogenic enzymes, were significantly more suppressed by corn oil compared with tripalmitin in male C57BL/6J mice fed for 8 days. Corn oil was more effective than tripalmitin at all dietary fat levels tested (between 50 and 250 g fat/kg diet).

Clarke et al. (106) fed male Sprague-Dawley rats a moderate fat diet (20 E% fat) containing safflower oil, fish oil (menhaden) or tripalmitin for 8 days and found that both safflower oil and fish oil strongly suppressed hepat-
ic abundance of FAS mRNA compared with tripalmitin, which was not different from the fat-free control diet. Applying the reverse approach, Pachikian et al. (76) showed that removing n-3 PUFA from the diet resulted in increased expression of hepatic SREBP-1c, FAS and SCD in male C57BL/6J mice fed for 3 months with otherwise similar diets. In concordance, Pettinelli et al. (107) showed that livers of obese patients are depleted in n-3 PUFA in conjunction with higher expression of SREBP-1c and FAS compared with lean subjects.

Comparing unsaturated fats, Ronis et al. (69) demonstrated that corn oil was more effective than olive oil (5 E% fat) in reducing hepatic lipogenesis during 21 days of total enteral overfeeding in male Sprague-Dawley rats. The hepatic expressions of three fatty acid transporters (FATP-2, FATP-5 and CD36) were lower after corn oil than olive oil. Furthermore, the hepatic expression of FAS, ACC and SCD were lower after corn oil compared with olive oil. Similar results were found for SREBP-1c and ChREBP expressions. The suppressive effect of PUFA on lipogenesis appear to occur rather rapidly, as Xu et al. (108, 109) showed that both n-3 and n-6 PUFA strongly suppressed the hepatic mRNA and protein levels of SREBP-1c (and FAS) compared with triolein after only 5 meals in male meal-trained Sprague-Dawley rats. Also, Levy et al. (66) showed that the postprandial hepatic mRNA expression of SREBP-1c and FAS were completely blunted in Fischer 344 rats fed fish oil compared with lard, implying less activation of lipogenesis despite the newly-fed condition. Further support of the rapid effects were provided by Dentin et al. (110) who refed 24-h-fasted male C57BL/6J mice a fat-free diet or the same diet supplemented with either triolein or PUFA for 18 hours and found that the hepatic mRNA levels of ChREBP were suppressed by 64% by PUFA but not by triolein. In parallel, the expression of SREBP-1c and FAS were decreased by PUFA but not by triolein. Interestingly, cytosolic levels of ChREBP were unaffected but PUFA totally prevented the nuclear translocation. Additional experiments in cultured hepatocytes showed that linoleic acid, EPA and DHA strongly suppressed ChREBP mRNA whereas stearate and oleate had no effect. Linoleic acid was found to accelerate the rate of mRNA decay (of both ChREBP and SREBP-1c) and shortened the half-life of ChREBP mRNA by ~50%, whereas neither stearate nor oleate had any effect.

In another early study, Reiser et al. (111) fed male albino rats high-fat diets based on various fatty acids/dietary fats for 2 weeks and assessed hepatic lipogenesis by injecting radiolabeled acetate. The recovery of the tracer in hepatic fatty acids (as a measure of de novo synthesized fatty acids) was higher for lard, butter oil and tripalmitin compared with safflower oil and trilinolein. Similarly, Sabine et al. (112) fed male C57BL/J mice diets containing corn oil, safflower oil, coconut oil, tripalmitin, oleic acid and triolein for 3 days and assessed hepatic lipogenesis by incubating liver slices with
radiolabeled acetate. A suppressive effect (compared with a fat-free diet) on hepatic lipogenesis was seen only by corn oil and safflower oil, the other fats had no effect. Further, Bartley et al. (113) fed male and female Long-Evans rats and C3H mice diets containing tripalmitin, triolein or safflower oil for 5 days and estimated the hepatic lipogenesis by measuring the incorporation of labeled acetate into fatty acids in liver slices. Safflower oil clearly showed the lowest conversion of acetate into fatty acids, in both rats and mice. This was paralleled by decreased activities of lipogenic enzymes (e.g. FAS).

Flick et al. (114) showed that safflower oil (15 weight%) markedly decreased hepatic FAS activity compared with coconut oil in male Sprague-Dawley rats when switched from a fat-free diet to the fat-enriched diet. The results confirmed earlier observations by Allmann et al. (115) who fed male C57BL/6J mice a normal low-fat diet, a fat-free diet and the same fat-free diet supplemented with various fatty acids and oils and found that addition of either linoleate or corn oil to the fat-free diet strongly suppressed hepatic FAS activity whereas addition of coconut oil, palmitate or oleate had no suppressive effects.

Toussant et al. (116) fed male Sprague-Dawley rats moderate fat diets (~20E%) containing either safflower oil, beef tallow or tripalmitin for 7 days, and found that hepatic fatty acid synthesis and ACC were significantly lower by safflower oil compared with both beef tallow and tripalmitin. The same results were observed when the diets only contained ~10E% fat and were fed for 28 days. The suppressing effect of safflower oil compared with beef tallow on hepatic fatty acid synthesis and FAS has also been shown in male Sprague-Dawley rats rendered diabetic by streptozotocin (117). Finally, Sanz et al. (46) showed that the hepatic activity of FAS was significantly lower by sunflower oil than beef tallow also in chickens.

In contrast, Sealls et al. (77) found no differential effects by canola oil and lard on hepatic expression of FAS, SCD, SREBP1, PPAR alpha and PPAR gamma in male C57BL/6 mice fed a relatively low-fat diet (15 E% fat) for 8 weeks. Further, some studies in mice (118-120) have shown that linoleic acid (compared with SFA) could increase endocannabinoids and thereby induce lipogenesis and weight gain. However, this seems to be dependent on the conversion of linoleic acid to arachidonic acid, which does not seem to occur in humans despite large changes in linoleic acid (121).

A general conclusion from the studies described above is that high-fat diets based on SFA are inefficient in suppressing lipogenesis compared with high-fat diets based on PUFA.

**Fat oxidation, thermogenesis and energy metabolism**

Fatty acids are sequentially degraded in 2-carbon units (acetyl-CoA) in a multistep process called beta-oxidation, which occurs inside the mitochon-
Fatty acids cannot pass the mitochondrial membrane by themselves but need to be transported. This is done by the enzyme carnitine palmitoyltransferase (CPT) and is considered to be the rate limiting step in fatty acid oxidation. The generated acetyl-CoA units can then enter the citric acid cycle, where carbon dioxide (CO₂) is generated. The implication of this is that fatty acid oxidation can be assessed by measuring the release of CO₂.

The citric acid cycle also generates electron carriers, transporting electrons to the ‘electron transport chain’ residing in the mitochondrial membrane. The flow of electrons through this chain of enzymes produces an electrochemical gradient over the mitochondrial membrane by exporting protons. The proton gradient can be decreased by letting the protons back in again via an ATPase-enzyme in the membrane, simultaneously producing ATP. However, in brown adipose tissue there is another enzyme in the mitochondrial membrane called uncoupling protein-1 (UCP1), which lets the protons back in again without producing ATP; the energy instead being released as heat. A simplified overview of the fatty acid oxidation pathway and thermogenesis is shown in Figure 9.

The generation of energy from dietary substrates requires oxygen, and the metabolism of fatty acids requires more oxygen compared with carbohydrates. One implication of this is that metabolism can be quantified by measuring the ratio of CO₂ production to oxygen consumption (the respiratory quotient, RQ), which is lower for fat than for carbohydrates. Therefore, a decreased RQ or increased oxygen consumption indicates higher fat oxidation.

![Figure 9. Simplified overview of fatty acid oxidation and thermogenesis.](image-url)
**Animal studies**

Shimomura et al. (31) observed that postprandial oxygen consumption was higher, and the RQ lower, when rats were fed safflower oil compared with beef tallow, indicating that diet-induced thermogenesis and fat oxidation were higher after safflower oil. Similarly, Matsuo et al. (122) showed that postprandial oxygen consumption was lower after beef tallow compared with safflower oil, i.e. diet-induced thermogenesis was higher after safflower oil. The reduced thermogenesis by beef tallow seems to be due to reduced sympathetic activity as chemical sympathectomy almost completely abolished the differential effects of beef tallow and safflower oil. Further, Takeuchi et al. (36) reported that postprandial oxygen consumption was lower after lard compared with safflower oil, high oleic acid safflower oil and linseed oil, indicating lower diet-induced thermogenesis. They also found that norepinephrine turnover in brown adipose tissue was significantly lower after lard compared with the other three groups, indicating decreased sympathetic activity. From a repeated experiment, Takeuchi et al. (123) also reports that serum triiodothyronine was significantly lower after lard compared with the other three groups. Furthermore, as a general measure of energy metabolism, the activity of the sodium pump (Na⁺K⁺-ATPase) was significantly lower (~40%) in the liver and skeletal muscle after lard compared with the three other groups.

Matsuo et al. (124) showed that 9 weeks of feeding a beef tallow-based diet in 5-week old male Sprague-Dawley rats resulted in lower content of mitochondrial uncoupling protein (UCP1) in brown adipose tissue, compared with isoenergetic feeding of a safflower oil-based diet. Additionally, Matsuo et al. (33) repeated this finding in male Wistar rats showing that UCP1 mRNA level and UCP1 content in brown adipose tissue was lower after beef tallow compared with both safflower oil and soybean oil. The potential of n-6 PUFA to increase UCP1 have been indicated earlier by Nedergaard et al. (125), showing that a mixture of sunflower seed oil and linseed oil (10 E% 18:2n-6+18:3n-3) increased mitochondrial UCP1 content in 6-week old male and female Sprague-Dawley rats (compared with 3 E% 18:2n-6+18:3n-3). Further, Sadurskis et al. (126) fed 6-week old male NMRI mice high-fat diets (20E% fat) with either a normal PUFA-content (4 E% fat) or high PUFA-content (10 E% fat) for 2 weeks. Food intake and body weight gain were similar between groups, but mice fed the high PUFA diet had a greater thermogenic capacity and a 40% higher content of UCP in brown adipose tissue compared with mice fed the normal PUFA diet. Interestingly, Ferramosca et al. (67) showed that addition of n-3 PUFA (krill oil) strongly up-regulated (3-fold) CPT-1 activity also within a high-fat (35 E% fat) background diet based on lard in male Sprague-Dawley rats fed for 12 weeks.

Levy et al. (66) showed that the postprandial decrease in hepatic mRNA expression of PPARα and CPT1 were blunted in Fischer 344 rats fed with
fish oil compared with lard, implying less inhibition of fatty acid oxidation despite being in postprandial condition. Applying the reverse approach, Pachikian et al. (76) showed that removing n-3 PUFA from the diet resulted in lower hepatic fatty acid oxidation, as evidenced by decreased expression of PPARα and decreased carbon dioxide production in liver slices incubated with palmitate, in male C57BL/6J mice fed for 3 months with otherwise similar diets. In concordance, Pettinelli et al. (107) showed that livers of obese patients are depleted in n-3 PUFA in conjunction with lower expression of PPARα and CPT-1 compared with lean subjects.

Kabir et al. (64) observed that hepatic fatty acid oxidation (both mitochondrial and peroxisomal) was significantly lower in rats fed palm oil compared with safflower oil, linseed oil or perilla oil. Congruently, Mercer and Trayhurn (40) demonstrated that lean and obese (ob/ob) mice fed with corn oil had substantially greater mitochondrial mass and oxidative capacity of brown adipose tissue compared with mice fed beef tallow.

Cenedella et al. (127) showed that oxidation (as assessed by respiratory CO₂) of linoleic acid was about twice as high as palmitic acid in rats fed labeled fatty acids. Cenedella also showed that both liver and muscle oxidation was about twice as high for linoleic acid compared with palmitic acid. Yet another early study by Dupont et al. (128) showed that the postprandial cumulative recovery of labeled carbon in exhaled air was highest for linoleic acid, followed by oleic acid, followed by stearic acid in rats intraperitoneally injected with labeled fatty acids. Finally, Leyton et al. (129) showed that linoleic, linolenic and oleic acid were all oxidized at a significantly faster rate than palmitic and stearic acid in female Sprague-Dawley rats, as assessed by the recovery of the exhaled tracers during 24 hours.

By using the same semistarvation-refeeding model as Dulloo et al. (37) and Yepuri et al. (38), Crescenzo et al. (130) showed that refeeding with a high-fat diet based on safflower oil (compared with an isoenergetic lard-based diet) resulted in a higher hepatic mitochondrial proton leak, i.e. greater mitochondrial uncoupling. This was suggested to be due to increased incorporation of arachidonic acid in the inner membrane of liver mitochondria.

Adding some nuance regarding SFA, Van den Berg et al. (44) showed that energy expenditure and fat oxidation rate was significantly lower by lard and palm oil with added stearate compared with palm oil alone in C57Bl/6J mice fed ad libitum for 5 weeks.

In contrast, Rodriguez et al. (131) fed (ad libitum) male Wistar rats high-fat diets (40 E% fat) based on either olive oil, sunflower oil, palm oil or beef tallow for 4 weeks, and found that olive oil caused the highest level of UCP1 mRNA in brown adipose tissue, and that UCP1 mRNA were not different in rats fed sunflower oil, palm oil or beef tallow. Olive oil also caused the highest levels of UCP2 and UCP3 in brown adipose tissue, and the other three groups were not different from each other. Further, rats fed olive oil had the highest mRNA level of UCP3 in skeletal muscle, and the other three
groups were not different from each other. Importantly though, the increased mRNA levels of UCP in brown adipose tissue were not accompanied by increased UCP protein levels. However, total body oxygen consumption was significantly higher in rats fed olive oil than in rats fed the other diets.

Kawada et al. (132) compared the effects of high-fat diets (27 E% fat) based on fish oil (bonito), lard, linseed oil or perilla oil on UCP1 expression in brown adipose tissue in 4-week old male Sprague-Dawley rats ad libitum-feb for 9 weeks. UCP1 protein levels were increased by fish oil compared with the three other diets, which did not differ from each other. Further, Takahashi et al. (133) fed 6-week old male Sprague-Dawley rats high-fat diets (20 E% fat) containing palm oil or sunflower oil for 3 weeks, and observed no differences in UCP1 mRNA levels in brown adipose tissue between groups.

Human studies
Kien et al. (51) fed healthy young adults liquid formula diets (40 E% fat) for 4 weeks. Diets differed only with regard to fatty acid composition, one being high in palmitic acid (16.8 E%) and one being low in palmitic acid and high in oleic acid (31.4 E%). The rate of fat oxidation was higher during the oleic acid-rich diet and the daily energy expenditure was considerably decreased during the palmitic acid-rich diet. Kien et al. (134) complemented this study with an acute study, feeding 20 healthy adults every 2 hours during a 14-hour period and found no differences in substrate oxidation between high palmitic acid or oleic acid diets, implying that differential effects on fat oxidation requires longer time to occur. Kien et al. (135) proceeded to investigate potential gender effects, and found that the increased fatty acid oxidation (both in the fed and fasted state) during the oleic acid-rich diet were statistically significant in women only. However, the decreased daily energy expenditure during the palmitic acid-rich diet was statistically significant in men only. Kien et al. (136) further showed that resting energy expenditure in both fasted and fed states was higher during a diet rich in oleic acid (28.8 E%) compared with palmitic acid (16.0 E%), in two 3-week crossover studies all foods were provided.

A small randomized crossover study by van Marken Lichtenbelt et al. (137) compared the effects of diets with a high (1.67) or low (0.19) (P:S) ratio for 14 days in 6 male subjects, and found that both resting metabolic rate and diet-induced thermogenesis were significantly higher after the diet with a high P:S ratio.

Piers et al. (138) demonstrated a significantly higher postprandial fat oxidation in 14 healthy males after a high-fat breakfast meal (43 E%) based on olive oil compared with an isoenergetic meal based on dairy cream, as assessed by indirect calorimetry during 5 hours after the meal. Soares et al. (139) repeated this experiment in 12, predominantly obese, postmenopausal women and found similar results. In a sub-analysis based only on the obese
subjects, diet-induced thermogenesis was also higher after olive oil compared with cream. Similarly to Piers, Casas-Agustench et al. (140) compared the effects of three isoenergetic high-fat breakfast meals (~52 E% fat) containing either walnuts, olive oil or cheese and butter on thermogenesis and substrate oxidation in 29 healthy men. Diet-induced thermogenesis was ~25% higher after the walnut and olive oil meals compared with the dairy meal, but postprandial fat or carbohydrate oxidation were not significantly different between meals.

DeLany et al. (141) compared the extent of oxidation of individual fatty acids in 4 normal-weight healthy men by giving meals with labeled fatty acids and measuring the recovery of the label (as CO₂) in exhaled air. The cumulative oxidation 9 hours after the meal was higher for linolenic, linoleic and oleic acid than for palmitic and stearic acid. Interestingly, the highest cumulative oxidation was found for lauric acid. Using a similar methodology, Schmidt et al. (142) also showed, in a cross-over study, that oleate was oxidized to a greater extent than palmitate in 10 healthy adults fed small frequent meals during 7 hours. Similarly, Jones et al. (143) showed that postprandial oxidation of both oleic acid and linoleic acid were markedly higher than for stearic acid, by feeding labeled fatty acids, mixed in a high-fat meal (40 E% fat), to 6 healthy males and measuring the recovery of labeled carbon in exhaled CO₂ during 9 hours after the meal. Jones et al. (144) proceeded in a cross-over study to compare the effects of three unsaturated oils on substrate oxidation and energy expenditure by using indirect calorimetry in 15 healthy males receiving high-fat breakfast meals (60 E% fat) based on either olive oil, sunflower oil or flaxseed oil. No differential effects on postprandial fat or carbohydrate oxidation were found, but olive oil increased total energy expenditure compared with the other oils.

In contrast, Cooper et al. (145) found no difference in average 24-h energy expenditure by high-fat diets (50 E%) based on either SFA or MUFA in 8 healthy men residing in a metabolic chamber for 5 days. Further, Piers et al. (53) found no differences in resting metabolic rate, diet-induced thermogenesis and fasting- and postprandial fat oxidation after 4 weeks of a MUFA-rich diet compares with a SFA-rich diet. However, the study population consisted of only 8 men (although in a crossover design). Counterintuitively, Jones et al. (146) showed that a diet with a low PUFA:SFA (P:S) ratio (created with lard and safflower oil), fed to 8 males for 7 days, resulted in a lower respiratory quotient and a higher basal fat oxidation rate compared with a diet with a high P:S ratio. However, the high P:S ratio diet caused a higher cumulative postprandial fat oxidation compared with the low P:S ratio diet.

Lovejoy et al. (147) performed a double-blind randomized crossover study in 25 healthy men and women, comparing the effects of diets enriched in either palmitic or oleic acid on substrate oxidation. All foods were provided and each treatment lasted for 4 weeks. No differential effects on substrate oxidation were observed between groups. However, the diets were relatively
low in total fat (28 E%) and the percent energy from SFA and MUFA in the respective diet were surprisingly low, potentially affecting the results.

Gillingham et al. (56) observed no differences in fasting or postprandial energy expenditure or substrate oxidation (assessed by indirect calorimetry) in a randomized cross-over study in 34 overweight women, comparing diets enriched in oleic acid or alpha-linolenic acid with a ‘Western diet’ containing higher amounts of SFA. Each diet was tested for 28 days and all food was provided. Further, Clevenger et al. (148) found no differential effects on diet-induced thermogenesis or postprandial substrate oxidation in 16 healthy obese women fed liquid high-fat breakfast meals (70 E% fat) based on either SFA, MUFA or PUFA. Clevenger et al. (149) also tested the same protocol in 15 premenopausal normal-weight women, and found that PUFA significantly increased diet-induced thermogenesis during the 5-hour postprandial period compared with MUFA and SFA, but substrate utilization was not different between meals.

Taken together, unsaturated fatty acids seem to be oxidized at a higher rate and stimulate fat oxidation and energy expenditure compared with long-chain SFA. This is most compellingly shown in animal studies, whereas results from human studies involve more discrepancies. Firm conclusions regarding differential effects of dietary fatty acids on fat oxidation and energy expenditure in humans should be drawn with caution due to the conflicting data.

**Lean tissue and protein synthesis**

Positive or negative changes in skeletal muscle mass are dependent on protein balance, i.e. the net effect of protein breakdown and protein synthesis. The metabolic pathways underlying protein synthesis are yet to be fully elucidated, but one central “signaling hub” is the kinase mechanistic target of rapamycin (mTOR), as illustrated in Figure 10.

![Figure 10](image-url)

*Figure 10. The enzyme mTOR is central for integrating various signals affecting protein synthesis*
Smith et al. (150) evaluated the effect of 8-week supplementation of high doses of EPA and DHA (4 g Lovaza/day) on muscle protein synthesis (using isotope-labelled tracers) in 9 young and healthy but inactive men and women. Muscle protein synthesis was measured both in the fasted state and during a hyperinsulinemic-hyperaminoacidemic clamp. The protein concentration and the protein/DNA ratio (a measure of cell size) in muscle biopsies increased after n-3 PUFA. Fasting muscle protein synthesis was not affected by n-3 PUFA, but the anabolic response during the clamp was ~50% greater after n-3 PUFA compared with before supplementation. In conjunction, n-3 PUFA increased the activation of the mTOR signaling pathway. Noteworthy is that this study did not include a control group, and the amount of n-3 PUFA provided was very high.

Smith et al. (151) also investigated the same intervention in 16 healthy older (age >65 years) men and women, but this time half the subjects received corn oil capsules thus acting as a control group. Again, n-3 PUFA had no effect on the basal rate of muscle protein synthesis but increased the anabolic response during the clamp. The increased activation of the mTOR signaling pathway was seen also in this study. The increase in mTOR by dietary fish oil supplementation is supported by McGlory et al. (152), who also showed that 2 weeks seems to be enough to reach maximal increases in mTOR.

Tardif et al. (153) tested the effects of palmitic and oleic acid (physiological concentration) on protein synthesis in C2C12 myotubes, and found that palmitic acid decreased protein synthesis by 30%, whereas oleic acid had no inhibitory effect. Addition of palmitic acid doubled the content of ceramides, whereas oleic acid had no effect on ceramide content compared with control. Furthermore, when ceramide synthesis was blocked, palmitic acid had no inhibitory effect on protein synthesis, and addition of ceramides recapitulated the inhibitory effects on protein synthesis observed after addition of palmitic acid. This study implies that palmitic acid can directly inhibit protein synthesis in muscle cells, by the generation of ceramides. Similarly, Woodworth-Hobbs et al. (154) treated cultured C2C12 myotubes with palmitate, DHA or palmitate and DHA combined and found that palmitate increased proteolysis by 31%. Palmitate also suppressed the activity of Akt and upregulated proteolytic systems. These responses were rescued by cotreatment with DHA. Further, Bryner et al. (155) treated cultured C2C12 myotubes with palmitate or DHA and found that palmitate decreased myotube diameter whereas DHA instead increased it. Concordantly, You et al. (156) showed that a fish oil-enriched diet could partly counteract skeletal muscle atrophy in male Sprague-Dawley rats subjected to 10 days of hindlimb immobilization, possibly due to maintained Akt activation and reduced proteolysis. Furthermore, Bergeron et al. (157) showed that a fish-oil enriched diet decreased amino acid oxidation and increased body protein accretion in male neonatal piglets. Finally, Liu et al. (158) compared the effects of diets...
containing corn oil or fish oil for 21 days in piglets and found that fish oil increased muscle protein mass in conjunction with increased Akt activity and decreased proteolytic systems.

Gingras et al. (159) showed that abomasal infusion of fish oil in growing steers for 35 days increased amino acid disposal and decreased amino acid oxidation compared with control oil, effects associated with increased activation of Akt and mTOR. Interestingly, the lipid metabolite phosphatidic acid is an activator of mTOR and it has been shown that phosphatidic acid needs to be composed of at least one unsaturated fatty acid in order to stimulate mTOR (160, 161). Whether mTOR can distinguish between different unsaturated fatty acids (i.e. n-3 and n-6 PUFA) remains to be determined.

Collectively, n-3 PUFA seems to have clear modulating effects on muscle protein synthesis, also in humans. The underlying mechanisms are unclear, and whether the same stimulatory effects can be seen also for other PUFAs (e.g. n-6 PUFA) remains to be investigated.

**Physical activity**

Physical activity can exert strong modulatory effects on body composition, both by increasing lean tissue and decreasing fat mass.

Kien et al. (51) found no difference in physical activity in young healthy subjects fed with liquid formula diets high in either palmitic acid or oleic acid for 4 weeks. However, physical activity was only assessed during 7 days using a uniaxial accelerometer. Kien et al. (136) proceeded to investigate this more thoroughly using two separate crossover studies. Study 1 consisted of 18 healthy normal weight men and women, and study 2 included 12 healthy normal weight to obese men and women. The dietary intervention was identical in both studies, and consisted of 3-week periods of a diet high in palmitic acid (16.0 E%) or oleic acid (28.8 E%). Total fat content in both diets was 40 E% and all foods were provided. Physical activity was assessed continuously during the intervention by a biaxial accelerometer. In both studies, physical activity was significantly higher during the diet rich in oleic acid compared with palmitic acid. Mean physical activity was 12% and 15% higher by oleic acid in study 1 and 2, respectively. However, Wong et al. (162) showed a strong suppressive effect on spontaneous locomotor activity by corn oil compared with olive oil in 8-week old female C57/Bl6 mice fed high-fat diets (40 E% fat) for 6 weeks.

**Hypothalamic insulin resistance**

High insulin sensitivity is important not only in skeletal muscle, liver and adipose tissue but apparently also in the brain and hypothalamus. In the brain, insulin is anorectic and reduces food intake and body weight (163). Interestingly, intranasal insulin administration during 8 weeks has been
shown to decrease body weight and body fat in healthy human subjects (164). Further, Gancheva et al. (165) demonstrated in a cross-over study that a single dose of intranasal insulin transiently decreased liver fat content by 35% (as assessed by MRS) in lean healthy subjects, but not in subjects with type 2 diabetes. Also, brain insulin application has been shown to increase cerebrocortical activity and promote locomotor activity (i.e. physical activity), but that this effect is blunted in obesity due to insulin resistance (166), findings that have also been observed in humans (167).

Sartorius et al. (168) fed 4-week old male C57BL/6J mice diets containing either canola oil or milk fat for 8 weeks and found that the SFA-enriched diet impaired insulin sensitivity in the brain and decreased cerebrocortical activity compared with the MUFA-enriched diet. Further, an acute brain insulin application stimulated locomotion in MUFA-fed mice whereas this effect was blunted in SFA-fed mice. The same authors also fed 24 lean healthy subjects with yoghurt enriched with either milk fat or canola oil for 12 weeks, and observed that the SFA-enriched yoghurt decreased the intrinsic brain activity compared with the MUFA-enriched yoghurt as assessed by functional MRI.

Interestingly, Tschritter et al (169) observed that the ability of insulin to stimulate cerebrocortical activity in humans was negatively associated with BMI, VAT and liver fat content. Further, saturated but not unsaturated free fatty acids were strongly and independently inversely associated with insulin-mediated stimulation of cerebrocortical activity. Tschritter et al. (170) followed this up and found that higher cerebral insulin sensitivity at baseline correlated with greater loss of body fat and VAT during lifestyle intervention in overweight humans, and that dietary intake of SFA was strongly associated with cerebral insulin sensitivity.

Benoit et al. (171) have previously shown that palmitic acid (both direct infusion and oral gavage) impaired hypothalamic insulin (and leptin) signaling compared with oleic acid in rodents, and that these effects are mediated through protein kinase C. Inflammation may indeed be a causative mechanism underlying hypothalamic insulin resistance, and SFA compared with unsaturated fat has been shown to induce hypothalamic inflammation (172, 173).

In conclusion, fatty acids may not only have local signaling effects in target organs (e.g. binding to nuclear receptors in the liver) and behave differently regarding lipogenesis and oxidation, but may also affect central signaling pathways in the brain and thereby affecting both fat storage and behavior (e.g. physical activity).
Gut microbiota

The composition of the gut microbiota, and how that affects our health, has really stepped into the limelight during recent years. The degradation and production of molecules by the gut microbiota may have broad health effects, and the microbial composition in the gut has been associated with a myriad of health conditions, of which some may be causal.

Kishino et al. (174) showed that bacteria from the gut microbiota can generate specific types of fatty acids, in particular hydroxy and oxo fatty acids, from common dietary fatty acids such as linoleic acid. These particular fatty acids were also found in the plasma of mice. Goto et al. (175) subsequently showed that hydroxy and oxo fatty acids could activate PPARα and PPARγ. Finally, Nanthirudjanar et al. (176) showed that these microbial-derived fatty acids could markedly decrease the mRNA expression of SREBP-1c, SCD, FAS and ACC in cultured liver cells and also reduce triacylglycerol accumulation. Further, oral administration of one of the fatty acids to sucrose-fed mice resulted in decreased hepatic expression of SREBP-1c, SCD and ACC. Taken together, these results imply that species of the gut microbiota can synthesize rather potent signaling molecules from common dietary fatty acids, and that these fatty acids reach the circulation and may affect lipogenic signaling in the liver. Except for the production of particular fatty acids, there are other potential mechanisms for how the microbiota may affect liver fat content (177). Dietary PUFA can affect ectopic fat storage, but it remains to be shown how much of the effect, if any, that is mediated by the gut microbiota.

Interestingly, de Wit et al. (72) fed male C57BL/6J mice high-fat diets based on either palm oil, olive oil or safflower oil for 8 weeks and found that palm oil reduced the gut microbial diversity and induced a microbial pattern previously associated with overweight and metabolic disorders. Further, palm oil caused significantly higher levels of liver fat and body fat compared with safflower oil.

Further, Caesar et al. (178, 179) showed that metabolic differences between lard- and fish oil-fed mice could be partly ascribed to the gut microbial composition.

Collectively, the gut microbial composition appears to be of significance for the health effects of (at least some) dietary compounds. With the advent of better technology, the microbial composition of the host will probably have to be taken into account in the future in order to fully understand and explain the molecular mechanisms underlying dietary health effects.

LPL activity

Lipoprotein lipase (LPL) is an enzyme attached to the luminal surface in endothelial cells in capillaries, responsible for the hydrolysis of triglycerides in lipoproteins and thereby making the fatty acids available for uptake to the
tissues. The activity of LPL therefore modulates the amount of fatty acids available for tissue uptake. Differential effects on LPL activity of fatty acids could potentially dictate their distribution among organs and fat depots, which in turn could influence if they are oxidized or stored.

Shimomura et al. (31) reported that postprandial LPL activity in adipose tissue increased more after beef tallow compared with safflower oil, and that postprandial LPL activity in the heart decreased after beef tallow compared with safflower oil. LPL activity in muscle tended to be higher after safflower oil compared with beef tallow both pre- and postprandial but was not statistically significant. Further, Matsuo et al. (32) observed that both fasting and postprandial LPL activity in the heart and brown adipose tissue were higher after safflower oil compared with beef tallow. LPL activity in muscle followed the same pattern but was statistically significant only in fasting. The decreasing effect of beef tallow on LPL activity could be explained by reduced activity of the sympathetic nervous system, i.e. reduced content and turnover of norepinephrine in the tissues. Matsuo et al. (122) repeated this experiment also in chemically sympathectomized rats and found that sympathectomy abolished the differences in body fat accumulation between beef tallow and safflower oil treatment, suggesting that reduced sympathetic activity is an important mechanism for the fattening effect of SFA. Finally, Takeuchi et al. (123) reported that the activity of LPL in subcutaneous abdominal (but not intra-abdominal) adipose tissue was higher after lard compared with safflower oil, linseed oil and high oleic safflower oil.

**Overall conclusion regarding potential mechanisms**

In conclusion, the underlying mechanisms explaining the differential effects of fatty acids on body composition and ectopic fat accumulation have not been fully established. The differential effects on lipogenesis and fat oxidation are the most well investigated, and may be the most important, but several other mechanisms (both peripheral and central) are likely to be involved as well.
Aims

The overall aim of this thesis was to investigate the effects of dietary fatty acids on body composition and body fat distribution (including ectopic fat) in humans, with emphasis on the role of the omega-6 PUFA linoleic acid (18:2n-6) and the SFA palmitic acid (16:0). The overall hypothesis was that linoleic acid would be beneficial compared with palmitic acid during overfeeding.

Specific aims

- To investigate if sunflower oil (rich in linoleic acid) could decrease the accumulation of liver fat during overfeeding in young, lean and healthy subjects compared with palm oil (rich in palmitic acid) (Paper I).
- To investigate potential mechanisms underlying the effects in Paper I by using NMR-metabolomics (Paper II).
- To investigate the cross-sectional associations between circulating linoleic acid and palmitic acid and body fat content in 70-year-old men and women (Paper III)
- To investigate if the findings from Paper I could be replicated in overweight and obese subjects (Paper IV).
Subjects and Methods

Participants

Papers I and II – The LIPOGAIN study

Participants were recruited by advertisements in local newspapers and billboards. Fifty-five individuals were screened and 41 of those were randomized to PUFA or SFA groups. The randomized participants (13 women and 28 men) were between 20 and 38 years of age and had a BMI between 18 and 27 kg/m². Exclusion criteria included diabetes, liver disease and other relevant chronic diseases, abnormal clinical chemistry, medications influencing energy metabolism, pregnancy or lactation, use of omega-3 supplements and frequent heavy/intense exercise.

Paper III – The PIVUS cohort

Subjects participated in the PIVUS (Prospective Investigation of the Vasculature in Uppsala Seniors) cohort study. The primary aim was to investigate the predictive power of vascular function on cardiovascular disease. Persons aged 70 living in the community of Uppsala, Sweden, were invited. Between April 2001 and June 2004, 2025 subjects were randomly invited within one month of their 70th birthday in order to standardize for age, and 1016 (50.1%) chose to participate. MRI was performed in 287 randomly selected participants, which represents the current study population.

Paper IV – The LIPOGAIN-2 study

Participants were recruited by advertisements in local newspapers and billboards. Eighty-one individuals were screened and 61 of those were randomized toPUFA or SFA groups. The randomized participants (24 women and 37 men) were between 22 and 59 years of age and had a BMI between 19 and 39 kg/m² (the vast majority was overweight or obese). Exclusion criteria included diabetes, liver disease and other relevant chronic diseases, abnormal clinical chemistry, medications influencing energy metabolism, pregnancy or lactation, use of omega-3 supplements and frequent heavy/intense exercise.
Methods
Study designs and interventions

Papers I and II
The LIPOGAIN study was a 7-week, double-blinded, randomized, controlled trial with parallel group design in free-living subjects (see Figure 11). The study was carried out from August through December 2011 at the Uppsala University Hospital, Uppsala, Sweden. Subjects were randomized by drawing lots, with a fixed block size of 4 and allocation ratio 1:1. Subjects were stratified by sex, and were unaware of the block size. Subjects were randomized to eat muffins containing either sunflower oil (high in the major dietary PUFA, linoleic acid, 18:2 n-6) or palm oil (high in the major SFA, palmitic acid, 16:0). Body weight was measured and muffins were provided to participants weekly at the clinic. Muffins were baked in large batches under standardized conditions in a metabolic kitchen at Uppsala University. Muffins were added to the habitual diet, and the amount was adjusted to individual energy requirements to achieve a 3% weight gain. The amount of muffins consumed per day was individually adjusted weekly, i.e. altered by +/- 1 muffin/day depending on the rate of weight gain of the individual. Subjects were allowed to eat the muffins anytime during the day. Except for fat quality, the muffins were identical with regard to energy, fat, protein, carbohydrate, and cholesterol content, as well as taste and structure. The composition of the muffins provided 51% of energy from fat, 5% from protein, and 44% from carbohydrates. The sugar to starch ratio was 55:45. Except for the addition of muffins, subjects were instructed to maintain their habitual diet and physical activity level.

*Figure 11. Design of the LIPOGAIN study*
**Paper III**

Cross-sectional analyses were performed between fatty acid composition in serum cholesterol esters and total body fat content, visceral adipose tissue content and abdominal subcutaneous adipose tissue content. Participants filled in a lifestyle questionnaire including e.g. smoking status and education. Assessments of body fat content were performed on average two years after the baseline investigation.

![The PIVUS cohort illustration](image)

*Figure 12. Illustration of the PIVUS cohort including the subcohort that constitutes the study population of Paper III*

**Paper IV**

The LIPOGAIN-2 study was a 12-week, double-blinded, randomized controlled trial with parallel group design in free-living subjects (see Figure 13). The study was carried out from August 2014 through June 2015 at the Uppsala University Hospital, Uppsala, Sweden. Subjects were randomized by computer generated lists stratified by sex, age and BMI. During the first 8 weeks of the study subjects were overfed (intentional weight gain) and during the last 4 weeks of the study subjects were calorically restricted (intentional weight loss). The data included in this thesis presents results from the first 8 weeks (effects of overfeeding). Subjects were randomized to eat muffins of identical composition as in the first LIPOGAIN study, and the procedure was also identical.
**Figure 13.** Design of the LIPOGAIN-2 study

Assessment of body composition and fat depots

**Air displacement plethysmography (ADP; BodPod)**
Total body fat mass and total body FFM were assessed by whole-body air displacement plethysmography (COSMED). Subjects were measured in a fasting state wearing only their underwear, and a swim cap. Body density values were converted to percent body fat by using the Siri equation (180). Thoracic gas volume was predicted.

**Magnetic resonance imaging (MRI)**
Liver fat, pancreas fat, VAT, abdominal SAT, total adipose tissue and total lean tissue were assessed by MRI using a 1.5T Achieva clinical scanner (Philips Healthcare, Best, the Netherlands) modified to allow arbitrary table speed. Subjects were measured in a fasting state, in a supine position with their arms above their heads. Liver fat content was measured using a dedicated scan. The liver volume was delineated manually from the images using the software ImageJ (version 1.42q). Pancreas fat content was measured using the same scan as for liver fat. In Paper I, liver fat content was measured by two operators and the average was used. The coefficient of variation between the two operators was 2.14±2.14%. In Paper IV, liver fat content was measured by one operator.

**Dual-energy X-ray absorptiometry**
In Paper III, total body fat content was estimated by DXA (DPX, Lunar Prodigy, Lunar Corp., Madison, WI, USA). By triple measurements in 15 subjects, the precision error was 1.5% for total fat mass.
Bioimpedance analysis
Total body water content was assessed by bioimpedance analysis (Tanita BC-558; Tanita Corporation, Tokyo, Japan). Subjects were measured in a fasting state wearing only their underwear.

Assessment of fatty acid composition
Fatty acid composition in plasma/serum cholesterol esters (CE) and phospholipids (PL) was measured by gas chromatography. Plasma/serum (0.5 mL) was mixed with 2.5 mL methanol, 5 mL chloroform (with 0.005% added butylated hydroxytoluene, BHT) and 7.5 mL NaH₂PO₄ (0.2 mol/l) and stored overnight in 4°C for lipid extraction. The chloroform phase was then removed with a syringe and evaporated to dryness on a 30°C heating block using nitrogen gas. The lipid residue was dissolved in chloroform and the lipid esters were separated by thin-layer chromatography (TLC); the adsorbent containing POPOP as fluorescent agent. The TLC-plates were eluted at room temperature with the solvent system petroleum ether/diethyl ether/acetic acid (81:18:1 by volume). The lipid fractions were visualized in UV light and the spots containing cholesterol esters were scraped off into vials and the lipid esters were then methylated at 60°C overnight after addition of 2 mL H₂SO₄ (5%) in methanol. The fatty acid methyl esters were extracted into 3 mL petroleum ether (0.005% BHT) after addition of 1.5 mL distilled water. The phases were separated after thorough mixing and centrifugation at 1500g for 10 minutes. The petroleum ether phase was pipetted off and the solvent was evaporated under nitrogen gas on a 30°C heating block. The fatty acid methyl esters were dissolved in 120 µL hexane and placed in vials. The fatty acid methyl esters were separated by gas-liquid chromatography on a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, USA) with helium gas as a carrier gas. An Agilent Technologies system consisting of model GLC 6890N, autosampler 7683 and Agilent ChemStation was used. The temperature was programmed to 150-260°C. The fatty acids were identified by comparing each peak’s retention time with fatty acid methyl ester standards Nu Check Prep (Elysian, MN, USA). Fatty acids are presented as the relative sum of the fatty acids analysed. Desaturase activities were estimated as product-to-precursor ratios of individual fatty acids in serum CE as follows: SCD-1; 16:1n-7/16:0, delta-5 desaturase; 20:4n-6/20:3n-6 and delta-6 desaturase; 18:3n-6/18:2n-6.

NMR-based metabolomics
NMR measurements were performed at 298°C on a Bruker Avance 600 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a cry-
oprobe. Non-lipid metabolites (mixing plasma with acetonitrile for precipitation) were identified using a non-targeted approach.

Adipose tissue biopsies
Adipose tissue biopsies were taken subcutaneously, 3-4 cm below and lateral to the umbilicus by needle aspiration under local anesthesia (1% lidocaine). Samples were washed with saline and quickly frozen using dry ice covered with ethanol, and stored at -70°C until analysis.

Gene expression
Hybridized biotinylated complementary RNA was prepared from total RNA and hybridized to a GeneChip Human Gene 1.1 ST Array (Affymetrix Inc., Santa Clara, CA, USA) using standardized protocols. Absolute differences in gene expression were calculated for each gene in each subject, comparing post-value with pre-value. Differences between groups were compared using ‘significance analysis of microarray’ (false detection rate 25%). Differences were adjusted for weight gain.

Dietary intake and physical activity
In Paper I and IV, dietary intake was assessed by 4-day weighed food records, and processed with Dietist XP version 3.1 dietary assessment software. In Paper III, dietary intake was assessed by self-report in a pre-coded 7-day optical readable food record. Quantities were reported in household measurements or portion sizes (guided by pictures). Non-pre-coded items could be reported in free text.

Ethics and trial registration
The intervention trials were conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent prior to inclusion and the trials were approved by the regional ethical review board in Uppsala (Dnr 2011/095 for Lipogain, and Dnr 2014/186 for Lipogain-2). Both trials are registered at clinicaltrials.gov (NCT01427140 for Lipogain and NCT02211612 for Lipogain-2).

Statistics
**Paper I**
Differences in changes between groups were analyzed per protocol with Student t-test. Variables with non-normal distributions were log-transformed or analyzed with non-parametric test (Mann-Whitney U test) if normality
was not attained. Data is given as mean (SD) or median (IQR) and correlations are given as Pearson $r$ (or Spearman rho for variables with non-normal distributions).

**Paper II**
Orthogonal partial least square discriminant analysis (OPLS-DA) was used to identify variables contributing to group classification, and interpretation of the models was done by using s- and VIP-plots. Metabolite changes during the intervention and between groups were analyzed with student t-test, after spectral peaks had been manually integrated and normalized to each spectrums total intensity.

**Paper III**
Correlations are given as Pearson $r$. Variables with non-normal distribution were log-transformed to attain normality. Multiple regression analyses were performed with energy intake, height, alcohol intake, sex, smoking, education and physical activity as covariates.

**Paper IV**
Differences in mean values between groups after the intervention were analyzed with ANCOVA adjusted for baseline values. Data is given as mean (SD) or median (IQR) and correlations are given as Pearson $r$. 
Results

Paper I

All 39 participating subjects completed the intervention, however two subjects were excluded from the analyses due to considerable weight loss. Presented data are thus based on 37 subjects (11 women and 26 men). Mean age was 26.9 years (IQR 24 to 29.5, range 20 to 36) and mean BMI was 20.7 (IQR 19.2 to 21.5, range 17.8 to 26.9). Baseline characteristics and changes are shown in Table 1. Both groups increased similar in body weight but the SFA group gained more liver fat, total body fat and visceral fat, but less lean tissue, when compared with the PUFA group. The ratios of lean/fat tissue gained in the PUFA and SFA groups were ~1:1 and 1:4 respectively.

Table 1. Baseline characteristics and changes during the intervention

<table>
<thead>
<tr>
<th></th>
<th>PUFA (n=18) Baseline</th>
<th>Mean Absolute Change</th>
<th>SFA (n=19) Baseline</th>
<th>Mean Absolute Change</th>
<th>Mean Difference in Change (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight, kg</td>
<td>67.4 ± 8.2</td>
<td>1.6 ± 0.85</td>
<td>63.3 ± 6.8</td>
<td>1.6 ± 0.96</td>
<td>-0.02 (-0.63 to 0.58)</td>
<td>0.94</td>
</tr>
<tr>
<td>Waist Girth, cm</td>
<td>79.4 ± 5.6</td>
<td>0.97 ± 2.2</td>
<td>76.1 ± 5.1</td>
<td>1.0 ± 2.3</td>
<td>-0.03 (-1.53 to 1.47)</td>
<td>0.97</td>
</tr>
<tr>
<td>Liver Fat, % (MRI)</td>
<td>0.75 (0.65 to 1.0)</td>
<td>0.04 ± 0.24</td>
<td>0.96 (0.79 to 1.1)</td>
<td>0.56 ± 1.0</td>
<td>-0.52 (-1.0 to -0.01)</td>
<td>0.033</td>
</tr>
<tr>
<td>Lean Tissue, L (MRI)</td>
<td>43.4 ± 8.4</td>
<td>0.86 ± 0.62</td>
<td>41.8 ± 6.9</td>
<td>0.31 ± 0.68</td>
<td>0.55 (0.11 to 0.98)</td>
<td>0.015</td>
</tr>
<tr>
<td>VAT, L (MRI)</td>
<td>0.99 (0.50 to 1.6)</td>
<td>0.11 ± 0.21</td>
<td>0.81 (0.52 to 1.0)</td>
<td>0.22 ± 0.16</td>
<td>-0.12 (-0.24 to 0.01)</td>
<td>0.035</td>
</tr>
<tr>
<td>VAT:SAT ratio (MRI)</td>
<td>0.08 ± 0.04</td>
<td>0.00 ± 0.01</td>
<td>0.07 ± 0.03</td>
<td>0.01 ± 0.01</td>
<td>-0.01 (-0.02 to 0.00)</td>
<td>0.073</td>
</tr>
<tr>
<td>Abdominal SAT, L (MRI)</td>
<td>2.2 (1.9 to 3.1)</td>
<td>0.25 ± 0.32</td>
<td>1.8 (1.5 to 2.8)</td>
<td>0.34 ± 0.23</td>
<td>-0.09 (-0.27 to 0.10)</td>
<td>0.32</td>
</tr>
<tr>
<td>Total Body Fat, L (MRI)</td>
<td>14.4 (12.6 to 19.6)</td>
<td>0.97 ± 1.0</td>
<td>12.9 (10.4 to 18.2)</td>
<td>1.5 ± 0.70</td>
<td>-0.57 (-1.2 to 0.01)</td>
<td>0.013</td>
</tr>
<tr>
<td>Lean Tissue, % (BodPod)</td>
<td>81.9 ± 6.3</td>
<td>-0.81 ± 1.2</td>
<td>85.6 ± 7.4</td>
<td>-1.7 ± 1.1</td>
<td>0.93 (0.15 to 1.70)</td>
<td>0.021</td>
</tr>
<tr>
<td>Total Body Fat, % (BodPod)</td>
<td>18.1 ± 6.3</td>
<td>0.81 ± 1.2</td>
<td>14.4 ± 7.4</td>
<td>1.7 ± 1.1</td>
<td>-0.93 (-1.70 to -0.15)</td>
<td>0.021</td>
</tr>
</tbody>
</table>
The main results are visualized in Figure 14, showing the percent change from baseline. Both groups consumed similar amounts of muffins (3 per day, equaling 750 kcal/day) and the total energy intake (3136 kcal/day and 3035 kcal/day for PUFA and SFA groups respectively) was not significantly different between groups. Macronutrient composition (E%) of the diets is shown in Figure 15. Further, physical activity did not differ between groups. The relative proportion of palmitic acid and linoleic acid in plasma cholesterol esters and adipose tissue changed in the expected direction, indicating good compliance (Figure 16). Furthermore, the proportion of palmitoleic acid (16:1n-7) and the SCD1-index in plasma cholesterol esters decreased by PUFA compared with SFA. Changes in linoleic acid in plasma cholesterol esters was inversely associated with changes in liver fat content (rho=-0.38, P=0.02) and total body fat (r=-0.55, P=0.001) but positively associated with changes in lean tissue (r=0.55, P=0.001). Changes in palmitic acid in plasma cholesterol esters was positively associated with changes in VAT (r=0.37, 0.023), body fat (r=0.53, P=0.001) but negatively associated with changes in lean tissue (r=-0.53, P=0.001). Further, changes in the plasma SCD1-index was positively associated with changes in liver fat (rho=0.44, P=0.006).

Figure 14. Visualization of changes in body weight, fat depots and body composition during the intervention. The y-axes are percent change from baseline.
Figure 15. Dietary composition (E%) during the intervention

Figure 16. Changes in relative proportions of palmitic acid and linoleic acid in plasma cholesterol esters and adipose tissue during the intervention

Analysis of gene expression in adipose tissue revealed that 20 genes were differentially regulated between SFA and PUFA groups (see Table 5 in Paper I).
Fasting plasma glucose was unchanged in both groups during the intervention. Both fasting serum insulin and HOMA-IR (homeostasis model of assessment insulin resistance) increased to a similar extent in both groups (P=0.97 and P=0.79 for difference between groups, respectively). The ketone-body β-hydroxybutyrate decreased markedly in both groups but did not differ between groups (P=0.14).

Paper II

Two subjects were excluded due to abnormal peaks in the spectra, results are thus based on data from 35 subjects. The OPLS-DA identified the metabolites contributing to group classification as 3-hydroxybutyrate, creatine/creatinine, acetoadetate, acetate, alanine, the aromatic amino acids (AAA; tryptophan, tyrosine, phenylalanine; integrated as one integral) and the branched chain amino acids (BCAA; leucine, isoleucine, valine; integrated as one integral). Acetate decreased by PUFA and increased by SFA, whereas the opposite was found for lactate. These two changes were the only ones to reach statistical significance between the groups. PUFA tended to decrease 3-hydroxybutyrate to a greater extent than SFA (P=0.097). Relative metabolite changes are shown in Figure 17.

![Figure 17. Relative metabolite changes during the intervention. Asterisks denote significant differences between groups.](image-url)
Changes in several metabolites correlated with changes in fat depots and lean tissue (MRI), as shown in Table 2.

Table 2. Metabolites correlating with fat depots and lean tissue

<table>
<thead>
<tr>
<th>MRI assessment</th>
<th>Metabolite</th>
<th>r²</th>
<th>p</th>
<th>Metabolite</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean tissue</td>
<td>Lactate</td>
<td>0.10</td>
<td>0.07</td>
<td>Leucine</td>
<td>0.16</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT</td>
<td>Leucine</td>
<td>0.16</td>
<td>0.02</td>
<td>Alanine</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>0.16</td>
<td>0.02</td>
<td>Lactate</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>Liver fat</td>
<td>3-methyl-2-oxovalerate</td>
<td>0.09</td>
<td>0.09</td>
<td>Alanine</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Total body fat</td>
<td>3-methyl-2-oxovalerate</td>
<td>0.16</td>
<td>0.02</td>
<td>Alanine</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>0.11</td>
<td>0.06</td>
<td>Lactate</td>
<td>0.06</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>0.14</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Paper III

The study population of 287 subjects (52% men) was overweight (mean BMI 26.8±4.1) but generally healthy. Self-reported energy intake was 1887±467 kcal/day and the macronutrient proportions (E%) were 16.3, 48.9, 31.1 and 2.0 for protein, carbohydrates, fat and alcohol, respectively.

Palmitic acid in serum cholesterol esters was positively associated with VAT and total body fat (but not SAT); Figure 18 panel A. In contrast, linoleic acid was inversely associated with all three depots; as was alpha-linolenic acid. Surprisingly, EPA and DHA were positively associated with VAT and total body fat. Furthermore, the SCD-index was positively associated with SAT and total body fat (but only borderline with VAT), whereas delta-5 desaturase was inversely associated with all fat depots and delta-6 desaturase positively associated with all fat depots; Figure 18 panel B. Associations remained statistically significant also in the multivariate models. Associations were not clearly sex-specific, although some associations (palmitic acid with VAT) were stronger in men and other (delta-5 desaturase and all fat depots) were stronger in women.

All fat depots were positively associated with insulin resistance (HOMA-IR) although VAT showed the strongest association, tightly followed by total body fat; Figure 18 panel C. Palmitic acid in cholesterol esters was positively associated with HOMA-IR, whereas linoleic acid was inversely associated.
Figure 18. Associations between fatty acids in serum cholesterol esters, desaturase activity indices and HOMA-IR with body fat depots. Y-axes show Pearson r. PA, palmitic acid; LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Asterisks denote significant correlations.
Paper IV

Of the 61 subjects enrolled in the study, only 1 subject dropped out. Presented data are thus based on 60 subjects (23 women, 37 men). The mean age was 41.9±8.4 years (IQR 36.3 to 48, range 22 to 59) and the mean BMI was 28.0±3.6 (IQR 25.4 to 29.3, range 19.3 to 39.1). Baseline characteristics and changes are shown in Table 3.

Table 3. Baseline characteristics and changes during the intervention

<table>
<thead>
<tr>
<th></th>
<th>PUFA (n=30) Mean Absolute Change</th>
<th>SFA (n=30) Mean Absolute Change</th>
<th>Difference between groups (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight, kg</td>
<td>84.3±14.1 2.01±1.90</td>
<td>88.6±13.6 2.31±1.38</td>
<td>0.15 (-0.29 to 0.59)</td>
<td>0.50</td>
</tr>
<tr>
<td>Waist Girth, cm</td>
<td>94.6±10.5 3.05±4.43</td>
<td>97.4±10.1 3.57±4.25</td>
<td>0.46 (-0.63 to 1.55)</td>
<td>0.41</td>
</tr>
<tr>
<td>Liver Fat, % (MRI)</td>
<td>2.02 (1.36 to 4.56)</td>
<td>1.46 (0.96 to 3.89)</td>
<td>1.54±2.0 0.80 (0.32 to 1.27)</td>
<td>0.001</td>
</tr>
<tr>
<td>Liver Fat, L (MRI)</td>
<td>0.03 (0.02 to 0.07)</td>
<td>0.02 (0.01 to 0.06)</td>
<td>0.03±0.04 0.02 (0.01 to 0.02)</td>
<td>0.000</td>
</tr>
<tr>
<td>Liver Volume, L (MRI)</td>
<td>1.55±0.27 0.03±0.11</td>
<td>1.57±0.24</td>
<td>0.05±0.08 0.01 (-0.01 to 0.04)</td>
<td>0.39</td>
</tr>
<tr>
<td>Pancreas Fat, % (MRI)</td>
<td>3.58 (2.00 to 7.63)</td>
<td>3.02 (1.36 to 7.55)</td>
<td>0.49±2.29 0.05 (-0.48 to 0.58)</td>
<td>0.85</td>
</tr>
<tr>
<td>VAT, L (MRI)</td>
<td>3.75 (1.52 to 4.45)</td>
<td>3.14 (1.83 to 4.81)</td>
<td>0.37±0.29 0.05 (-0.02 to 0.13)</td>
<td>0.17</td>
</tr>
<tr>
<td>VAT:SAT ratio (MRI)</td>
<td>0.49 (0.32 to 0.73)</td>
<td>0.42 (0.26 to 0.67)</td>
<td>0.01±0.03 0.005 (-0.003 to 0.01)</td>
<td>0.23</td>
</tr>
<tr>
<td>Abdominal SAT, L (MRI)</td>
<td>5.60 (4.59 to 8.70)</td>
<td>7.63 (5.78 to 9.32)</td>
<td>0.72±0.60 0.07 (-0.08 to 0.22)</td>
<td>0.35</td>
</tr>
<tr>
<td>Total Body Fat, L (MRI)</td>
<td>27.30 (22.89 to 35.13)</td>
<td>1.77±1.63</td>
<td>31.44 (26.33 to 36.68)</td>
<td>2.22±1.57 0.23 (-0.19 to 0.65)</td>
</tr>
<tr>
<td>Total Body Fat, kg (Bod-Pod)</td>
<td>23.6 (18.4 to 32.7)</td>
<td>1.75±2.07</td>
<td>26.4 (22.3 to 31.0)</td>
<td>2.18±1.70 0.21 (-0.29 to 0.70)</td>
</tr>
<tr>
<td>Total Body Fat, % (Bod-Pod)</td>
<td>30.4±10.2 1.22±1.89</td>
<td>32.2±8.6</td>
<td>1.59±1.78 0.22 (-0.26 to 0.69)</td>
<td>0.36</td>
</tr>
<tr>
<td>Lean Tissue, L (MRI)</td>
<td>46.83±9.82 0.54±0.93</td>
<td>47.22±8.44</td>
<td>0.67±1.21 0.07 (-0.21 to 0.35)</td>
<td>0.61</td>
</tr>
<tr>
<td>Lean Tissue, kg (Bod-Pod)</td>
<td>58.0±10.6 0.36±1.5</td>
<td>59.6±9.7</td>
<td>0.15±1.4 -0.08 (-0.46 to 0.29)</td>
<td>0.66</td>
</tr>
<tr>
<td>Total Body Water, % (BIA)</td>
<td>52.7±6.6 -0.75±1.6</td>
<td>51.2±5.7</td>
<td>-0.45±1.2 0.10 (-0.26 to 0.46)</td>
<td>0.59</td>
</tr>
</tbody>
</table>
Both groups gained similar in body weight, but PUFA caused less accumulation of liver fat compared with SFA. Accumulation of pancreas fat, VAT, abdominal SAT, total body fat, lean tissue and total body water did not differ between groups. However, sex-specific analyzes showed that VAT increased significantly more by SFA compared with PUFA in men, and total body fat tended to increase more by SFA than PUFA in women (P=0.06).

At baseline, carriers of the PNPLA3 risk allele tended to have higher liver fat content compared with non-carriers (2.8% vs 4.7%; P=0.07). The effect of SFA on liver fat accumulation during the intervention was independent of genotype, however the potential decreasing effect of PUFA is possibly driven by carriers of the risk allele (Figure 19).

![Figure 19. Change in liver fat content by PUFA and SFA according to if subjects were carriers (CG+GG) or non-carriers (CC) of the PNPLA3 risk allele.](image)

Both groups consumed similar amounts of muffins (3 per day, equaling 750 kcal/day) and the total energy intake (2727 kcal/day and 2906 kcal/day for PUFA and SFA groups respectively) was not significantly different between groups. Macronutrient composition (E%) of the diets is shown in Figure 20. Further, physical activity did not differ between groups.
Figure 20. Dietary composition (E%) during the intervention

The relative proportion of palmitic acid and linoleic acid in plasma cholesterol esters, adipose tissue and phospholipids changed in the expected direction, indicating good compliance (Figure 21). Furthermore, the proportion of palmitoleic acid (16:1n-7) and the SCD1-index in plasma cholesterol esters decreased by PUFA and increased by SFA. Change in SCD1-index was positively associated with change in liver fat content ($r=0.49$, $P=0.0001$).
Figure 21. Changes in relative proportions of palmitic acid and linoleic acid in plasma cholesterol esters, adipose tissue and phospholipids during the intervention.
Discussion

In this thesis, diets enriched in n-6 PUFA (linoleic acid) were shown to have beneficial effects on body composition and ectopic fat in humans during overfeeding compared with diets enriched in SFA (palmitic acid). A higher proportion of linoleic acid in plasma was further associated with lower levels of both abdominal and total adipose tissue in a population-based sample of elderly individuals. The underlying mechanisms are unclear (and likely multifactorial) but exploratory analyses indicated differential effects on intermediary metabolism. These results support current dietary guidelines and provide further incentives to let dietary fat intake be based on unsaturated rather than saturated fat.

Paper I

In Paper I, overfeeding SFA and PUFA caused distinct effects on body composition and body fat distribution, despite similar overall weight gain between groups. SFA caused higher deposition of liver fat, VAT and total body fat, whereas PUFA caused higher deposition of lean tissue. The effects on liver fat support the results from a previous randomized study by Bjermo comparing similar treatments, although iso-calorically (78). In addition to replicating the differential effects of SFA and PUFA on liver fat content, one of the main conclusions from Paper I was that PUFA can prevent the accumulation of liver fat also during induced weight gain. However, the most surprising finding was the clear difference in the accumulation of lean tissue between groups, which was observed when assessed both with MRI and ADP. Our interpretation was that increased lean tissue corresponds to increased skeletal muscle mass. A frequently asked question has been if the difference in lean tissue could have been due to differences in intramuscular fat? However, this seems unlikely as the MRI and ADP yielded similar results and the ADP-technique is based on whole-body density, i.e. it captures changes in fat mass independent on location. It could also be argued that differences in lean tissue could arise due to different tissue hydration status. This also seems unlikely as no differences could be found in total body water content, and the results remained when total body water was accounted for in a three-compartment model. A third possibility is that the increase in lean tissue corresponds to something else than skeletal muscle mass, such as connective tissue or organ weight. This cannot be disproven by our data. How-
ever, as fatty acid-specific effects on skeletal muscle protein synthesis has been observed in both cultured muscle cells (153) and humans (151) it is not unreasonable that the increase in lean tissue actually corresponds to skeletal muscle mass. If so, the differences in muscle mass in Paper I did not seem to be mediated by differences in physical activity as assessed by accelerometers. Although a tightly controlled study by Kien (136) has indeed shown fatty acid-specific effects on physical activity.

The differential effects on VAT and total adipose tissue were not as controversial considering the fairly large amount of animal studies suggesting this. Also, the human studies by Summers (50) and Norris (54) indicated beneficial effects on abdominal obesity by PUFA. However, the underlying mechanisms responsible for these effects on partitioning remain to be discerned. Perhaps the differential effects of SFA and PUFA on tissue-specific activity of LPL are involved (see subsection ‘LPL activity’ in the Introduction), but no measures on either LPL activity or sympathetic nervous system activity were assessed in Lipogain so no tentative conclusions can be drawn from the present data.

The changes in fatty acid composition seen in both plasma and adipose tissue reflected the intervention and thereby compliance, i.e. linoleic acid increased by sunflower oil and palmitic acid by palm oil. Furthermore, the changes in fatty acid composition also reflected aspects of endogenous metabolism. Palmitoleic acid (16:1n-7) and the SCD-1 activity index decreased by PUFA compared with SFA, which indicates a suppressed lipogenic activity, congruent with the results on body composition. The suppressive effect on lipogenesis by PUFA compared with SFA is well established in various experimental models and settings (see Introduction).

The differential gene expression in adipose tissue observed between groups was in general modest, and difficult to interpret. Perhaps the most interesting change was the 20% downregulation of ALDH1A1 by PUFA (and 9% upregulation by SFA). ALDH1A1 has in animal studies been shown to inhibit energy dissipation and promote fat storage, and ALDH1A1-deficient mice are protected from diet-induced liver fat accumulation (181).

As a proxy measure of fatty acid oxidation, the ketone body beta-hydroxybutyrate was measured in fasting plasma, and was found to decrease in both groups during the intervention. The decrease tended to be larger by PUFA, although this was not statistically significant. This was counterintuitive to what could be expected based on previous literature, however the results should be interpreted cautiously considering the experimental conditions (e.g. overfeeding and a single fasting value).

Fasting plasma glucose was unchanged in both groups whereas fasting serum insulin and HOMA-IR increased modestly in both groups (to a similar extent). At first glance the similar increases in insulin and HOMA-IR between groups may seem counterintuitive, as a higher deposition of liver fat by SFA could be expected to result in higher fasting insulin and insulin re-
istance. Importantly though, the absolute increase in liver fat by SFA was small (~0.5%) and all subjects were still lean (BMI ~21) and far below the cut-off for fatty liver (~5.5%) also after the intervention. Therefore, the lack of differences is not surprising and the increase in liver fat content was likely too small in absolute amounts to produce significant metabolic deterioration in this healthy group.

Paper II

In Paper II we aimed to identify non-lipid metabolites differentially affected by SFA and PUFA, and to correlate these with changes in body composition data. Surprisingly few (only two) metabolites were differentially regulated between groups. Acetate decreased by PUFA and increased by SFA and lactate increased by PUFA and decreased by SFA. Beta-hydroxybutyrate decreased numerically in both groups and tended to decrease more by PUFA thus replicating the finding in Paper I (where beta-hydroxybutyrate was measured enzymatically), indicating reliability of the method. Although not significant, some interesting trends were observed. The BCAAs and AAAs increased more by SFA compared with PUFA. This was interesting as the BCAA and AAA repeatedly have been associated with obesity, insulin resistance and a dysregulated metabolism in general (182, 183). Several of the metabolites were correlated to changes in body composition, e.g. the BCAAs leucine and valine were positively associated with VAT accumulation. Overall, it is challenging to interpret what these changes really means and the results are rather hypothesis-generating. However, and as discussed more thoroughly in Paper II, the metabolite changes could potentially reflect alterations in tissue-specific insulin sensitivity not large enough to be detected by proxy-measures such as HOMA-IR (which did not differ between groups). For example, higher hepatic insulin sensitivity by PUFA might explain stronger suppression of ketogenesis. Furthermore, insulin is a regulator of circulating BCAA and promotes their degradation in the liver (184). Importantly, although subjects were overfed and increased in body weight and fat mass, the weight gain was small and subjects were still very lean after overfeeding. Thus, no major deteriorations in insulin sensitivity or metabolic state could be expected. Overall, no firm conclusions about the underlying mechanisms can be drawn, but it seems safe to conclude that SFA and PUFA have differential effects on intermediary metabolism (at least during overfeeding).

Paper III

In Paper III, we aimed to investigate the associations between fatty acid composition in serum cholesterol esters with the amount of total adipose tissue, VAT and SAT in a population-based setting of 287 elderly (70 years
Linoleic acid was inversely associated with all three depots whereas palmitic acid was positively associated with VAT and total adipose tissue. The associations remained when adjusted for energy intake, height, alcohol intake, sex, smoking, education and physical activity. These results support our previous findings from Lipogain, implying that fatty acid composition may be important for adipose tissue distribution also in elderly individuals and in the general free-living population. Importantly though, despite being statistically significant, all associations were rather weak (Pearson r ~0.10 to 0.30), and in the regression model the partial $r^2$ were between 2% and 9%, implying that fatty acids only explained a small degree of the variance in fat depots.

Furthermore, the SCD-1 index was positively associated with SAT and total adipose tissue, but surprisingly not with VAT (although the association was “borderline” significant in the regression model). The SCD-1 index has previously been associated with BMI, waist girth and sagittal abdominal diameter (58) and thus this study is in line with previous findings although the association for VAT did not reach statistical significance. Indices for delta-5 and -6 desaturases were also congruent with previous literature, i.e. delta-5 desaturase was inversely and delta-6 desaturase was positively associated with fat mass (185).

We also investigated the associations for omega-3 fatty acids of plant (alpha-linolenic acid, 18:3n-3) and animal origin (EPA, DHA) and found that 18:3n-3 was inversely associated with VAT and total fat whereas EPA and DHA were, surprisingly, positively associated with fat depots. The latter observations may seem counterintuitive, but similar observations (for EPA) have previously been found in another Swedish population (58).

The associations for palmitic acid should be interpreted cautiously. Palmitic acid is not a reliable biomarker of dietary intake as it can also be formed by de novo lipogenesis from carbohydrates. The proportion of palmitic acid in cholesterol esters was not correlated with the dietary intake of palmitic acid. However, there were no associations between palmitic acid in cholesterol esters and dietary intake of either total carbohydrates or sugar.

Another caveat to bear in mind is that MRI measurements and blood sampling were separated by an average of two years, i.e. some individuals could have made significant dietary changes during this time and thereby introducing bias, but this is unlikely to alter the results on a group level. Furthermore, fatty acid composition in cholesterol esters have been shown to be rather stable within Swedish middle-aged and elderly individuals (186).

Paper IV

In Paper IV, we repeated the same intervention as in Paper I, but this time the subjects were older and had considerably higher BMI, and the duration of the intervention was 8 weeks instead of 7 weeks. The purpose was to in-
vestigate if the same beneficial results found in Paper I could be replicated in a population more representative of the general population, and the primary outcome was change in lean tissue rather than liver fat. As in Paper I, the intervention succeeded in that both groups gained equal in body weight, however we failed to replicate the differential effects on lean tissue accumulation between groups. Change in lean tissue was similarly non-significant between groups when assessed both with MRI and ADP, as well as when corrected for total body water. Furthermore, change in lean tissue was not correlated with change in linoleic acid in plasma. The reasons for these discrepancies between Paper I and Paper IV are unclear, but may be due to the different study populations, e.g. excess body fat have detrimental effects on skeletal muscle protein synthesis (187). Interestingly, both total adipose tissue and VAT have been shown to predict an accelerated loss of lean tissue in longitudinal studies (188, 189). Age is also a negative modulator of muscle protein metabolism (190), however it can be discussed if this population was “old enough” for these changes to occur, but it should not be disregarded. The sex-distribution of subjects in Paper I and Paper IV was slightly different which potentially could have affected the results, however sex-specific analyses did not clearly indicate this. Interestingly, it has been shown that positive energy balance during inactivity accelerates muscle atrophy by increasing inflammation (191). However, our population was probably not inactive enough to evoke this metabolic state and no signs of increased inflammation were observed (data not shown). Perhaps the potential lean tissue-promoting effect by n-6 PUFA can only by observed in younger individuals, who may have higher potential for muscle growth. Although n-3 PUFA are effective also in older individuals (55, 151), the mechanisms of n-3 vs n-6 PUFA-mediated increases in lean tissue may be different.

The differential effects on liver fat accumulation seen in Paper I was at least as impressive in Paper IV, and liver fat actually tended to decrease by PUFA (in line with the results by Bjermo (78)), despite weight gain, whereas SFA caused a ~50% relative increase. The decrease by PUFA was driven by subjects carrying the PNPLA3 risk allele. In contrast, SFA increased liver fat similarly in carriers and non-carriers. The percentage increase in liver fat content by SFA was calculated to represent an actual increase of 30 mL. The changes in liver fat content were reflected by changes in plasma ALT levels, which increased significantly by SFA compared with PUFA.

Disappointingly, we could not replicate the differential accumulation of total body fat or VAT seen in Paper I. This was surprising, more so than the absence of differential effects on lean tissue, and more difficult to explain. The VAT- and body fat-promoting effects of SFA compared with PUFA have repeatedly been suggested in the literature, and, to the best of my knowledge, no negative modulating effects of age or baseline body fat content have been proposed. However, sex-specific analyzes showed that VAT
increased significantly more by SFA in men only, as did total body fat, although with borderline significance.

General discussion
In both Paper I and Paper IV, similar amounts of muffins were required for both groups in order to gain comparably in weight, implying that the net available energy is similar for SFA and PUFA (assuming equal absorption), but that they are merely directed to different processes within the body. This was not unexpected, although results from animal studies are not completely consistent. Additionally, both the lean subjects in Paper I and the overweight/obese subjects in Paper IV required more or less the same amount of muffins (~3 per day) in order to achieve (or at least approach) target weight gain. Interestingly though, ~3 muffins per day in Paper I caused a weight gain of 1.6 kg whereas the increase in Paper IV was 2.2 kg.

The beneficial effects of PUFA compared with SFA on body fat seem to be large enough to be observed also in the general population as indicated in Paper III where linoleic acid was inversely associated with body fat, including VAT. Further, among the fat depots, VAT showed the strongest association to HOMA-IR in Paper III, implying that the fatty acid-specific effects on VAT accumulation observed in Paper I may have negative long-term consequences for glucose homeostasis if sustained.

In both Paper I and Paper IV, PUFA decreased the SCD1-index in plasma compared with SFA, and changes in the SCD1-index were positively associated with changes in liver fat content. This supports the suppressive effects of PUFA on hepatic lipogenesis and is in line with previous literature (78). Subjects with NAFLD have been shown to have elevated levels of 16:1n-7 and the SCD1-index and lower levels of 18:2n-6 compared with subjects without NAFLD (4, 90, 91, 192).

What this thesis adds
The most novel finding, and maybe the most clinically relevant, was that liver fat accumulation can be counteracted also during induced weight gain. With the addition of this thesis, the beneficial effect on liver fat content by n-6 PUFA compared with SFA in humans has now been consistently shown, and seems to be a general effect applicable to both lean and overweight/obese subjects, in both male and females, over a wide age range.

Further, the results also imply that the general body composition (i.e. amount of fat- and lean tissue) and fat distribution (intra-abdominally vs. subcutaneously) could be affected by the dietary fat composition, although these results does not seem as general and may only be valid for certain groups (i.e. young and lean subjects).
The importance of food source and a nuanced view

It is important not to generalize “too much”. Commonly, dietary fatty acids are classified as SFA, MUFA and PUFA. Also, the awareness has increased of the importance of distinguishing between n-6 and n-3 PUFA. A similar awareness and distinction of fatty acids needs to be made also for SFA, i.e. all SFA do not have the same effects, which is obvious when reviewing the literature. Furthermore, the food source seems to be important even when the fatty acid composition is identical. The most compelling evidence for the latter are the randomized controlled trials comparing various dairy products, e.g. cheese with butter (193-196) or cream with butter oil (197), showing differential effects despite equal amounts of fat with similar fatty acid compositions. Examples with vegetable fats can also be found (198). However all these studies were made with “cholesterol-centric” outcomes; the same question has not been investigated in humans in the context of body composition and body fat distribution, although there are animal studies implying the same phenomenon (37, 44, 68). With regard to public recommendations, focus should shift from single nutrients to complete foods and dietary patterns. Fortunately, this shift has already begun.

Strengths and limitations

The major strengths are that both intervention trials (Paper I and IV) were randomized and double-blinded, and also stratified by gender thereby providing balanced groups. All study personnel remained blinded throughout the intervention and statistical analyses. Further, all subjects received a high level of attention (visit at the clinic once a week) and compliance to the intervention was objectively assessed by changes in fatty acid composition in plasma. Body composition and ectopic fat were measured by accurate and reliable methods. Almost no drop-outs occurred, minimizing the risk of attrition bias (Paper I and IV).

In Paper III, the major strengths are that the study population was population-based and both exposure and outcome variables were measured by accurate and reliable techniques. Further, all individuals were of the same age and the sex-distribution was balanced.

There are also limitations. In Paper I and IV, the MRI methods used relied on fixed-spectrum models and thus did not allow full characterization of all lipid resonances of the liver spectra to detect changes in liver lipid saturation (i.e. the assumption was made that tissue lipid saturation was unchanged). Regarding lean tissue, neither the MRI nor the ADP can guarantee that changes consist of muscle mass; this is an assumption. Further, the results gained by overfeeding may not necessarily translate to the general population, being more weight-stable. Also, the relatively high doses of n-6 PUFA
used resulted in a PUFA intake above the general recommendations, making extrapolations to the general population more difficult; i.e. the marked effects on body composition and ectopic fat may occur at intakes higher than what can be recommended for long-term use. In both interventions (Paper I and IV), sunflower oil was used as the source of PUFA and palm oil as the source of SFA. Extrapolating the results to other dietary sources of PUFA and SFA should be done with caution. However, for liver fat, the effects seem to be similar for butter (78), at least in an iso-caloric trial. Since both butter and palm oil cause increased liver fat as compared with n-6 PUFA, such effects might be due to SFA in general (at least sources rich in palmitic acid).

In Paper III, the relatively long lag-time between blood sampling and measurement of body composition could have introduced errors for some individuals. Further, the cross-sectional design prevents conclusions about causal relationships.

Clinical and societal implications

“Let food be thy medicine and medicine be thy food” – Hippocrates

What we choose to eat, and not to eat, has been convincingly shown to affect future risk of disease. The type of fat in the diet has since long been associated with long-term health outcomes, mainly regarding blood lipids and cardiovascular disease. A growing number of studies now also suggest that dietary fatty acid composition may affect other aspects as well, such as body composition and accumulation of ectopic fat. These findings may partly explain the epidemiological associations between dietary fatty acid composition and e.g. incidence of type 2 diabetes. These findings further support existing dietary guidelines (i.e. that dietary fat should be mainly unsaturated), but also provides another rationale for this (except blood lipids). Based on Paper I and Paper IV, as well as previous data (78), the effects of dietary SFA and n-6 PUFA on liver fat content seems rather potent and applicable in both genders and over a wide age range. As NAFLD is the most common liver disorder in developed countries, a rather simple dietary shift from SFA to more PUFA could have preventive potential. The widespread use of palm oil by the food industry is therefore of concern, and efforts should be made to try to incorporate higher proportions of unsaturated fat in food items at the expense of SFA.

The potential muscle mass-promoting effect of n-6 PUFA could, if established, be clinically exploited in the treatment of specific groups of patients, e.g. in the elderly, malnourished or bedridden individuals. Further, an increased skeletal muscle mass would probably also be of importance for gen-
eral public health, and for the maintenance of self-independence when growing old.

However, although single nutrients may have large effects in experimental studies, it is important not to be blinded by these as the overall dietary pattern will likely have precedence for long-term health.
Conclusions

- Overfeeding n-6 PUFA (sunflower oil) in young and lean subjects reduced the accumulation of liver fat, visceral fat and total body fat and instead promoted the accumulation of lean tissue compared with SFA (palm oil), despite similar weight gain.

- n-6 PUFA and SFA exerted differential effects on intermediary metabolism, but the mechanisms underlying the effects on body composition remain unclear.

- The proportion of linoleic acid in serum cholesterol esters was inversely associated with contents of visceral fat, subcutaneous abdominal fat and total body fat in a population-based sample of elderly men and women, whereas the proportion of palmitic acid was positively associated with contents of visceral fat and total body fat.

- Overfeeding n-6 PUFA (sunflower oil) prevented the accumulation of liver fat also in slightly older, overweight and obese individuals compared with SFA (palm oil), despite similar weight gain. However, the differential effects on lean tissue, visceral fat and total body fat could not be replicated in this population.

Future perspectives

As with all interesting research findings, they generate not only answers but also new questions. The following are some questions worthy of further investigations:

Despite being provided with high-caloric muffins daily for ~2 months, some individuals had considerable difficulties in gaining weight. These individuals seemed more or less “resistant” to weight gain despite forcing the system. What characterizes these individuals? Do they increase their metabolism, oxidizing the extra energy? Or do they (involuntarily) compensate by eating less of their habitual diet? This subgroup of individuals would be highly interesting to study further.
Which molecular mechanisms and signals can explain the potential lean tissue-promoting effect of PUFA? Is it a direct effect, i.e. specific signals inducing growth, or is it an indirect effect due to difficulties storing large amounts of PUFA and thereby making more energy available for growth of other tissues?

Can the hypertrophic effects of resistance training be enhanced by a higher dietary intake of n-6 PUFA as seen for n-3 PUFA?

Can the liver fat-promoting effect of SFA be inhibited by minor dietary components (e.g. phytochemicals; as suggested in animals) provided in ample amounts of fruit and vegetables etc?

Do other sources of SFA, e.g. coconut fat, consisting of ~50% lauric acid (12:0), behave similarly as butter and palm oil on body composition and ectopic fat?

Although n-6 PUFA decreased liver fat on a group level, some individuals receiving n-6 PUFA showed an increase in liver fat content. The same, but reverse, reasoning also goes for SFA. What characterizes these individuals?

An important outstanding question is of course if the beneficial effects observed on both ectopic fat and body composition are valid for more moderate intakes of n-6 PUFA (i.e. within the current dietary recommendations) during energy balance.

I denna avhandling var syftet att studera hur sammansättningen av fettet vi äter kan påverka hur mycket fett som lagras in i kroppen, samt var i kroppen det hamnar. Då den vuxna befolkningen i allmänhet tenderar att långsamt gå upp i vikt över tid ville vi studera denna frågeställning under dessa förhållanden, nämligen genom att låta frivilliga personer gå upp i vikt genom att berika kosten med fett av olika sammansättning (mättat eller fleromättat fett). Vi ville också undersöka om de resultat vi fann från dessa experiment kunde överföras till en större grupp människor ur den allmänna befolkningen.

I det första delarbetet (Paper I) slumpades 39 unga och friska män och kvinnor till att äta muffins bakade på antingen palmolja (mättat fett) eller
solrosolja (fleromättat fett) under sju veckors tid. Muffinsen var avsedda att ätas som ett komplement, utöver deltagarnas vanliga kost, med syftet att skapa en måttlig viktpåkast. Båda grupper gick upp lika mycket i vikt (vilket var tanken), men den extra vikten hamnade på olika ställen över kroppen i de båda grupperna. Deltagarna som åt muffins med palmolja (mättat fet) lagrade in mer fet i levern och bukhålan, samt mer kroppsfett totalt, jämfört med deltagarna som åt muffins med solrosolja (fleromättat fet). Deltagarna som åt muffins med solrosolja fick istället en större ökning av mängden muskelmassa jämfört med deltagarna som åt muffins med palmolja. Dessa resultat visar att en relativt enkel förändring i kosten (ändrad fettkälla) kan ha förvånansvärt stora effekter på kroppens sammansättning och kroppsfettets fördelning över kroppen.

I det andra delarbetet (Paper II) analyserade vi blodproverna från Paper I med syftet att förstå förstå vad som händer mer i detalj med ämnesomsättningen. Vi fann att några ämnen (metaboliter) påverkades olika i de båda grupperna. Flera av dessa ämnen ingick i ett ”centralt nav/nätverk” som är involverat i väldigt många olika processer relaterade till ämnesomsättningen, vilket innebar att resultaten var svårtolkade. Dessa förändringar kan dock tyda på att olika organ (t.ex. levern, underhudsfettet och musklerna) blev olika känsliga för kroppens hormoner (t.ex. insulin) av de olika fettsorterna (palmolja och solrosolja). Sammantaget kunde vi dock inte dra några starka slutsatser om vilka mekanismer som låg bakom de effekter vi såg på kroppens sammansättning i Paper I.

I det tredje delarbetet (Paper III) ville vi undersöka om de relativt markanta effekterna av olika sorters fet i kosten på kroppsfettets mängd och fördelning som vi såg i Paper I även avspeglades i den allmänna befolkningen. Vi hade tillgång till blodprover som tidigare samlats in från 287 st äldre (alla 70 år gamla) män och kvinnor. Vi använde dessa blodprover för att analysera hur mycket mättat respektive fleromättat fet varje individ hade i blodet, vilket avspeglar hur mycket mättet respektive fleromättat fet de ätit i kosten. Dessa mängder relaterades sedan till hur mycket kroppsfett varje individ hade samt hur det var fördelat över kroppen. I linje med resultaten i Paper I såg vi att personer som hade mer fleromättat fet i blodet hade mindre kroppsfett i buken samt mindre kroppsfett totalt, det vill säga det fanns ett ”omvänt/negativt samband” mellan mängden fleromättat fet i blodet och mängden kroppsfett. Personer som hade större mängd mättat fet i blodet hade istället mer kroppsfett i buken samt kroppsfett totalt, det vill säga det fanns ett ”direkt/positivt samband” mellan mängden mättet fet i blodet och mängden kroppsfett. Denna studie kan enskilt inte användas för att dra orsakssamband då mätningarna (blodprov och kroppsfett) bara gjorts vid ett enda tillfälle och det sannolikt finns andra faktorer som skiljer individerna åt. Men resultaten stämmer förvånansvärt väl överens med de effekter som visades i delarbete I.

Sammantaget kan vi säga att fleromättat fett verkar motverka inlagring av fet i levern jämfört med måttat fett, och detta verkar gälla över ett brett åldersspän, hos både män och kvinnor samt hos både smala och överviktiga/feta individer. Detta skulle kunna vara en delförklaring till de positiva hälsoeffekter på lång sikt som tidigare visats av en kost med mer fleromättat och mindre måttat fett. Fleromättat fett har möjligen även andra positiva effekter på kroppens sammansättning jämfört med måttat fett, till exempel på mängden bukfett, totalt kroppsfett samt muskelmassa men dessa effekter verkar inte vara lika generella och kanske bara gäller vissa grupper och/eller under vissa förhållanden. Fler detaljerade studier behövs för att belysa dessa frågeställningar innan säkra slutsatser kan dras.
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