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Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat

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Abstract

Δ9-Desaturase (stearoyl-CoA desaturase 1, SCD-1) regulates the desaturation of SFA, mainly stearic and palmitic, to MUFA. Δ6-Desaturase (D6D) and Δ5-desaturase (D5D) are involved in the metabolism of linoleic and α-linolenic acid to polyunsaturated metabolites. The objective of the present study was to study the effects of different types of dietary fat on indices of fatty acid desaturase (FADS) activity (evaluated as product:precursor ratios) in plasma and skeletal muscle in human subjects. A high SCD-1 index has been related to obesity and metabolic disorders, while the D5D index is associated with insulin sensitivity. Fatty acid composition of serum and skeletal muscle lipids was analysed by GLC during a randomised, controlled, 3-month dietary intervention in healthy subjects. A comparison of the effects of a diet containing butter fat (SFA, *n* 17) with a diet containing monounsaturated fat (MUFA, *n* 17), keeping all other dietary components constant, showed a reduced SCD-1 activity index by 20 % on the MUFA diet compared with the SFA diet assessed in serum cholesteryl esters. The D6D and D5D indices remained unaffected. Supplementation with long-chain *n*-3 fatty acids reduced the SCD-1 index by a similar magnitude while the D6D index decreased and the D5D index increased. It is concluded that changes in the type of fat in the diet affect the indices of FADS activity in serum and skeletal muscle in human subjects. The desaturase activity indices estimated from the serum lipid ester composition are significantly related to corresponding indices studied in skeletal muscle phospholipids.

Key words: Fatty acid desaturase index: Dietary fat: Serum lipid fatty acid composition: Skeletal muscle

The fatty acid composition of the diet is one important determinant of the health effects of food. A low proportion of unsaturated fat and a high proportion of saturated fat in the diet have been related to an increased risk for atherosclerotic CVD[1] and diabetes[2]. The assessment of fat intake from different food sources is, however, associated with substantial measurement errors. The fatty acid composition of the diet is reflected in the fatty acid composition of body tissues, and analysis of the plasma fatty acid composition is probably a more objective and accurate way to mirror dietary fat quality[3,4].

Not only the content of fatty acids in the diet, but also the endogenous metabolism of fatty acids, e.g. by elongation and desaturation, will influence their effects in the body[5,6]. Δ9-Desaturase (stearoyl-CoA desaturase 1, SCD-1) activity has an important role in modulating the intracellular effects of SFA, as demonstrated *in vitro* in human adipocytes[7] and myotubes[8]. A high SCD-1 activity index has been associated with obesity[9–11], hypertriacylglycerolaemia[12] and the metabolic syndrome[13], as well as with an increased risk to develop insulin resistance[14] and cardiovascular death and total death[15]. In contrast, the Δ5-desaturase (D5D) index in serum[10,11] and skeletal muscle[9] is associated with insulin sensitivity. A high D5D index in serum predicts a reduced risk to develop the metabolic syndrome[13] and total as well as cardiovascular death[15].

Much of our knowledge regarding the nutritional regulation of fatty acid desaturases (FADS) is derived from experimental studies in animal models, while information from human studies is sparse. The aim of the present study was to investigate how a change in dietary fat quality only, keeping all other aspects of the diet unchanged, influences the indices of FADS activity in human serum lipids and skeletal muscle tissue. To measure mRNA or protein expression of these enzymes in human tissues in a clinical study is not readily performed. We have studied the relationships (ratios) between fatty acid products and

Abbreviations: CE, cholesteryl ester; D5D, Δ5-desaturase; D6D, Δ6-desaturase; E%, percentage of energy; FADS, fatty acid desaturase; PL, phospholipid; SCD-1, stearoyl-CoA desaturase 1; SREBP, sterol regulatory element-binding protein.

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precursors in lipid esters in serum and skeletal muscle tissue to estimate the enzyme activities. There are animal studies, *in vitro* data[16–20] as well as human studies[21–24] supporting the assumption that these ratios may be used as indices of actual *SCD-1* gene expression. However, the ratios cannot be assumed to directly reflect desaturase activities. The rationale for using product:precursor ratios in tissue and blood lipids to reflect SCD-1 activity has recently been reviewed[25]. Polymorphisms in the genes *FADS1* and *FADS2*, encoding D5D and Δ6-desaturase (D6D), respectively, are associated with the ratios of arachidonic acid (20 : 4*n*-6) to linoleic acid (18 : 2*n*-6) and EPA (20 : 5*n*-3) to α-linolenic acid (18 : 3*n*-3) in erythrocyte membranes[26], with the fatty acid composition in serum phospholipids (PL)[27] and with plasma fatty acids[28]. They are also associated with fatty acid profiles in erythrocytes, reflecting altered D5D and D6D activities evaluated as product:precursor ratios[29].

The present study concerns the effects of a diet based on butter fat compared with a diet containing monounsaturated fat, as well as the effects of the addition of long-chain *n*-3 fatty acids on the indices of SCD-1, D5D and D6D activities in serum lipid esters and skeletal muscle lipids in healthy human subjects. The main interest concerned the comparison between a diet rich in butter fat and a diet containing MUFA. The hypothesis was that the butter-rich diet would induce a higher SCD-1 activity index than the diet containing monounsaturated fat.

Materials and methods

Design of the study

For the studies of the effects of dietary fat on desaturase activities, we have used earlier findings (B Vessby, I-B Gustafsson, S Tengblad and L Berglund, unpublished results) from the KANWU study[30], a controlled parallel study lasting 90 d. The study design and methods have been described in detail earlier[30]. Participants were randomly allocated to a butter-rich diet containing a high proportion of SFA (SFA diet) or to a diet containing MUFA (MUFA diet) due to a high content of oleic acid. Within the groups, there was a second random assignment to supplements of capsules containing fish oil (3·6 g *n*-3 fatty acids/d containing 2·4 g EPA and DHA, i.e. three capsules twice per d of Pikasol; Lube A/S) or placebo capsules containing the same amount of olive oil. The test period was preceded by a 2-week 'stabilisation period' on the habitual diet when all subjects received placebo capsules. Clinical tests were carried out during this period and participants completed a 3 d dietary record (two weekdays and one weekend day) to document pretrial dietary habits. In addition, two 3 d dietary records were completed at the beginning of the second and third months of the study period.

Subjects

The present study is restricted to the Swedish arm of the KANWU study, performed at Uppsala University, including twenty-seven men and seven women. Table 1 presents the clinical characteristics of the study subjects. Subjects with impaired glucose tolerance were included but those with diabetes were excluded. The degree of physical activity and alcohol intake did not change throughout the study. Subjects using lipid-lowering drugs, thiazide diuretics, β-blockers and corticosteroids were excluded. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee at the Medical Faculty of Uppsala University. Verbal informed consent was obtained from all subjects. Verbal consent was witnessed and formally recorded.

Table 1. Clinical characteristics of the participants
(Mean values and standard deviations, *n* 34)

	Mean	SD
Age (years)	50·2	8·0
BMI (kg/m^2)	25·7	2·5
SBP (mmHg)	125	13
DBP (mmHg)	74	10
P-glucose (mmol/l)	5·5	0·4
P-insulin (pmol/l)	40	28
S-TAG (mmol/l)	1·25	0·65
S-cholesterol (mmol/l)	5·26	0·71

SBP, systolic blood pressure; DBP, diastolic blood pressure; P, plasma; S, serum.

Diets

Participants were instructed to eat isoenergetic diets, with the same proportions of the main nutrients including similar amounts of total fat, but with a high proportion of SFA (SFA diet) or MUFA (MUFA diet). The diets were calculated to contain 37 % energy (E%) of fat with 17, 14 and 6 E% of SFA, MUFA and PUFA, respectively, in the SFA diet and 8, 23 and 6 E% in the MUFA diet using the database from the Swedish Food Administration. The estimated proportion of *trans*-fatty acids was low and similar in both diets. All participants were instructed before the study by trained dietitians on the preparation of their diets, with repeated contacts every second week thereafter to assure good adherence to the diet. They were all supplied with main dishes prepared to contain either mainly saturated or monounsaturated fat and edible fats to be used as spreads on bread, for cooking and in dressings. Core foods such as butter, margarine, oils and a range of other staple items were provided. Subjects were not informed about the type of diet they were following. The SFA diet included butter and table margarine containing a relatively high proportion of SFA. The MUFA diet included a spread and margarine containing high proportions of oleic acid derived from high-oleic sunflower oil with negligible amounts of *trans*-fatty acids and *n*-3 fatty acids.

The intake during the test period was calculated as the mean values of the dietary records provided during the second and third months of the study. Data on margarines and other specially prepared foods were entered onto the database for inclusion in the analyses. Serum lipid fatty acid composition was measured to confirm the validity of the reported dietary fatty acid intake.

Clinical tests and laboratory analyses

Blood samples were drawn after a 12 h overnight fast from an antecubital vein. A number of clinical tests and analyses were performed before and at the end of the study. In the Uppsala cohort, a skeletal muscle biopsy was performed at day 90 for determination of the fatty acid composition in the skeletal muscle tissue. A muscle sample was obtained from the musculus quadriceps femoris (vastus lateralis) by an incision through the skin and fascia from the mid-lateral part of the muscle under local anaesthesia using a Bergstrom needle[31], and then immediately frozen and stored at −70°C until analysis. Thereafter, one part of the sample (15–30 mg) was homogenised, extracted overnight and separated by TLC for analysis of the fatty acid composition.

The fatty acid composition of serum and skeletal muscle lipids was determined by GLC, after separation of the fatty acid fractions by TLC, using a 25 m NB-351 silica capillary column, essentially as described earlier[32]. For plasma fatty acids, the CV between successive GC runs was 0·2–5 %. For determination of the proportions of fatty acids in skeletal muscle PL, based on duplicate samples, the CV was less than 10 % for all the fatty acids with the exception of palmitoleic acid (16 : 1*n*-7), heptadecanoic acid (17 : 0) and α-linolenic acid (18 : 3*n*-3), which were present in small amounts with larger variations between the analyses (CV 20, 28 and 44 %, respectively). For the proportions of fatty acids in skeletal muscle TAG, the CV was 10 % or less for all fatty acids with proportions larger than 0·5 % with the exception of α-linolenic acid (CV 13 %). The relative amount of fatty acids was expressed as a percentage of the total amount of fatty acids reported.

To estimate the desaturase activities, we used the product: precursor ratios of individual fatty acids in lipid esters according to the following: Δ9-desaturase (SCD-1) activity index, 16 : 1*n*-7/16 : 0 or 18 : 1/18 : 0 (see below); D6D activity index, 18 : 3*n*-6/18 : 2*n*-6 (or 20 : 3*n*-6/18 : 2*n*-6 in PL due to a very low proportion of 18 : 3*n*-6); D5D activity index, 20 : 4*n*-6/20 : 3*n*-6.

Statistical methods

Results for continuous variables are presented as means and standard variations. For variables with a skewed distribution, a logarithmic transformation was made before the statistical analysis. For each outcome variable, treatment effects were estimated from a statistical model in which treatment categories (SFA diet/MUFA diet and the presence/absence of *n*-3 fatty acids) and their interactions were analysed. Factors including age, sex and the baseline value of the outcome variable were considered as covariates. For outcome variables where the interaction between the analysed factors and the presence or absence of *n*-3 fatty acids was non-significant, a limited model was used excluding those terms. Results of the analyses are presented as adjusted mean treatment effects within groups and their *P* values. Furthermore, the difference between the treatment groups for adjusted mean treatment effects is presented. Due to the large number of statistical tests performed, $P<0{\cdot}01$ was chosen for statistical significance to reduce the risk for false mass significances.

Results

Clinical characteristics

The clinical characteristics of the participants randomised to the SFA and MUFA diets were closely similar, as were the characteristics of those randomised to supplementation with *n*-3 fatty acids (Pikasol) and placebo (Table S1, available online). Body weight remained unchanged during the study.

Diet composition

The only significant difference between the two diets concerned the content of SFA (17·6 E% on the SFA diet and 9·2 E% on the MUFA diet) and MUFA (10·3 and 19·5 E%, respectively), which were close to the targeted values, and cholesterol which was higher in the SFA diet. Importantly, the proportions of other nutrients were similar during the two dietary periods, including the amount of total fat and the proportion of PUFA (Table S2, available online). This was also true for the choice of foods in the diets during the test periods, including the type of carbohydrate-rich foods, with an identical content of dietary fibre. The nutrient composition of the diets of the subjects randomised to achieve *n*-3 fatty acids (Pikasol) and placebo capsules was close to identical (Table S3, available online).

Fatty acid composition in serum

Fatty acid compositions in serum in the SFA and MUFA groups were similar before the test periods (Table S4, available online) as were those of subjects randomised to supplementation with *n*-3 fatty acids or placebo (Table S5, available online).

There was an expected reduction in the proportion of most SFA after the MUFA period compared with the SFA period in both cholesteryl esters (CE) and PL (Table S4, available online). The exception was stearic acid (18 : 0), which remained unchanged. During the MUFA diet, there was a substantial increase in oleic acid (18 : 1) in both CE and PL ($P<0{\cdot}0001$). Although the difference in changes in palmitic acid (16 : 0) in serum during the two periods was not clearly seen in CE ($P=0{\cdot}03$, NS), these changes were significantly different in PL ($P=0{\cdot}0009$). A clear difference regarding the changes in palmitoleic acid (16 : 1*n*-7) (which decreased on the MUFA diet) was seen in CE ($P=0{\cdot}002$). This difference was not significant in PL. There was a difference between the changes in linoleic acid during the SFA and MUFA periods in CE ($P=0{\cdot}009$) with a certain decrease during the MUFA period compared with the SFA period. Such a difference was not apparent in PL. There were no differences between the two periods regarding all long-chain PUFA with the exception of an increase in docosapentaenoic acid (22 : 5*n*-3) in PL during the SFA period, which significantly differed from that during the MUFA period ($P=0{\cdot}002$).

Supplementation with *n*-3 fatty acids caused no significant changes in any SFA or MUFA in serum CE (Table S5, available online). In PL, there was, on the other hand, a significant increase in stearic acid ($P=0{\cdot}009$) and a clear reduction in

Table 2. Fatty acid desaturase activities estimated from the serum cholesteryl ester (CE) and phospholipid (PL) fatty acid compositions in subjects randomised to the SFA (*n* 17) and MUFA (*n* 17) diets, respectively*

(Mean values and standard deviations)

	Before the test period				Changes during the test period				
	SFA diet		MUFA diet		SFA diet		MUFA diet		Difference between the changes
Desaturase ratios	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
Δ9-Desaturase (16 : 1*n*-7 to 16 : 0)									
CE	0·32	0·07	0·31	0·08	−0·002	0·059	−0·065	0·084	0·009
PL	0·026	0·005	0·025	0·007	−0·001	0·004	−0·005	0·007	NS
Δ6-Desaturase (18 : 3*n*-6 to 18 : 2*n*-6)									
CE	0·016	0·008	0·016	0·006	−0·002	0·006	0·000	0·007	NS
Δ6-Desaturase (20 : 3*n*-6 to 18 : 2*n*-6)									
PL	0·16	0·05	0·14	0·03	−0·014	0·034	0·011	0·047	NS
Δ5-Desaturase (20 : 4*n*-6 to 20 : 3*n*-6)									
CE	7·89	1·87	8·08	1·7	2·09	3·02	1·68	2·84	NS
PL	2·68	0·65	2·70	0·54	0·70	0·96	0·50	0·99	NS

*Treatment effects were estimated from a statistical model in which treatment categories (SFA diet/MUFA diet and the presence/absence of *n*-3 fatty acids) and their interactions were analysed.

oleic acid ($P<0·0001$) after supplementation with *n*-3 fatty acids compared with placebo. Linoleic acid levels were reduced in both CE ($P<0·0001$) and PL ($P<0·0001$). The proportions of dihomo-γ-linolenic acid (20 : 3*n*-6) were reduced in both CE and PL, while arachidonic acid (20 : 4*n*-6) was significantly reduced only in PL ($P<0·0001$). All long-chain *n*-3 fatty acids increased during Pikasol supplementation as expected ($P<0·0001$ for all).

Effects of dietary fat changes on desaturase indices in serum

Tables 2 and 3 show the FADS indices estimated from the CE and PL fatty acid compositions in serum before the diet periods, respectively, and the changes in indices during the test periods as related to the changes in dietary fat quality. There was a significantly reduced SCD-1 index ($P=0·009$), as mirrored by the ratio 16 : 1*n*-7 to 16 : 0 in CE, by 20 % on the MUFA diet compared with the SFA diet (Table 2). The difference was not significant when analysing PL. An increase in 18 : 1 to 18 : 0 during the MUFA diet seen in both CE and PL was, on the other hand, directly related to the high proportion of oleic acid in the MUFA diet and cannot be related to SCD-1 activity. There was no indication of any changes in the D6D or D5D index due to the different fat types in the SFA and MUFA diets.

Supplementation with long-chain *n*-3 fatty acids (Table 3) caused significant changes in the indices of SCD-1, D6D and D5D activities in both serum CE and PL. A reduction in the SCD-1 index during *n*-3 supplementation, compared

Table 3. Fatty acid desaturase activities estimated from the serum cholesteryl ester (CE) and phospholipid (PL) fatty acid compositions in subjects randomised to supplementation with Pikasol (3·4 g/d) (*n* 17) or placebo (*n* 17), respectively*

(Mean values and standard deviations)

	Before the test period				Changes during the test period				
	Pikasol		Placebo		Pikasol		Placebo		Difference between the changes
Desaturase ratios	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
Δ9-Desaturase (16 : 1*n*-7 to 16 : 0)									
CE	0·32	0·07	0·31	0·08	−0·069	0·063	0·002	0·074	0·003
PL	0·027	0·008	0·025	0·007	−0·006	0·004	−0·001	0·007	NS
(18 : 1*n*-9 to 18 : 0)									
CE	23·5	5·2	24·7	2·9	3·27	8·05	3·38	8·01	NS
PL	0·94	0·10	0·89	0·09	−0·09	0·155	0·068	0·183	<0·0001
Δ6-Desaturase (18 : 3*n*-6 to 18 : 2*n*-6)									
CE	0·015	0·008	0·017	0·006	−0·005	0·005	0·004	0·005	<0·0001
(20 : 3*n*-6 to 18 : 2*n*-6)									
PL	0·15	0·04	0·16	0·05	−0·031	0·030	0·028	0·033	<0·0001
Δ5-Desaturase (20 : 4*n*-6 to 20 : 3*n*-6)									
CE	7·87	1·6	8·11	2·0	3·99	2·42	−0·21	1·15	<0·0001
PL	2·68	0·52	2·70	0·67	1·32	0·78	−0·12	0·42	<0·001

*Treatment effects were estimated from a statistical model in which treatment categories (SFA diet/MUFA diet and the presence/absence of *n*-3 fatty acids) and their interactions were analysed.

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with the placebo period, was seen as a significant lowering of the ratio 16:1*n*-7 to 16:0 (in CE) and 18:1 to 18:0 (in PL). Here, the ratio 18:1 to 18:0 could be considered to reflect SCD-1 activity, as oleic acid intake in the diet did not differ between the groups. The magnitude of the reduction in the SCD-1 index during Pikasol supplementation, compared with placebo, was similar to that on the MUFA diet when compared with the SFA diet. In addition, there was a significant decrease in the D6D index and an increase in the D5D index due to the addition of *n*-3 seen in both CE and PL.

Fatty acid composition in skeletal muscle

Skeletal muscle PL after the SFA diet showed a similar scenario to that in serum lipid esters with significantly higher 14:0 ($P<0{\cdot}0001$), 15:0 ($P<0{\cdot}0002$) and 17:0 ($P<0{\cdot}0001$) compared with the MUFA diet, while palmitic and stearic acids were not significantly different (Table S6, available online). The concentration of 16:1*n*-7 was significantly higher after the SFA diet ($P=0{\cdot}002$), while 18:1, due to high dietary intake, was higher after the MUFA period. With the exception of a higher concentration of 22:5*n*-3 after the SFA diet ($P=0{\cdot}002$), there were no significant differences between the two diets regarding all the PUFA. Similar differences between the two diets were seen in skeletal muscle TAG (Table S7, available online) regarding 14:0, 16:1*n*-7 and 18:1. In addition, palmitic acid ($P=0{\cdot}008$) was significantly higher in skeletal muscle TAG after the SFA diet compared with the MUFA diet.

Supplementation with *n*-3 fatty acids (Table S7, available online) caused a significant reduction in oleic acid and all *n*-6 PUFA in skeletal muscle PL, while the proportions of long-chain *n*-3 fatty acids increased (all $P<0{\cdot}0001$). The only effect of Pikasol seen in skeletal muscle TAG was a significant increase in the proportions of 22:5*n*-3 ($P=0{\cdot}0007$) and 22:6*n*-3 ($P=0{\cdot}0002$).

Effects of dietary fat changes on desaturase indices in skeletal muscle fatty acids

The desaturase indices studied in skeletal muscle PL after the test periods are shown in Table 4. The SCD-1 index, as estimated from the ratio between 16:1*n*-7 and 16:0, was significantly different when comparing the SFA and MUFA diets (higher after the SFA diet). Again, the higher 18:1 to 18:0 ratio on the MUFA diet is ascribed to the dietary intake of 18:1. No diverging effects of SFA and MUFA were seen on the D6D and D5D indices. Long-chain *n*-3 fatty acids reduced the SCD-1 index, demonstrated by a significantly reduced ratio between 18:1 and 18:0 in PL. The same tendency (NS) was seen for the ratio 16:1*n*-7 to 16:0. There was a significant reduction in D6D while the D5D index increased, in accordance with the changes seen in serum. Neither diet affected the desaturase indices calculated from the skeletal muscle TAG fatty acid compositions.

Associations between the desaturase indices estimated from serum lipid ester and skeletal muscle phospholipid fatty acid compositions

There were strong associations between the desaturase indices estimated from the fatty acid composition of serum lipids at the end of the intervention period and corresponding indices calculated from the skeletal muscle PL. Thus, there were positive correlations between the SCD-1 index assessed in serum CE and PL, on the one hand, and the corresponding index in skeletal muscle PL, on the other hand (r 0·58, $P<0{\cdot}001$ and r 0·56, $P<0{\cdot}01$, respectively). The ratio 16:1*n*-7 to 16:0 in CE and PL was also strongly associated with the D6D index (r 0·59, $P<0{\cdot}001$ and r 0·55, $P<0{\cdot}01$, respectively) and the D5D index (r −0·51, $P<0{\cdot}01$ and r −0·53, $P<0{\cdot}01$) as mirrored in skeletal muscle PL. The D6D activity index assessed in serum CE and PL was closely correlated with the D6D index in skeletal muscle PL (r 0·65, $P<0{\cdot}001$ and r 0·80, $P<0{\cdot}001$,

Table 4. Indices of fatty acid desaturase activities in skeletal muscle phospholipids (PL) and TAG after the change in dietary fat type (SFA *v.* MUFA) and supplementation with Pikasol (3·4 g/d) or placebo, respectively*

(Mean values and standard deviations)

	After the dietary intervention					After *n*-3 supplementation				
	SFA diet		MUFA diet			Pikasol		Placebo		
Desaturase ratios	Mean	SD	Mean	SD	Difference (*P*)	Mean	SD	Mean	SD	Difference (*P*)
Δ9-Desaturase (16:1*n*-7 to 16:0)										
TAG	0·30	0·08	0·25	0·10	NS	0·27	0·10	0·28	0·08	NS
PL	0·034	0·01	0·026	0·01	0·0052	0·027	0·008	0·032	0·013	NS
(18:1*n*-9 to 18:0)										
TAG						10·1	3·3	11·2	3·6	NS
PL						0·86	0·20	0·97	0·16	0·0008
Δ6-Desaturase (20:3*n*-6 to 18:2*n*-6)										
TAG	0·015	0·003	0·017	0·007	NS	0·015	0·005	0·017	0·005	NS
PL	0·038	0·007	0·036	0·008	NS	0·032	0·004	0·042	0·007	0·0001
Δ5-Desaturase (20:4*n*-6 to 20:3*n*-6)										
TAG	2·42	0·51	2·20	0·56	NS	2·29	0·42	2·36	0·66	NS
PL	9·1	1·4	10·1	2·1	NS	10·6	1·7	8·70	1·4	0·0085

*Treatment effects were estimated from a statistical model in which treatment categories (SFA diet/MUFA diet and the presence/absence of *n*-3 fatty acids) and their interactions were analysed.

respectively), as was the D5D index in both serum CE and PL with the D5D index in skeletal muscle PL (r 0·78, $P<0·001$ and r 0·81, $P<0·001$, respectively).

Discussion

The present study investigates the effects of the changes in dietary fat quality on the indices of FADS activities (product: precursor ratios), estimated in serum lipids and skeletal muscle, in healthy subjects. FADS are important regulators of the endogenous metabolism of fatty acids, derived from exogenous as well as endogenous sources[5,6,33]. Δ9-Desaturase (SCD-1) regulates the desaturation of SFA, mainly stearic (18 : 0) and palmitic (16 : 0) acid, to the corresponding MUFA, by introducing a double bond in the Δ9 position. D6D and D5D regulate the desaturation of linoleic acid (18 : 2*n*-6) and α-linolenic acid (18 : 3*n*-3) to their polyunsaturated metabolites of the *n*-6 and *n*-3 series.

The desaturases are widely expressed in many tissues in the body including liver, skeletal muscle, adipose tissue, skin and the pancreatic β-cell. The expression of desaturase activities is regulated by a number of different nutrients including fatty acids, hormones and environmental factors as reviewed elsewhere[5,6,25,33]. While PUFA are known to influence desaturase activities, there is little information, if any, directly comparing the effects of dietary fats rich in SFA and MUFA in human subjects. In the present study, we compared the effects on the indices of desaturase activities of a diet containing butter, with a high proportion of SFA and more cholesterol, with those of a diet rich in monounsaturated oleic acid. The proportions of PUFA in the diets were the same. In addition, we recorded the consequences of supplementation with long-chain *n*-3 PUFA.

In healthy human subjects on a high-fat diet, the majority of SFA in body tissues are derived from the diet, while net *de novo* lipogenesis is restricted[34–36]. The major MUFA in plasma, oleic (18 : 1*n*-9) and palmitoleic (16 : 1*n*-7) acids, may either come from food or be formed in the body through the action of SCD-1. The content of 16 : 1*n*-7 is usually low in the diet with small variations. The only known major dietary source of 16 : 1*n*-7 is macadamia nuts, while other nuts are shown to be rich sources of 18 : 1 with a very low content of 16 : 1*n*-7[37]. Macadamia nuts were not included, and minor dietary sources of 16 : 1*n*-7, e.g. fatty fish, were identical in both diets. This means that the proportion of 16 : 1*n*-7, when related to 16 : 0, may be considered to reflect SCD-1 activity[25]. Oleic acid (18 : 1), on the other hand, is mostly not a useful indicator of SCD-1 activity as the Western diet contains considerable, and variable, amounts of oleic acid. The enrichment of monounsaturated fat in the MUFA diet in the present study was achieved by the inclusion of spreads, oils and margarines with a high content of oleic acid.

In the present study, the comparison between two diets rich in SFA and MUFA, respectively, showed that a diet containing a high proportion of butter fat, compared with a diet rich in monounsaturated oleic acid, was accompanied by a higher SCD-1 activity index as estimated from the serum lipid ester fatty acid composition (Table 2). The D6D and D5D indices remained unchanged. Suppression of SCD by dietary fat is unique to *n*-6 and *n*-3 PUFA[5,25,33]. Oleic acid has no effect on SCD-1 expression or protein levels[38]. Experimental studies have shown that SCD-1 mediates the pro-lipogenic effects of SFA in the diet[39]. SFA induce an increase in *SCD-1* mRNA levels in mice[40] and palmitate increases *SCD-1* expression in cultured myotubes[8]. Rodent models indicate that SCD-1 activity is up-regulated in skeletal muscle cells, under conditions of saturated fat exposition, or in obesity[41]. It has been suggested that SCD-1 has an important role in modulating the intracellular effects of SFA by converting them to less harmful MUFA[7,8]. Thus, a high SCD-1 activity index in plasma may probably be considered as a reflection of high exposure to (exogenously or endogenously synthesised) saturated fat. This is compatible with the associations with obesity[9–11], hypertriacylglycerolaemia[12] and the metabolic syndrome[13], as well as with an increased risk to develop insulin resistance[14] and cardiovascular death[15]. A high D5D activity index in skeletal muscle[9] and serum[10,11], on the other hand, is associated with good insulin sensitivity and a reduced risk to develop the metabolic syndrome[16] and cardiovascular death[15]. An extensive review of the evidence for SCD and the risk of disease has recently been published[25].

SFA could mediate their effects on the SCD-1 promoter by directly increasing sterol regulatory element-binding protein (SREBP)-1c expression, or increasing the expression of peroxisome proliferator-activated receptor-γ coactivator-1β (PGC1-β), a SREBP-1c co-activator[42]. In addition, it is possible that the higher content of cholesterol in the SFA diet may have contributed to the increased SCD-1 activity, by opposing the repression of the gene mediated by PUFA, by a SREBP-1-independent mechanism[43]. Supplementation with long-chain *n*-3 fatty acids (Table 3) reduced the D6D index and increased the D5D activity index, in addition to a decrease in the SCD-1 index. Stable isotope studies in human subjects[44,45] have indicated a feedback control of D6D by dietary long-chain *n*-3 fatty acids. A transcriptional suppression of SCD-1 and D6D by dietary factors has been ascribed to long-chain PUFA of the *n*-6 and *n*-3 series, probably through the suppressed activity of SREBP-1c[5]. Experimental studies have indicated that both D6D and D5D are suppressed by dietary PUFA[5]. Whether the increase in the D5D index in the present study reflects a true increase in enzyme activity, or rather is a consequence of a reduced availability of the substrate due to the suppression of D6D activity, remains to be clarified.

Similar effects on the desaturase indices were seen not only in serum CE and PL, but also in skeletal muscle PL (Table 4). Associations between the indices of desaturase activities in serum lipid esters and in skeletal muscle PL were strong and highly significant. It suggests that estimations of the effects of dietary fat on desaturase activity indices in plasma lipids may closely reflect the effects on desaturase indices in other body tissues, as shown here for skeletal muscle. This is of practical importance as serum or plasma is readily available, while more invasive techniques are needed to obtain samples from other body tissues.

If the desaturation index is used to estimate enzyme activity, it should preferentially be used in defined lipid

fractions[24,25,46]. The specificity of PL and CE biosynthesis will have an important impact on the interpretation of fatty acid ratios as markers of fatty acid desaturation. Each hepatic lipid fraction has a characteristic fatty acid ratio[24]. Comparing the SCD-1 activity index 16 : 1/16 : 0 in hepatic lipid fractions with hepatic *SCD-1* mRNA expression showed strong associations with the SCD-1 activity indices of hepatic TAG, NEFA, CE and PL. The relationships to the 18 : 1/18 : 0 index were weaker or absent[23]. A high ratio between 16 : 1*n*-7 and 16 : 0 in serum CE presumably mainly reflects a high SCD-1 activity index in the liver. Changes in the SCD-1 index in serum cholesterol esters were closely associated with a reduction in liver fat on a PUFA-rich diet[47]. In the present study, changes in the ratio 16 : 1*n*-7 to 16 : 0 were more clearly seen in serum CE than in PL, probably at least partly explained by higher levels and less variation in the proportions of 16 : 1*n*-7 in CE. It has been suggested[25] that plasma PL, which reflect a number of lipid pools, may be less useful as a biomarker of SCD-1 activity.

Changes in SCD-1 activity in adipose tissue, e.g. related to increased neolipogenesis[7], may, on the other hand, probably preferentially be studied in fasting plasma NEFA[11] when adipose tissue samples are not at hand. SCD-1 activity in adipocytes[7] and skeletal muscle cells[8] is positively associated with insulin sensitivity. Accordingly, a high proportion of 16 : 1*n*-7 in plasma NEFA mirroring a high SCD-1 activity index in adipose tissue may be directly related to insulin sensitivity[48].

The present study indicates that an increased intake of butter fat rich in SFA and cholesterol, when exchanged for oleic acid rich in MUFA, in human subjects is also associated with a higher SCD-1 activity index – when other components of the diet are kept unchanged. An alternative cause of increased SCD-1 activity is *de novo* lipogenesis from carbohydrates in the liver due to an excessive intake of sugar and refined carbohydrates[49,50]. In the present study, however, the amount of carbohydrates, as well as the type of carbohydrate-rich foods, was the same during the test periods.

There is a sex-specific effect on SCD-1 expression, and the ratio 16 : 1 to 16 : 0 in CE seems to be higher in women than in men[25]. In the present study, men and women were randomly divided into the two intervention groups (Table S1, available online). The small number of women included does not allow a study of possible sex differences in response to the changes in the type of dietary fat, but sex is used as a covariate in the statistical analyses. Induction of SCD-1 is insulin responsive[5,25]. Metabolic variables, including fasting insulin and insulin sensitivity index, were well comparable in the intervention groups (Table S1, available online). In order to reduce the risk that different levels before the intervention (e.g. due to insulin resistance) would confound the results, baseline values of the outcome variables have been used as covariates in the statistical analyses. The desaturase indices in the two groups before the intervention were closely similar (Tables 2 and 3).

Studies of the indices of FADS activity are of value to understand how fatty acid metabolism and related health effects are influenced by exogenous as well as endogenous factors. This is, as far as we know, the first controlled study in human subjects where a comparison of the effects of a diet based on butter fat with a diet containing monounsaturated fat, keeping all other dietary components constant, shows a higher SCD-1 activity index on a diet rich in butter fat than on a diet containing monounsaturated fat. This was apparent when assessed in serum lipid esters as well as in skeletal muscle PL. The indices of D6D and D5D activities remained unaffected. Supplementation with long-chain *n*-3 fatty acids also showed, in addition to a reduced SCD-1 index, a lower D6D activity index and a significantly increased D5D activity index.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114512005934

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