

Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men

L. S. Piers^{1*}, Karen Z. Walker², Rachel M. Stoney³, Mario J. Soares⁴ and Kerin O'Dea¹

¹Menzies School of Health Research, Casuarina, Northern Territory, Australia

²Nutrition and Dietetics, Department of Medicine, Monash University, Monash Medical Centre, Clayton, Victoria, Australia

³The Nutrition Department, The Alfred Hospital, Prahran, Victoria, Australia

⁴Department of Nutrition, Dietetics and Food Science, Curtin University of Technology, School of Public Health, Perth, Western Australia, Australia

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A randomised crossover study of eight overweight or obese men (aged 24–49 years, BMI 25.5–31.3 kg/m²), who followed two diets for 4 weeks each, was performed to determine whether substitution of saturated fat with monounsaturated fat affects body weight and composition. Subjects were provided with all food and beverages as modules (selected *ad libitum*) of constant macronutrient composition, but differing energy content. The % total energy from saturated fat, monounsaturated fat and polyunsaturated fat was 24, 13 and 3 % respectively on the saturated fatty acid (SFA)-rich diet and 11, 22 and 7 % respectively on the monounsaturated fatty acid (MUFA)-rich diet. MUFA accounted for about 80 % of the unsaturated fats consumed on both diets. Body composition, blood pressure, energy expenditure (resting and postprandial metabolic rates, substrate oxidation rate, physical activity), serum lipids, the fatty acid profile of serum cholesteryl esters and plasma glucose and insulin concentrations were measured before and after each diet period. Significant ($P \leq 0.05$) differences in total cholesterol and the fatty acid composition of serum cholesteryl esters provided evidence of dietary adherence. The men had a lower weight (-2.1 (SE 0.4) kg, $P=0.0015$) and fat mass (-2.6 (SE 0.6) kg, $P=0.0034$) at the end of the MUFA-rich diet as compared with values at the end of the SFA-rich diet. No significant differences were detected in energy or fat intake, energy expenditure, substrate oxidation rates or self-reported physical activity. Substituting dietary saturated with unsaturated fat, predominantly MUFA, can induce a small but significant loss of body weight and fat mass without a significant change in total energy or fat intake.

Obesity: Dietary fat: Weight loss: Body composition: Energy expenditure

Despite their established health benefits, diets with a high monounsaturated fatty acid (MUFA) content are often not promoted due to concerns that their relatively high dietary total fat content might promote obesity (Garg, 1994; deLorgeril & Salen, 2000), as fat *per se* not only forms highly palatable and energy-rich foods (Blundell & MacDiarmid, 1997), but is also efficiently stored in the body (Stubbs, 1998). Although population and intervention studies have suggested the importance of high-fat diets in the promotion of obesity (Lissner & Heitmann, 1995), a consensus establishing the relationship between a high fat intake and obesity has not yet been established (Bray & Popkin, 1998; Willet, 2002). Recent evidence implicates saturated fatty acids (SFA), rather than the unsaturated fats, in the development of obesity, due to the greater propensity for SFA to be stored in adipose tissue rather than being oxidised (Storlien *et al.*

1998, 2001). Studies using indirect calorimetry in human volunteers have indicated that polyunsaturated fatty acids (PUFA) are better oxidised than SFA in both normal weight (Jones & Schoeller, 1988) and obese men (Jones *et al.* 1992). Fewer studies, however, examine the fate of the nutritionally important MUFA. Jones *et al.* (1985), using stable isotope-labelled fatty acids of similar chain length, demonstrated that monounsaturated oleic acid (18:1) is more readily oxidised than saturated stearic acid (18:0) or polyunsaturated linoleic acid (18:2) (stearic:linoleic:oleic oxidation ratio 1.0:4.5:14.0). We too have previously reported a greater postprandial fat oxidation rate ($P < 0.05$) in fourteen men after consumption of a breakfast meal rich in MUFA, as compared with a SFA-rich meal (Piers *et al.* 2002). Together, these results indicate that the predominance of certain fatty acids in a given dietary fat

Abbreviations: CE, cholesteryl ester; EE, energy expenditure; FM, fat mass; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

* **Corresponding author:** Dr L. S. Piers, present address, Health Surveillance and Evaluation Section, Rural and Regional Health and Aged Care Services, Department of Human Services, Level 18, 120 Spencer Street, Melbourne, Victoria 3000, Australia, fax +61 3 9637 4763, email leonard.piers@dhs.vic.gov.au

influences its *in vivo* partitioning between oxidation and storage.

We, therefore, hypothesised that if a MUFA-rich diet were compared with a SFA-rich diet, the higher rate of fat oxidation on the MUFA-rich diet would result in reduced energy intake and/or the loss of body fat mass (FM). Reduced food intake would compensate for the greater amount of energy liberated from ingested food high in MUFA. Loss of FM would result if the rate of fat deposition declined while the rate of endogenous fat mobilisation remained stable. Furthermore, on a MUFA-rich diet, an enlarged body fat store would no longer be required to achieve high fat oxidation rates, as would be the case when poorly oxidisable SFA was consumed (Flatt, 1995).

To test these hypotheses, we invited all subjects from our previous acute study (Piers *et al.* 2002) to participate in a longer intervention study examining the influence of the type of dietary fat on energy intake, body weight and composition. Subjects were required to consume two diets of constant macronutrient composition and with a fat content reflecting that of an average western diet (about 40% total energy), but differing in fatty acid composition (one being rich in SFA and the other in MUFA) in a randomised crossover study. Each diet was consumed for 4 weeks.

Subjects and methods

Subjects

Subjects for the present study were recruited from participants of our earlier acute study investigating fat oxidation rates by indirect calorimetry (Piers *et al.* 2002). As the results of the earlier study were not available at that time, subjects were only told that we were interested in seeing if the consumption of two special diets, for 4 weeks each, resulted in any change in fat oxidation rates in the longer term. Subjects remained unaware that a change in body weight or composition might be expected. Eight of the fourteen volunteers agreed to participate in the intervention study and the remaining men declined due to the time commitment required. The eight male subjects (seven of European and one of South Asian ancestry) were aged between 24 and 49 years and were resident in Melbourne, Victoria, Australia. Inclusion criteria were as in the previous study (Piers *et al.* 2002) including: (1) absence of clinical signs or symptoms of chronic disease; (2) weight stability (± 2 kg for preceding 12 months); (3) not on medication affecting body composition; (4) BMI within the range 25–32 kg/m²; (5) resting diastolic blood pressure < 90 mmHg.

Study design

Subjects consumed two high-fat diets (40% energy) of fixed macronutrient composition. The diets were designed to differ only in the type of fat provided, one diet predominantly containing MUFA and the other predominantly containing SFA. In order to determine whether total energy intake varied with the type of dietary fat

consumed, each diet was administered in a modular form; this kept the proportion of macronutrients constant while permitting *ad libitum* energy intake. The order of administration of the diets was randomly assigned and there was no intervening washout period. Anthropometric measurements, body composition and blood pressure measurements were made initially and at the end of each diet period. Resting and postprandial metabolic rate were also measured in response to an isoenergetic test meal of constant macronutrient composition. However, the type of fat in the test meal reflected the type of fat that had been predominantly consumed in the associated diet period. Fasting and 2 h postprandial venous blood samples were collected on these occasions. In addition, subjects were required to maintain a self-reported physical activity diary daily for the entire duration of the two diet periods.

Diets

Sources of fat for the SFA-rich diet were milk, butter, cream, cheese and fatty meat, while fat in the MUFA-rich diet was provided from olive oil, nuts and avocados. Each diet comprised forty modules, paired between diets for similar energy, protein, carbohydrate, total fat and fibre content, and with the exception of the fat source, based on largely similar foods. Each diet contained ten breakfast modules (0.7–2.2 MJ), ten lunch modules (0.9–4.1 MJ), ten dinner modules (3.1–5.3 MJ) and ten snack modules (1.0–4.2 MJ). The forty modules provided for the SFA-rich diet contained on average (% energy): fat 40.3 (SD 0.8), SFA 24.4 (SD 2.6), MUFA 12.5 (SD 1.5), carbohydrate 41.9 (SD 3.7). The forty modules provided for the MUFA-rich diet were designed to contain on average (% energy): fat 40.1 (SD 1.1), SFA 11.0 (SD 0.9), MUFA 22.3 (SD 1.7), carbohydrate 42.2 (SD 3.6). An exception was two paired snack modules containing wine or beer (% energy): alcohol 19.2, fat 40.7, carbohydrate 27.2. The MUFA-rich diet modules provided slightly more dietary fibre than the SFA-rich diet modules (3.4 (SD 1.8) v. 2.8 (SD 1.9) g/MJ respectively). Examples of the modules are provided in Table 1. The macronutrient composition of each module, and the module recipe can be obtained by contacting the corresponding author (L. S. P.).

Each week, each subject chose freely from the module menu and could consume their chosen modules in any order and in any quantity, each day. Modules were always provided in excess of intake requirements. Subjects were instructed that once the consumption of a module was started, the entire module had to be consumed. No food other than that provided was allowed. All food was prepared under the supervision of a research dietitian at The Kingston Centre (Cheltenham, Victoria, Australia), a hospital for geriatric patients with large food service facilities. Modules were given to subjects mainly in a ready-to-eat form. Some modules required reheating or simple cooking. Detailed storage and preparation instructions were provided for each module. Each subject completed, on a daily basis, a record detailing all modules consumed each day, over the four weeks of each diet.

Table 1. Examples of modules provided in the saturated fatty acid-rich and monounsaturated fatty acid-rich test diets

| Module | SFA-rich modules | MUFA-rich modules |
|--------|---|---|
| 1 | Natural muesli with creamy milk | Toasted muesli with skimmed milk |
| 2 | Tomatoes on buttered toast | Tomatoes fried in olive oil, toast |
| 4 | Poached egg, toast and butter, orange juice | Egg fried in olive oil, toast, skimmed milk, orange juice |
| 5 | Cornflakes and creamy milk | Cornflakes, cashews and skimmed milk |
| 14 | Muffins, butter and cottage cheese | Muffins, high monounsaturated fat margarine, cottage cheese |
| 15 | Vegetable soup and toast with butter | Soup made with vegetables fried in olive oil, toast |
| 22 | Rump steak stir-fried with celery and butter, boiled rice | Rump steak stir-fried with celery, almonds and olive oil, boiled rice |
| 24 | Fish cooked in butter, peas, carrots, potato, oranges | Fish fried in olive oil, peas, carrots, potato, oranges |
| 27 | Roast chicken with carrots and beans, baked potato with butter and soured cream, canned apricots with cream | Roast chicken with carrots and beans, baked potato with high-monounsaturated fat margarine, canned apricots |
| 31 | Cheesecake and ice cream, a glass of creamy milk and a banana | Fruit cake, ice cream, almonds, and a glass of skimmed milk |
| 33 | Chocolate biscuit with glass of creamy milk | Ginger biscuit, almonds and a glass of skimmed milk |
| 37 | One glass of white wine, cheese and sultanas | One glass of white wine, almonds and sultanas |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid.

Breakfast meals used for postprandial studies

The SFA-rich breakfast comprised 92 g natural Swiss muesli (Uncle Toby's; Wahgunyah, Victoria, Australia), 57 g cream (Bulla, Regal Cream Products; Colac, Victoria, Australia) and 275 g skimmed milk (Australian Milk Marketing; Abbotsford, Victoria, Australia). The MUFA-rich breakfast consisted of 96 g muesli baked with 20 g extra virgin olive oil (Bertolli; Preston, Victoria, Australia) and served with 285 g skimmed milk. Both breakfast meals, which have been used previously (Piers *et al.* 2002), provided 2.5 MJ energy: 15% as protein, 43% as fat and 42% as carbohydrate. The fibre content of the SFA-rich breakfast was 12.0 g, while that of the MUFA-rich breakfast was 11.5 g. The SFA-rich breakfast contained 4.1 g PUFA, 8.8 g MUFA and 16.1 g SFA, while the MUFA-rich breakfast contained 5.6 g PUFA, 18.6 g MUFA and 4.9 g SFA, as determined from Australian food composition tables (NUTTAB95; English & Lewis, 1991).

Diet assessment questionnaire

At the end of each diet, subjects were given a questionnaire regarding their assessment of diet quality. For each question, they were asked to indicate their response by marking a cross on an unmarked 100 mm line extending between two extreme ratings (0, 100) and the length to the mark was then measured with a ruler. The questions were: (1) How do you rate this diet overall? (2) How did this diet satisfy your hunger? (3) How do you describe the taste of this diet? (4) How energetic did you feel while on this diet?

Anthropometry

Standing height was measured using a wall-fixed stadiometer and recorded to the nearest 1 mm. Body weight was measured immediately after voiding and after an overnight fast. Subjects wore light indoor clothing and no shoes. The measurement was taken on a beam balance and was recorded to the nearest 100 g. Waist and hip

circumferences were measured as described by Callaway *et al.* (1988).

Body composition

Dual-energy X-ray absorptiometry whole-body scans were performed as described by Piers *et al.* (2002). Total and regional body composition was determined with Lunar software (Lunar software version 1.3; Madison, WI, USA) used in the extended research mode to provide data on whole-body and regional (limbs and trunk) lean tissue mass and FM (g).

Resting and postprandial metabolic rate

Resting and postprandial metabolic rate was measured by indirect calorimetry using a Deltatrac II metabolic monitor (Datex Instrumentarium Corp., Helsinki, Finland), an open-circuit ventilated canopy measurement system. The measurement was conducted under standardised conditions using the same measurement protocol as employed in our previous study (Piers *et al.* 2002).

Substrate oxidation rates

Whole-body substrate oxidation rates were calculated using measures of O₂ consumption, CO₂ production, total urinary N excretion and the equations of Ben-Porat *et al.* (1983), with the assumption that N excretion was steady over the duration of urine collection (Elia & Livesey, 1988; Livesey & Elia, 1988).

Blood pressure and pulse rate

Systolic and diastolic blood pressure and pulse rate were measured before and after each diet period under standardised conditions (after an overnight fast, in the post-absorptive state, after having rested in the supine position for at least 1 h) using a Dinamap portable adult vital signs monitor (model 100; Critikon, Tampa, FL, USA).

Physical activity assessment

All subjects were asked to maintain a daily physical activity diary. The diary consisted of four 15 min blocks for each hour of the day from 06.00 to 23.00 hours. It was assumed that subjects slept between 23.00 and 06.00 hours, but they were asked to indicate their actual waking and sleeping times when there was any deviation from this pattern. Subjects were asked to write down the nature of the activity predominantly performed during each 15 min block. These activities were then coded depending upon the type and intensity of activity. The total number of 15 min blocks at each activity level was determined. Energy expenditure (EE) at each level of activity was obtained by multiplying the measured resting metabolic rate (kJ per 15 min) by an activity factor obtained from the literature (Food and Agriculture Organization/World Health Organization/United Nations University, 1985), appropriate for the type of activity being performed, and by the number of 15 min blocks for which the activity was performed during the day. EE during sleep was assumed to be equal to resting metabolic rate measured at the end of each diet period. The sum of the EE associated with the levels of activity over 24 h gave total EE (kJ/d). Physical activity levels were calculated by dividing the total EE (kJ/d) obtained from the self-reported activity diaries by measured resting metabolic rate (kJ/d).

Blood samples

Fasting and 2 h postprandial (following the standard breakfast meal) blood samples (10 ml) were collected from all subjects before and at the end of each diet period. The samples were centrifuged at 2000 g for 15 min before plasma was separated and stored at -70°C . Plasma glucose, insulin concentrations and the lipid profile were measured in the fasting sample as described by Piers *et al.* (2002).

Serum fatty acid analysis

Fatty acid analysis was carried out at the Child Nutrition Research Centre, Flinders Medical Centre, South Australia, Australia. Lipids were extracted from serum samples with chloroform-methanol (Bligh & Dyer, 1959). Serum phospholipids and cholesteryl esters (CE) were separated by TLC and evaporated to dryness under N_2 . The samples were methylated in H_2SO_4 (10 ml/l methanol) at 70°C for 3 h. When cooled, methyl esters were extracted into *n*-heptane and transferred to vials containing anhydrous Na_2SO_4 as the dehydrating agent. Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 6890 GC (Hewlett-Packard, Palo Alto, CA, USA) equipped with a 50 mm capillary column (internal diameter 0.33 mm) coated with BPX-70 (0.25 μm film thickness; SGE Pty Ltd, Victoria, Australia). The injector temperature was set at 250°C while the detector (flame ionisation) temperature was 300°C . The oven temperature commenced at 140°C and was programmed to rise at 5°C per min to 220°C . The carrier gas He was used at a velocity of

350 mm/s. Fatty acid methyl esters were identified based on retention time relative to authentic lipid standards (Nuchek Prep Inc., Elysian, MN, USA). Between-run variations of the same sample for major fatty acid peaks ($>2\%$) were $<1\%$.

Ethics

All subjects gave written informed consent to participate in the study. The Standing Committee on Ethics in Research on Humans, Monash University, approved the experimental protocol.

Statistical analysis

Data were analysed using the STATA Statistical Software (Release 7, College Station, TX, USA) statistical software package. All results are presented as mean values and standard deviations unless otherwise stated. The outcome variables of interest were body weight and FM. The covariates included energy intake, total fat intake, total EE associated with physical activity and physical activity level. The change in a variable of interest associated with a particular diet and the difference between the changes in a variable of interest between the two diet periods, in the same subject, were obtained as follows:

$$\begin{aligned} \text{change in variable} &= \text{measure after diet} \\ &\quad - \text{measure before diet} \end{aligned}$$

and

$$\begin{aligned} \text{difference in change} &= \text{change on SFA-rich diet} \\ &\quad - \text{change on MUFA-rich diet.} \end{aligned}$$

Where no pre-intervention measures were made, measures made at the end of each diet period were compared. Pearson correlation coefficients were computed to examine relationships between variables of interest. The difference between the changes in the variables of interest, or the difference in variables at the end of each diet period, were analysed using ANOVA for the 2×2 crossover study design. This is similar to repeated measures, except that the treatment factor (effects of the two diets in question) was assigned across the time periods. Each subject received both treatments in a particular sequence (sequence in which the diets were administered: SF-rich diet \rightarrow MUFA-rich diet = 1, MUFA-rich diet \rightarrow SFA-rich diet = 2). Within each subject, a period effect (the temporal order in which the diets were administered to each individual in each sequence) was also assessed. It was not possible to separate the sequence effect from any carry-over effect, as only two time periods were considered (Yandell, 1997). A significant ($P \leq 0.05$) sequence effect indicates that caution is required in interpreting treatment effects. When this occurred, we went on to compare the effects by treatment differences between diets in the first study period only, using an unpaired *t* test as has been recommended (Yandell, 1997). A paired *t* test was used to

compare paired data within individuals. Regression analysis with robust variance estimates (*Stata Reference Manual: Version 7*, 2001; STATA Corporation) was used to test for any treatment, sequence and period effects, after adjusting for confounding factors.

Results

Dietary intake

Dietary intake data for the two diet periods are presented in Table 2. On the SFA-rich diet, men consumed 24 % energy as SFA, 3 % energy as PUFA and 13 % energy as MUFA, whereas on the MUFA-rich diet the men consumed 11 % energy as SFA, 6 % energy as PUFA and 23 % energy as MUFA. Proportionately, MUFA accounted for approximately 80 % of the unsaturated fatty acids consumed on both diets. Although subjects had been asked to eat the dietary modules *ad libitum*, results indicated no significant differences between the SFA- and MUFA-rich diets in total energy ($P=0.16$), fat ($P=0.17$) or carbohydrate ($P=0.37$) intake (ANOVA). However, in the case of total energy and total fat intake, a significant sequence effect was apparent ($P=0.05$ and $P=0.04$ respectively). Consequently, differences in these variables were examined for the first diet period alone, a procedure recommended when a significant sequence effect is detected (Yandell, 1997). This analysis (using an unpaired *t* test) showed that during the first diet period, those individuals following an SFA-rich diet had consumed significantly more (14.3 (SD 2.3) MJ/d, $n=4$) energy than those individuals following the MUFA-rich diet (10.6 (SD 0.5) MJ/d, $n=4$; $P<0.05$). The best explanation for this difference would appear to be the greater amount of heavy physical activity by the four individuals receiving the SFA-rich diet in the first diet period (214 (SD 250) kJ/d) compared with those on the MUFA-rich diet (20 (SD 40) kJ/d; $P=0.22$, trend only), a 'between-subject' rather than 'within-subject' difference. A significant correlation was also evident between physical activity level and total energy intake in those on the SFA-rich diet ($n=4$, $r=0.99$, $P=0.002$), but not in those on the MUFA-rich diet ($P=0.10$).

In accordance with the study design, there were statistically significant differences in the consumption of different types of fat, intakes of SFA were substantially greater ($P<0.00005$), and MUFA ($P<0.00005$) and PUFA ($P=0.0002$) intakes substantially less, on the SFA-rich diet. However, once again a significant sequence effect ($P=0.04$) was evident in the ANOVA of SFA intake. Subsequent regression analysis of SFA intakes, with physical activity level as a covariate, showed a difference in SFA intakes between diets ($P<0.0005$); however, the sequence effect was no longer significant ($P=0.07$). Although subjects consumed less dietary fibre on the SFA-rich diet ($P=0.02$), once again there was a significant sequence effect ($P=0.003$). An independent *t* test of the fibre intake during the first dietary period showed that subjects ($n=4$) on the SFA-rich diet consumed a significantly greater amount of fibre (42.3 (SD 1.8) g/d) compared with subjects ($n=4$) on the MUFA-rich diet (34.5 (SD 1.6) g/d; $P=0.02$). There was no significant difference between diets in alcohol intake ($P=0.55$).

Table 2. Dietary intakes during the two diet periods*
(Mean values and standard deviations for eight subjects)

| Variables | SFA-rich diet | | MUFA-rich diet | | Difference between diets | | Statistical significance of effect: <i>P</i> |
|---------------------------|---------------|------|----------------|------|--------------------------|------------|--|
| | Mean | SD | Mean | SD | SFA - MUFA | 95 % CI | |
| Energy (kJ/d) | 12565 | 2475 | 11897 | 2319 | 668 | -325, 1660 | 0.16 |
| Total fat (g/d) | 137 | 27 | 130 | 26 | 7 | -4, 18 | 0.17 |
| Saturated fat (g/d) | 83 | 16 | 36 | 7 | 48 | -41, 54 | <0.00005† |
| Monounsaturated fat (g/d) | 43 | 9 | 73 | 14 | -30 | -96, -23 | <0.00005† |
| Polyunsaturated fat (g/d) | 11 | 3 | 21 | 6 | -10 | -13, -7 | 0.0002† |
| Carbohydrate (g/d) | 318 | 53 | 306 | 53 | 12 | -18, 43 | 0.37 |
| Fibre (g/d) | 36 | 8 | 40 | 8 | -5 | -8, -1 | 0.016† |
| Alcohol (g/d) | 11 | 12 | 12 | 15 | -1 | -6, 4 | 0.55 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid.

* For details of subjects, diets and procedures, see Table 1 and p. 718.

† Values were significantly different using an ANOVA for a 2 × 2 crossover study design, from intake during SFA-rich diet period.

Diet ratings

Subjects indicated (scale 0–100) that they felt more physically energetic on the MUFA-rich diet (81 (SD 18)) than on the SFA-rich diet (55 (SD 24)), a difference that was statistically significant (ANOVA, $P=0.01$). They also tended to prefer the MUFA-rich diet (78 (SD 12)) to the SFA-rich diet (60 (SD 29)), although this difference fell short of statistical significance ($P=0.09$). Otherwise, subjects found the two diets comparable in terms of satiety (SFA-rich diet 80 (SD 21) *v.* MUFA-rich diet 89 (SD 15); $P=0.32$) and taste (SFA-rich diet 66 (SD 23) *v.* MUFA-rich diet 69 (SD 17); $P=0.80$).

Anthropometry and body composition

All subjects had a BMI >25 kg/m². Six of the eight subjects had waist circumferences >0.99 m. Although there was no significant difference in the change in waist circumference ($P=0.15$) or change in hip circumference ($P=0.73$) during the two diet periods, the difference in the change in waist:hip ratio (0.03) was of borderline statistical significance ($P=0.05$, Table 3).

Body weight and composition, and the absolute change on the two diets, are presented in Table 3. ANOVA showed significantly higher body weight after the SFA than the MUFA-rich diet ($P=0.0015$). FM ($P=0.0037$), % body fat ($P=0.03$), trunk FM ($P=0.01$) and limb FM ($P=0.03$) (Fig. 1) were also significantly greater after the SFA-rich diet. However, a significant sequence effect ($P=0.03$) was evident in the ANOVA of the change in limb FM. Once again, an unpaired *t* test of the difference in the change in limb FM in the first dietary period only was carried out (Yandell, 1997). The change was significantly greater in the subjects on the SFA-rich diet (0.7 (SD 0.6) kg, n 4) compared with those on the MUFA-rich diet (−0.9 (SD 0.5) kg, n 4; $P=0.005$). No significant difference was found in the change in lean tissue mass ($P=0.20$) (Fig. 1). FM measured at the end of the SFA-rich diet was found to remain significantly greater than that measured at the end of the MUFA-rich diet after adjusting for energy intake (2.7 (robust SE 0.7) kg, $P=0.007$), total fat intake (2.7 (robust SE 0.7) kg, $P=0.007$), fibre intake (2.3 (robust SE 0.8) kg, $P=0.022$), self-reported total EE (2.7 (robust SE 0.6) kg, $P=0.004$) or physical activity level (2.7 (robust SE 0.6) kg, $P=0.004$).

Energy expenditure

No significant differences (ANOVA, $P>0.05$) between diets were evident in the change in resting metabolic rate ($P=0.93$) or change in diet-induced thermogenesis expressed in absolute terms ($P=0.54$), as % energy content in the meal ($P=0.55$) or as change in post-meal total energy output ($P=0.44$).

Total urinary nitrogen excretion and substrate oxidation rates

There were no significant differences on ANOVA of the change in total urinary N excretion ($P=0.80$), protein

oxidation rate ($P=0.80$), fasting fat oxidation rate ($P=0.98$), postprandial fat oxidation rate ($P=0.98$), fasting carbohydrate oxidation rate ($P=0.97$) or postprandial carbohydrate oxidation rate ($P=0.57$) between the two diet periods (Fig. 2). The change in FM was not significantly correlated with postprandial fat oxidation rates on either the MUFA-rich ($r -0.25$, $P=0.56$) or SFA-rich ($r 0.17$, $P=0.70$) diets. However, it tended to be inversely correlated with postprandial carbohydrate oxidation rates on the MUFA-rich ($r -0.65$, $P=0.08$), but not on the SFA-rich ($r 0.20$, $P=0.64$) diet.

Blood pressure and pulse rates

Table 3 shows the effect of diet on blood pressure and pulse rate. The difference in change in diastolic blood pressure ($P=0.08$) and mean arterial pressure (7.1 (95% CI −0.1, 14.4) mmHg; $P=0.05$) were of borderline significance (ANOVA). No significant difference was found in the change in systolic blood pressure ($P=0.13$) or in pulse rate ($P=0.92$).

Lipid profile, glucose and insulin concentrations

Lipid profile, glucose and insulin concentrations, fatty acid composition of serum CE are presented in Table 4. There was a significantly greater change in total cholesterol concentration on the SFA-rich as compared with the MUFA-rich ($P=0.004$) diet. However, the difference in the change in LDL-cholesterol was just short of statistical significance (1.1 mmol/l, $P=0.07$). No significant differences were observed in the change in other lipid fractions, or in fasting glucose or insulin concentrations.

Fatty acid profile in cholesteryl esters

The absolute changes in all classes of fat found in plasma CE are presented in Table 4. Total SFA in CE (g/100 g total fatty acids) was significantly greater on the SFA-rich as compared with the MUFA-rich ($P=0.0003$) diet. However, as a significant sequence effect was observed, an unpaired *t* test was performed to compare the subjects on the SFA-rich diet *v.* those on the MUFA-rich diet in the first diet period only (Yandell, 1997). This indicated a significantly ($P=0.02$) greater change in percentage SFA in CE had occurred in the four subjects on the SFA-rich diet (2.5 (SD 1.1) g/100 g total fatty acids) as compared with the four subjects on the MUFA-rich diet (−0.8 (SD 0.8) g/100 g total fatty acids). Total MUFA in CE ($P=0.04$), but not oleic acid (18:1n-9) content in CE ($P=0.61$), was greater on the SFA-rich diet. Total *n*-6 PUFA in CE was lower on the SFA-rich diet ($P=0.0001$), while total *n*-3 PUFA ($P=0.0008$) was higher. However, ANOVA indicated a significant ($P<0.05$) sequence effect occurring with both *n*-6 and *n*-3 PUFA content. Analysis of the change in *n*-6 PUFA in CE on the two diets, with an independent *t* test in the first study period, showed a decrease in the subjects on the SFA-rich diet (−6.1 (SD 2.2) g/100 g total fatty acids, n 4) and an increase in subjects on the MUFA-rich diet (0.9 (SD 1.1) g/100 g total fatty acids, n 4). The change in total *n*-6 PUFA in CE was

Table 3. Changes in anthropometry, body composition as measured by dual energy X-ray absorptiometry, blood pressure and pulse rate on the two diets†
(Mean values and standard deviations for eight subjects)

| Variable | SFA-rich diet | | | | | | MUFA-rich diet | | | | | |
|---------------------------------|---------------|-------|-------|-------|--------|-------|----------------|-------|-------|-------|--------|-------|
| | Before | | After | | Change | | Before | | After | | Change | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Waist circumference (m) | 0.995 | 0.085 | 0.996 | 0.084 | 0.0001 | 0.024 | 1.022 | 0.068 | 0.992 | 0.075 | -0.030 | 0.045 |
| Hip circumference (m) | 1.036 | 0.062 | 1.020 | 0.066 | -0.015 | 0.019 | 1.045 | 0.053 | 1.035 | 0.063 | -0.010 | 0.039 |
| Waist:hip ratio | 0.97 | 0.06 | 0.98 | 0.07 | 0.01 | 0.03 | 0.98 | 0.05 | 0.96 | 0.04 | -0.02* | 0.02 |
| Weight (kg) | 91.2 | 12.1 | 91.7 | 12.1 | 0.5 | 0.9 | 92.7 | 11.6 | 91.1 | 12.3 | -1.6* | 1.1 |
| FM (kg) | 24.9 | 5.6 | 25.9 | 5.9 | 1.0 | 1.6 | 26.6 | 5.3 | 25.0 | 5.9 | -1.7* | 0.8 |
| Trunk FM (kg) | 14.4 | 3.4 | 15.1 | 3.5 | 0.7 | 1.1 | 15.2 | 3.2 | 14.4 | 3.4 | -0.8* | 0.4 |
| Limb FM (kg) | 9.3 | 2.5 | 9.6 | 2.6 | 0.3 | 0.8 | 10.2 | 2.4 | 9.5 | 2.8 | -0.7* | 0.5 |
| Body fat (%) | 27.2 | 3.3 | 28.0 | 3.6 | 0.9 | 1.7 | 28.4 | 3.0 | 27.3 | 3.3 | -1.1* | 0.7 |
| Lean tissue mass (kg) | 62.9 | 7.1 | 62.1 | 6.7 | -0.8 | 1.5 | 62.6 | 6.8 | 62.5 | 6.9 | -0.1 | 0.7 |
| Systolic blood pressure (mmHg) | 116.6 | 11.8 | 117.4 | 13.2 | 0.8 | 6.7 | 122.4 | 10.9 | 114.4 | 8.9 | -8.0 | 7.1 |
| Diastolic blood pressure (mmHg) | 69.5 | 9.3 | 70.8 | 8.8 | 1.3 | 5.0 | 73.9 | 7.4 | 69.3 | 6.9 | -4.6† | 6.1 |
| Mean arterial pressure (mmHg) | 85.9 | 10.3 | 87.4 | 9.4 | 1.5 | 4.6 | 89.8 | 7.2 | 84.1 | 7.7 | -5.6* | 5.6 |
| Pulse rate (beats per min) | 57.3 | 4.6 | 56.1 | 6.3 | -1.1 | 8.5 | 57.0 | 6.0 | 56.4 | 3.6 | -0.6 | 4.0 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; FM, fat mass.

Mean values were significantly different from those of the SFA-rich diet (2 x 2 crossover study design, ANOVA; * $P \leq 0.05$).

Mean value had a trend towards a significant difference from that of the SFA-rich diet (2 x 2 crossover study design, ANOVA; † $P < 0.05$).

‡ For details of subjects, diets and procedures, see p. 718.

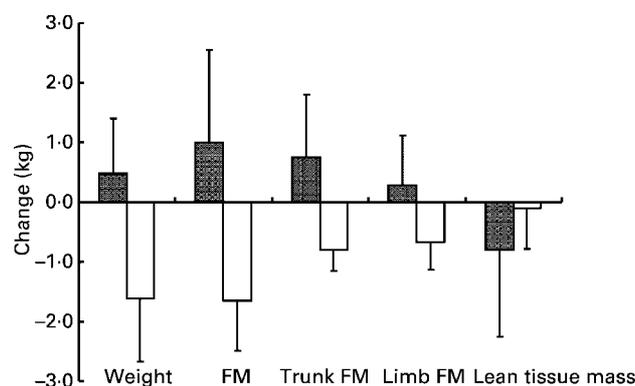


Fig. 1. Effect of saturated fatty acid-rich (■) and monounsaturated fatty acid-rich (□) diets on changes in body weight, fat mass (FM), trunk FM, limb FM and lean tissue mass. Values are means with standard deviations shown by vertical bars (n 8). For details of subjects, diets and procedures, see Tables 1 and 2 and p. 718.

predominantly due to a change in linoleic acid (18:2 n -6) ($P=0.001$).

Physical activity

ANOVA of self-reported physical activity diaries indicated no significant difference in total EE between diets (SFA-rich diet 10.1 (95% CI 9.2, 11.0) v. MUFA-rich diet 9.7 (95% CI 8.9, 10.6) MJ/d; $P=0.40$). There were no significant differences in self-reported EE associated with sleep ($P=0.71$), sitting ($P=0.42$) and light ($P=0.36$), moderate

($P=0.11$) or heavy physical activity ($P=0.99$) (ANOVA). There was also no significant difference in physical activity level between the two diet periods ($P=0.80$).

Discussion

Overall, subjects gained FM (1.0 (SD 1.6) kg) during the SFA-rich diet and lost FM (-1.7 (SD 0.8) kg) during the MUFA-rich diet (Table 3). These changes in body composition were observed despite the absence of statistically significant differences in total energy or fat intake during the two diet periods (Table 2). The changes in lean tissue mass in both diet periods were small and did not significantly affect the resting metabolic rate. Men on the SFA-rich diet gained FM predominantly on the trunk (0.7 (SD 1.1) kg) rather than on the limbs (0.3 (SD 0.8) kg). In contrast, on the MUFA-rich diet, similar amounts of body fat were lost both from the trunk (-0.8 (SD 0.4) kg) and from the limbs (-0.7 (SD 0.5) kg) (Fig. 1), a finding that is in accordance with our previous study (Walker *et al.* 1996).

The higher FM (+2.64 kg) at the end of the SFA-rich diet as compared with the MUFA-rich diet, when expressed as energy amounted to 82.8 MJ over 4 weeks, calculated at 31.4 kJ/g fat (Berrino *et al.* 2001). Differences in energy intake and physical activity data on the two diets explain only part of this difference. We can attribute 46.5 MJ of this difference to a greater energy intake on the SFA-rich diet (the upper limit of the 95% CI in the mean difference in energy intake: 1660 kJ/d for 4 weeks). A further 14 MJ can be attributed to a subtle increase in physical activity on the MUFA-rich diet (the difference between upper limit of the 95% CI of total EE reported for the MUFA-rich diet and the mean total EE reported for the SFA-rich diet: 10.6 v. 10.1 MJ/d for 4 weeks respectively). There is some subjective evidence for this, as questionnaires given to the subjects at the end of each diet period indicated that subjects felt 'more energetic' on the MUFA-rich diet ($P=0.01$), which is consistent with one of our earlier studies (Walker *et al.* 1995). The remaining difference can be explained on the basis of our previous results (Piers *et al.* 2002), relating to differences in fat oxidation rates between SFA-rich and MUFA-rich meals. In that study, we proposed that an individual whose energy intake was 10.0 MJ/d might oxidise an extra 670 kJ/d by consuming MUFA rather than SFA. In the current study, the observed mean energy intake on the MUFA-rich diet was 11.9 MJ/d. Extrapolating, an extra 797 kJ/d might be burnt on the MUFA-rich diet, or 22.3 MJ over 4 weeks, which is the energy difference otherwise unaccounted for.

The observed changes in body weight and body fat were accompanied by trends towards reduced waist:hip ratio and blood pressure (diastolic and mean arterial pressure) on the MUFA-rich diet (Table 4), indicative of physiological significance. It is well established that weight gain or loss, over as short a period as 1–2 months, is accompanied by an associated change in blood pressure (McCarron & Reusser, 1996). In addition, the waist:hip ratio is positively and independently correlated with systolic arterial pressure in male hypertensive patients (Raison *et al.* 1992).

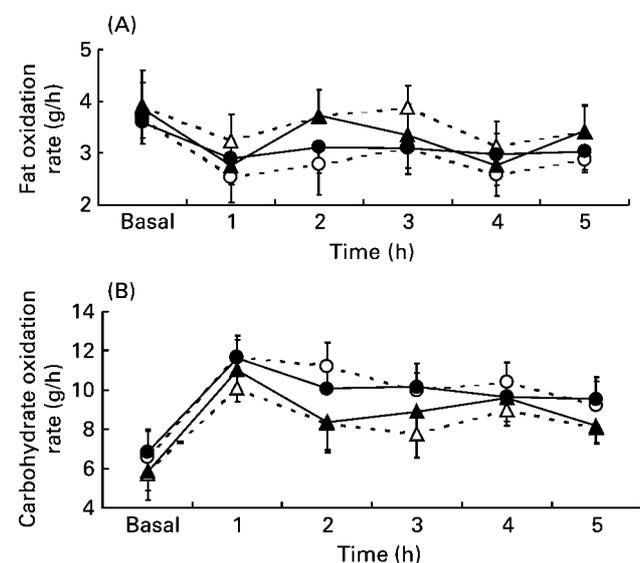


Fig. 2. Fat (A) and carbohydrate oxidation rates (B) measured in the basal state and following breakfast meals that differed only in the type of fat, one containing predominantly saturated fat and the other monounsaturated fat. The measurements were made prior to and at the end of two diet periods during which the macronutrient composition of the diet was held constant, but the type of fat was varied so that it was rich in saturated fatty acids (SFA) in one diet period and monounsaturated fatty acids (MUFA) during the other. —○—, Before SFA-rich diet; —△—, before the MUFA-rich diet; —●—, after the SFA-rich diet; —▲—, after the MUFA-rich diet. Values are means with standard deviations shown by vertical bars (n 8). For details of subjects, diets and procedures, see Tables 1 and 2 and p. 718.

Table 4. Changes in serum lipids, fatty acid profile of cholesteryl esters and plasma glucose and insulin concentrations on the two diets (Mean values and standard deviations for eight subjects)

| | SFA-rich diet | | | | | | MUFA-rich diet | | | | | |
|---|---------------|-----|-------|-----|--------|------|----------------|-----|-------|-----|--------|------|
| | Before | | After | | Change | | Before | | After | | Change | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Serum lipids | | | | | | | | | | | | |
| Total cholesterol (mmol/l) | 4.8 | 0.5 | 5.4 | 0.6 | 0.60 | 0.70 | 5.3 | 0.6 | 4.6 | 0.5 | -0.76* | 0.56 |
| Triacylglycerol (mmol/l) | 1.6 | 1.2 | 1.8 | 1.4 | -0.18 | 1.13 | 1.4 | 0.5 | 1.6 | 0.7 | 0.19 | 0.65 |
| LDL-cholesterol (mmol/l) | 3.0 | 0.4 | 3.5 | 0.6 | 0.44 | 0.82 | 3.4 | 0.5 | 2.8 | 0.4 | -0.56† | 0.43 |
| HDL-cholesterol (mmol/l) | 1.0 | 0.3 | 1.0 | 0.1 | 0.01 | 0.22 | 1.1 | 0.1 | 1.0 | 0.2 | -0.09 | 0.17 |
| Fasting glucose (mmol/l) | 5.4 | 0.6 | 5.4 | 0.5 | -0.04 | 0.60 | 5.5 | 0.5 | 5.4 | 0.5 | -0.15 | 0.52 |
| Fasting insulin (mU/ml) | 7.7 | 3.3 | 7.9 | 4.0 | 0.20 | 3.68 | 8.6 | 3.9 | 7.9 | 3.8 | -0.66 | 2.44 |
| Fatty acids in cholesteryl esters (g/100 g total fatty acids) | | | | | | | | | | | | |
| Total SFA | 11.6 | 1.2 | 14.5 | 1.2 | 2.9 | 1.1 | 13.2 | 1.9 | 11.2 | 0.9 | -2.0* | 1.9 |
| Total MUFA | 27.1 | 2.1 | 29.4 | 3.1 | 2.3 | 2.3 | 28.1 | 3.1 | 27.5 | 2.0 | -0.6* | 2.2 |
| Oleic acid (18:1 <i>n</i> -9) | 22.8 | 1.9 | 23.3 | 1.5 | 0.5 | 2.1 | 22.5 | 1.8 | 23.6 | 1.6 | 1.1 | 1.9 |
| Total <i>n</i> -6 PUFA | 58.7 | 3.5 | 52.4 | 3.4 | -6.3 | 2.3 | 55.2 | 4.7 | 58.9 | 3.1 | 3.7* | 3.6 |
| Linoleic acid (18:2 <i>n</i> -6) | 49.0 | 4.4 | 43.1 | 4.0 | -5.9 | 2.4 | 45.5 | 4.3 | 48.5 | 4.3 | 3.1* | 3.2 |
| Total <i>n</i> -3 PUFA | 2.2 | 0.8 | 3.1 | 0.6 | 0.9 | 0.4 | 3.0 | 0.6 | 2.1 | 0.8 | -0.9* | 0.4 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Mean values were significantly different from those of the SFA-rich diet (2×2 crossover study design, ANOVA; * $P \leq 0.05$).

Mean value had a trend towards a significant difference from that of the SFA-rich diet (2×2 crossover study design, ANOVA; † $P = 0.07$).

Observed resting and postprandial substrate oxidation rates at the end of the two diets did not differ from those observed with the same test meal before the diets (Fig. 2). The observed difference between SFA- and MFA-rich test meals given at the end of the diets, however, appeared smaller than observed previously (Piers *et al.* 2002). This was not unexpected, as the enlarged fat store at the end of the SFA-rich diet possibly promoted a chronic increase in fat oxidation unrelated to the meal consumed (Flatt, 1995). This chronic increase in fat oxidation rate, in response to a high fat intake, eventually permits a new plateau of fat balance and energy balance to be achieved at a higher body weight and FM; this is why body weight and FM do not keep increasing linearly (Flatt, 1995). While there is now considerable evidence in human subjects that dietary fat type can influence fat oxidation rates (Jones *et al.* 1985, 1992; Jones & Schoeller, 1988; DeLany *et al.* 2000; Piers *et al.* 2002), until the present study there has been no information on the impact this may have on body weight and body composition. However, animal studies indicate the potential differential impact of different dietary fats on body composition in the longer term (Matsuo *et al.* 1995; Takeuchi *et al.* 1995; Bell *et al.* 1997). Bell *et al.* (1997) compared MUFA-rich (rapeseed oil) and SFA-rich (beef fat) diets (40.8% energy from fat) in mice. Animals fed the MUFA-rich diet deposited significantly less body fat than mice fed the high-SFA diet: entirely consistent with our present results in human subjects.

Throughout our present study, foods and beverages were supplied as modules of fixed macronutrient composition, but differing energy content, as described by Stubbs *et al.* (1995). This procedure was adopted to determine if subjects would then consume more food on the SFA-rich diet. In fact, taste and satiety responses to the two diets did not differ and they allowed very similar total energy and total fat intake (Table 2). Hence, change in the type of fat *per se* did not

appear to affect total fat consumption. The observed significant sequence effect on fibre intake cannot be explained. Although all modules were designed to have similar fibre content, subject selection of individual modules resulted in a difference of 5 g/d between diets in fibre intake (Table 2). This difference, however, does not account for the differences in the change in FM, as this difference persisted after statistical adjustment for fibre intake. There were large differences between diets in the type of fat consumed. The difference in SFA intake between diets was 48 g/d (Table 2). These differences in fat intake were associated with blood lipid changes that provide objective evidence of dietary adherence (Table 4). On the SFA-rich diet, the plasma SFA fraction of plasma CE was 4.9% higher ($P = 0.001$), while total *n*-6 PUFA was 10.3% lower ($P = 0.0001$). In particular, the proportion of linoleic acid (18:2 *n*-6), an essential fatty acid, fell markedly on the SFA-rich diet and rose on the MUFA-rich diet, a difference of -9.0% ($P = 0.001$). The concentration of linoleic acid in plasma CE is well known to be reflective of dietary linoleic acid intake (Sinclair *et al.* 1987; Sanders *et al.* 1994; Sarkkinen *et al.* 1994). The difference in the change in oleic acid (18:1 *n*-9) was not significant ($P = 0.61$), but oleic acid is a non-essential fatty acid that can be synthesised from carbohydrate and is a poor indicator of dietary oleic acid intake (Sanders *et al.* 1994). Changes in the lipid profile (Table 4) were also consistent with dietary change (Table 2), with total and LDL-cholesterol rising on the SFA-rich diet and falling on the MUFA-rich diet, in accordance with the well established effects of SFA and MUFA (Hegsted *et al.* 1993). We did not include a washout period between the two diet periods, because previous studies have indicated that this is unnecessary when the dietary intervention period is > 3 weeks (Bonanome & Grundy, 1988; Fuentes *et al.* 2001).

There are several limitations in the present study. The first is the small number of subjects who participated.

The results need confirmation in larger studies of a similar design. Second, it is possible that subjects consciously restricted their intake on the MUFA-rich diet, as they were free-living and their meals were not supervised. Subjects had no reason to do this, however, as they were never told that the purpose of the study concerned changes in body weight. Throughout the study, they were constantly encouraged to eat to appetite and extra food modules were always made available. From their expressed preference for the MUFA-rich diet, we would possibly have expected more overeating in this phase, rather than the SFA-rich phase of the study. Third, it is possible that subjects were more physically active during the MUFA-rich diet. There was some subjective evidence for this from the questionnaire. Whether increased physical activity was the cause, or consequence, of the loss of FM on the MUFA-rich diet cannot be determined from the present study. Although it remains possible theoretically that the observed differences are the result of a pure coincidence, this seems unlikely given that all subjects systematically gained weight and FM on the SFA-rich diet and all subjects systematically lost weight and FM on the MUFA-rich diet.

The results of our current study are consistent with some recent findings in other dietary studies. An *ad libitum* diet low in animal fat and refined carbohydrates and rich in low-glycaemic-index foods, MUFA, *n*-3 PUFA and phyto-oestrogens favourably modified the hormonal profile of postmenopausal women when compared with a high-SFA control diet (Berrino *et al.* 2001). That MUFA-rich diet also significantly decreased body weight (4.06 *v.* 0.54 kg in the control group), waist:hip ratio, total cholesterol, fasting glucose level and area under the insulin curve after an oral glucose tolerance test. In another recent study (McManus *et al.* 2001), a moderate fat (35% energy), controlled energy, Mediterranean-style diet rich in MUFA (15–20% energy) has been shown to be superior to a low-fat (20% energy) high-carbohydrate diet for weight reduction. In that study, there was superior long-term participation and adherence with the MUFA-rich diet and greater weight loss. Other studies comparing SFA-rich to MUFA-rich diets, however, have not reported significant differences in the magnitude of weight loss (Mata *et al.* 1992; deLorgeril *et al.* 1999; Williams *et al.* 1999; Vessby *et al.* 2001). The mechanisms whereby SFA become more readily diverted to adipose tissue storage and the effects this may then have on body metabolism and energy balance remain to be elucidated. The peroxisome proliferator-activated receptors (α , δ (or β) and γ), a subfamily of the nuclear receptor gene family, have emerged recently as important physiological sensors of lipid levels and may provide a molecular mechanism whereby dietary fatty acids modulate lipid homeostasis. *In vitro* studies show that unsaturated fatty acids (both MUFA and/or PUFA) are more effective in stimulating peroxisome proliferator-activated receptor α than SFA (Kliwer *et al.* 1997; Halvorsen *et al.* 2001). Such results suggest a plausible mechanism, among others, for our present observations.

In conclusion, the results of our present study suggest that the previously observed greater fat oxidation rate following a MUFA-rich test meal, as compared with a

SFA-rich test meal, can result in a small but significant reduction in body weight and FM in men whose dietary SFA content is largely replaced with MUFA, within 4 weeks. This effect could possibly have been enhanced by subtle, unconscious, increases in physical activity and/or decreases in energy intake; however, there were no detectable changes in basal or postprandial EE. This is a novel result and it requires confirmation, perhaps using a whole-body calorimeter to measure EE and substrate oxidation rates in a larger number of subjects over a longer period of time, possibly in conjunction with the doubly-labelled water technique to measure free-living EE.

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References

- Bell RR, Spencer MJ & Sherriff JL (1997) Voluntary exercise and monounsaturated canola oil reduce fat gain in mice fed diets high in fat. *J Nutr* **127**, 2006–2010.
- Ben-Porat M, Sideman S & Bursztein S (1983) Energy metabolism rate equation for fasting and postabsorptive subjects. *Am J Physiol* **244**, R764–R769.
- Berrino F, Bellati C, Secreto G, *et al.* (2001) Reducing bioavailable sex hormones through a comprehensive change in diet: the diet and androgens (DIANA) randomized trial. *Cancer Epidemiol Biomarkers Prev* **10**, 25–33.
- Bligh EG & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Pharmacol* **37**, 911–917.
- Blundell JE & MacDiarmid JI (1997) Fat as a risk factor for over-consumption: satiety, satiety, and patterns of eating. *J Am Diet Assoc* **97**, S63–S69.
- Bonanome A & Grundy SM (1988) Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N Engl J Med* **318**, 1244–1248.
- Bray GA & Popkin BM (1998) Dietary fat intake does affect obesity! *Am J Clin Nutr* **68**, 1157–1173.
- Callaway CW, Chumlea WC, Bouchard C, *et al.* (1988) In *Anthropometric Standardisation Reference Manual*, pp. 39–54 [TG Lohman, AF Roche and R Martorell, editors]. Champaign, IL: Human Kinetics Books.
- DeLany JP, Windhauser MM, Champagne CM & Bray GA (2000) Differential oxidation of individual dietary fatty acids in humans. *Am J Clin Nutr* **72**, 905–911.

- deLorgeril M & Salen P (2000) Modified Cretan Mediterranean diet in the prevention of coronary heart disease and cancer. *World Rev Nutr Diet* **87**, 1–23.
- deLorgeril M, Salen P, Martin JL, Monjaud I, Delaye J & Mamelle N (1999) Mediterranean diet traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* **99**, 779–785.
- Elia M & Livesey G (1988) Theory and validity of indirect calorimetry during net lipid synthesis. *Am J Clin Nutr* **47**, 591–607.
- English R & Lewis J (1991) *Nutritional Values of Australian Foods*. Canberra: Australian Government Printing Service.
- Flatt JP (1995) Use and storage of carbohydrate and fat. *Am J Clin Nutr* **61**, 952S–959S.
- Food and Agriculture Organization/World Health Organization/United Nations University (1985) *Energy and Protein Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation*. Technical Report Series no. 724, Geneva: WHO.
- Fuentes F, Sanchez E, Sanchez F, *et al.* (2001) Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med* **134**, 1115–1119.
- Garg A (1994) High-monounsaturated fat diet for diabetic patients. Is it time to change the current dietary recommendations? *Diabetes Care* **17**, 242–246.
- Halvorsen B, Rustan AC, Madsen L, *et al.* (2001) Effects of long-chain monounsaturated and *n*-3 fatty acids on fatty acid oxidation and lipid composition in rats. *Ann Nutr Metab* **45**, 30–37.
- Hegsted DM, Ausman LM, Johnson JA & Dallal GE (1993) Dietary fat and serum lipids: an evaluation of the experimental data. *Am J Clin Nutr* **57**, 875–883.
- Jones PJ, Pencharz PB & Clandinin MT (1985) Whole body oxidation of dietary fatty acids: implications for energy utilization. *Am J Clin Nutr* **42**, 769–777.
- Jones PJ, Ridgen JE, Phang PT & Birmingham CL (1992) Influence of dietary fat polyunsaturated to saturated ratio on energy substrate utilization in obesity. *Metab Clin Exp* **41**, 396–401.
- Jones PJ & Schoeller DA (1988) Polyunsaturated:saturated ratio of diet fat influences energy substrate utilization in the human. *Metab Clin Exp* **37**, 145–151.
- Kliwer SA, Sundseth SS, Jones SA, *et al.* (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci USA* **94**, 4318–4323.
- Lissner L & Heitmann BL (1995) Dietary fat and obesity: evidence from epidemiology. *Eur J Clin Nutr* **49**, 79–90.
- Livesey G & Elia M (1988) Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *Am J Clin Nutr* **47**, 608–628.
- McCarron DA & Reusser ME (1996) Body weight and blood pressure regulation. *Am J Clin Nutr* **63**, 423S–425S.
- McManus K, Antinoro L & Sacks F (2001) A randomized controlled trial of a moderate-fat, low-energy diet compared with a low fat, low-energy diet for weight loss in overweight adults. *Int J Obes Relat Metab Disord* **25**, 1503–1511.
- Mata P, Garrido JA, Ordovas JM, *et al.* (1992) Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women. *Am J Clin Nutr* **56**, 77–83.
- Matsuo T, Shimomura Y, Saitoh S, Tokuyama K, Takeuchi H & Suzuki M (1995) Sympathetic activity is lower in rats fed a beef tallow diet than in rats fed a safflower oil diet. *Metab Clin Exp* **44**, 934–939.
- Piers LS, Walker KZ, Stoney RM, Soares MJ & O'Dea K (2002) The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) versus saturated fat (cream). *Int J Obes Relat Metab Disord* **26**, 814–821.
- Raison JM, Achimastos AM & Safar ME (1992) Sex-dependence of body fat distribution in patients with obesity and hypertension. *Clin Exper Hypertens* **14A**, 505–525.
- Sanders K, Johnson L, O'Dea K & Sinclair AJ (1994) The effect of dietary fat level and quality on plasma lipoprotein lipids and plasma fatty acids in normocholesterolemic subjects. *Lipids* **29**, 129–138.
- Sarkkinen ES, Agren JJ, Ahola I, Ovaskainen ML & Uusitupa MI (1994) Fatty acid composition of serum cholesterol esters, and erythrocyte and platelet membranes as indicators of long-term adherence to fat-modified diets. *Am J Clin Nutr* **59**, 364–370.
- Sinclair AJ, O'Dea K, Dunstan G, Ireland PD & Niall M (1987) Effects on plasma lipids and fatty acid composition of very low fat diets enriched with fish or kangaroo meat. *Lipids* **22**, 523–529.
- Storlien LH, Hulbert AJ & Else PL (1998) Polyunsaturated fatty acids membrane function and metabolic diseases such as diabetes and obesity. *Curr Opin Clin Nutr Metab Care* **1**, 559–563.
- Storlien LH, Tapsell LC, Fraser A, *et al.* (2001) Insulin resistance. Influence of diet and physical activity. *World Review Nutr Diet* **90**, 26–43.
- Stubbs RJ (1998) Nutrition Society Medal Lecture. Appetite, feeding behaviour and energy balance in human subjects. *Proc Nutr Soc* **57**, 341–356.
- Stubbs RJ, Harbron CG, Murgatroyd PR & Prentice AM (1995) Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum. *Am J Clin Nutr* **62**, 316–329.
- Takeuchi H, Matsuo T, Tokuyama K, Shimomura Y & Suzuki M (1995) Diet-induced thermogenesis is lower in rats fed a lard diet than in those fed a high oleic acid safflower oil diet, a safflower oil diet or a linseed oil diet. *J Nutr* **125**, 920–925.
- Vessby B, Unsitupa M, Hermansen K, *et al.* (2001) Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* **44**, 312–319.
- Walker KZ, O'Dea K, Johnson L, *et al.* (1996) Body fat distribution and non-insulin-dependent diabetes: comparison of a fiber-rich, high-carbohydrate, low-fat (23%) diet and a 35% fat diet high in monounsaturated fat. *Am J Clin Nutr* **63**, 254–260.
- Walker KZ, O'Dea K, Nicholson GC & Muir JG (1995) Dietary composition, body weight, and NIDDM. Comparison of high-fiber, high-carbohydrate, and modified-fat diets. *Diabetes Care* **18**, 401–403.
- Willet WC (2002) Dietary fat plays a major role in obesity: no. *Obes Rev* **3**, 59–68.
- Williams CM, Francis-Knapper JA, Webb D, *et al.* (1999) Cholesterol reduction using manufactured foods high in monounsaturated fatty acids: a randomized crossover study. *Br J Nutr* **81**, 439–446.
- Yandell BS (1997) *Practical Data Analysis for Designed Experiments*. Boca Raton, FL: CRC Press.