ORIGINAL ARTICLE
Dietary medium-chain saturated fatty acids induce gene expression of energy metabolism-related pathways in adipose tissue of abdominally obese subjects

JC Matualatupauw1,2, M Bohl3, S Gregersen3, K Hermansen3 and LA Afman1

BACKGROUND: Dietary medium-chain saturated fatty acids (MC-SFAs) have been shown to reduce total body fat. Previously, we showed that MC-SFAs prevent body fat accumulation, despite weight gain. Here, we aim to explore potential molecular mechanisms underlying the protective effect of MC-SFAs on body fat gain.

METHODS: The DairyHealth study examined the long-term effects of milk protein and milk fat with a low or high content of MC-SFA. In this 12 week, randomized, double-blind, diet intervention study, participants consumed 60 g milk protein (whey or casein) and 63 g milk fat (high MC-SFA or low MC-SFA) daily in a two by two factorial design. We used microarrays to measure whole genome gene expression changes in subcutaneous adipose tissue in a subpopulation of 12 participants, 6 in the low MC-SFA +casein group and 6 in the high MC-SFA+casein group. Gene expression of several genes that were found to be changed by MC-SFAs was confirmed in the full study population using qPCR.

RESULTS: High MC-SFA resulted in an upregulation of gene expression related to citric acid cycle and oxidative phosphorylation, and a downregulation of gene expression related to complement system and inflammation.

CONCLUSIONS: We hypothesize that the beneficial effects of MC-SFAs on prevention of fat accumulation are mediated via increased gene expression related to energy metabolism in the adipose tissue. Decreases in inflammation-related gene expression may have beneficial effects in relation to cardiometabolic diseases.

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INTRODUCTION
Medium-chain saturated fatty acids (MC-SFAs) are present in several types of foods including dairy products. Two recent meta-analyses show beneficial effects of intake of MC-SFAs on body weight and body composition and conclude that consumption of medium-chain triglycerides in exchange of long-chain triglycerides as part of a diet results in a reduction in total, visceral and subcutaneous body fat. In contrast to long-chain saturated fatty acids, MC-SFAs from foods are not incorporated into chylomicrons, but are transported directly to the liver as free fatty acids. In the liver, most of the MC-SFAs are oxidized directly. This is thought to be the cause of the post-prandial increase in energy expenditure observed after consumption of MC-SFAs. A smaller fraction of the MC-SFAs is distributed via the circulation to peripheral tissues such as the adipose tissue. In humans, MC-SFAs have been shown to be present in adipose tissue after a diet enriched in MC-SFAs. Moreover, in rats, medium-chain fatty acids (MCFAs) from the diet were shown to be incorporated into adipose tissue triglycerides. Furthermore, in this rat study a reduction in fat mass and a downregulation of adipogenic genes, including peroxisome proliferator activated receptor gamma (PPARγ) and CCAAT/enhancer-binding protein alpha (C/EBPa) was observed after the MCFa-diet compared with an isocaloric high-fat control diet. Moreover, in cultured murine and human adipocytes, incubation with the MC-SFA octanoic acid inhibited adipogenesis and expression of adipogenic genes. Taken together, these studies indicate that the effects dietary MC-SFAs on body weight and body composition are not only achieved by increased fatty acid oxidation in the liver, but suggest that MC-SFAs can affect adipose tissue as well. However, knowledge on the precise effects of dietary MC-SFAs on adipose tissue in humans is lacking. To investigate the effects of MC-SFAs on human adipose tissue, we studied whole genome gene expression profiles in subcutaneous adipose tissue biopsies of the DairyHealth study. This dietary intervention study examined the long-term effects of intake of milk fat with a low or high content of MC-SFAs and milk protein in abdominally obese participants. In this study, all intervention groups increased their body weight, though an increase in total body fat was only observed in the low MC-SFA groups. The high MC-SFA groups were protected against this body fat gain and these individuals displayed a gain in lean body mass.

We aim to increase our understanding of the effects of MC-SFAs on the adipose tissue that may explain the observed differences in body fat accumulation. To do this, we investigated the effects of a 12-week high MC-SFA versus a low MC-SFA diet on subcutaneous adipose tissue gene expression profiles.

MATERIALS AND METHODS
Participants
Participant recruitment procedures were described in detail previously. In short, participants were eligible for inclusion if ≥18 y of age, had an
abdominal circumference of ≥ 80 cm for women or ≥ 94 cm for men, and were weight stable for ≥3 months. Exclusion criteria were diagnosis with diabetes, pregnancy, lactating, or severe cardiovascular, renal, endocrine, or psychiatric disease. A total of 52 participants completed the study. Adipose tissue samples were not collected from three participants (two because of bleeding and one refrained from the post-intervention biopsy) and adipose tissue samples from two participants are missing because of early dropouts.

Study design

This study is a secondary analysis of a randomized, parallel-controlled, double-blinded, 12-week diet intervention study that was designed to examine the effects of milk fat, with a high or low MC-SFA content, and of milk protein, whey or casein, on post-prandial lipemia. Design of the original study was described in detail previously. Briefly, 52 participants were randomized in a 2 × 2 factorial design to one of four diets: high MC-SFA+casein, low MC-SFA+casein, high MC-SFA+whey, and low MC-SFA+whey. In this paper, we examined the effects of MC-SFAs on whole genome gene expression in subcutaneous adipose tissue in a subpopulation of 12 subjects, 6 in the high MC-SFA+casein group and 6 in the low MC-SFA+casein groups. We chose to perform the microarray analysis in the casein groups, as the MC-SFA-induced prevention of body fat accumulation was most pronounced in these groups. To validate findings of the microarray analyses and to examine gene expression changes in the whey groups, we performed targeted quantitative real-time polymerase chain reaction (qPCR) measurements on all adipose tissue samples.

All participants gave written informed consent to participate in the study and the study protocol was approved by the Central Denmark Region Committees on Health Research Ethics. The study was registered at clinicaltrials.gov as NCT01472666.

Dietary intervention

Participants consumed food products containing 63 g of milk fat and 60 g of milk protein daily. The milk fat contained 8.5 g and 6.9 g of MC-SFAs in the high MC-SFA and the low MC-SFA groups, respectively. Protein was either whey or casein. The milk fats were produced at the Danish Cattle Research Center (Foulum, Denmark) by feeding cattle high- or low-fat diets in order to produce low and high MC-SFA-containing milk, respectively. Butter was produced from these two types of milk and used as spread and in the production of rolls and cakes. Protein powder containing whey (Lacprodan DI-9224) or casein (Miprodan 30) was provided by Arla Foods Ingredients Group P/S (Viby J, Denmark). The daily food products were 2 rolls, 1 cake, and 25 g of butter (corresponding to a total of 63 g of milk fat/day) and two protein shakes (a total of 60 g/day of protein). Daily energy intake from the test products was 6200 KJ with 42 energy percentage (E%) as fat, 37 E% as carbohydrate, and 21 E% as protein.

A clinical dietician instructed the participants on how to incorporate the dietary supplementation into their habitual diet.

Dual-energy X-ray absorption scan

Whole body composition, total body fat, android fat percentage, gynoid fat percentage, and lean mass were measured by DEXA scan (Hologic Discovery A scanner, serial nr. 83986, Hologic Inc., Danbury, USA). To generate DEXA scan output we used the Hologic software Apex (Version 13.1.1.7).

Fat biopsies

Before and after intervention, fat biopsy specimens were taken from the abdominal subcutaneous adipose tissue. The biopsy specimens were taken with Bergstrom needle through a small skin incision under local anesthesia with 1-1.5 ml lidocaine (20 g/l)11. The adipose tissue was cleaned for blood with sterile saline, snap-frozen in liquid nitrogen, and stored at −80 °C.

RNA isolation

Adipose tissue samples were homogenized in TRIzol Reagent (Invitrogen, Breda, the Netherlands). RNA was extracted using chloroform and purified using Qiagen RNeasy Mini Kit (Qiagen, Venlo, The Netherlands). RNA concentration was measured on a Nanodrop ND 1000 spectrophotometer (Nanodrop) and integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent, Amstelveen, Netherlands). Samples with RIN values above 7.5 were considered suitable for microarray and qPCR analysis.

Microarray processing

Six subjects from the high MC-SFA+casein group and six from the low MC-SFA+casein group were selected for microarray analysis, so that each group contained the same number of males/females and subjects with the metabolic syndrome. Microarrays were performed before and after the intervention, resulting in a total of 24 microarrays. Total RNA was labeled using a one-cycle cDNA labeling kit (MessageAmp I-Biotin Enhanced Kit; Ambion) and hybridized to GeneChip Human Gene 1.1 ST arrays, containing 803487 probes (Affymetrix, Santa Clara, CA, USA). Sample labeling, hybridization to chips and image scanning was performed according to the manufacturers’ instructions.

Microarray analysis

Microarray analysis was performed using MADMAX pipeline for statistical analysis of microarray data. A custom annotation was used based on reorganized oligonucleotide probes, which combines all individual probes for a gene (custom CDF). This resulted in a total of 19 621 genes. Expression values were calculated using robust multichip average method, which includes quantile normalization.15 Probe sets with unlogged expression values higher than 20 on at least 1 array were selected for further statistical analysis. Significant differences in expression were assessed using limma. For all comparisons genes were defined as significantly different when the P-value was < 0.05. The response to the intervention in the high MC-SFA+casein and the low MC-SFA+casein groups was determined using paired t-tests with Bayesian correction as applied in limma. Differences in gene expression changes between the two groups were calculated from the individual log ratios and was calculated as the response to high MC-SFA+casein compared with the response to low MC-SFA+casein using unpaired t-tests with Bayesian correction, as implemented in limma. Microarray data are registered as GSE587382 in the Gene Expression Omnibus.

Pathway analyses

We performed pre-ranked gene set enrichment analysis (GSEA; http://www.broad.mit.edu/gsea). Briefly, genes were ranked based on the t-statistic and analyzed for over- or underrepresentation in predefined gene sets. Gene sets were derived from Biocarta, KEGG, Reactome and WikiPathways databases. To increase the strength of our findings, we used a relatively strict cut-off of FDR q < 0.1. The significant gene sets were visualized in Cytoscape using Enrichment Map.

Upstream regulator analysis

Upstream transcription regulators analyses were performed using QIA-GEN’s Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity). A P-value cutoff of 0.05 was used to determine differentially expressed genes. Upstream regulators were defined significant when the P-value was below 0.05 and the z score was > 2 or < −2. Upstream regulators with z scores > 2 were considered activated and regulators with z scores < 2 were considered inhibited.

QPCR

To confirm microarray results, we performed qPCR in samples in the full study population. RNA was reverse transcribed using a cDNA synthesis kit (First Strand cDNA Synthesis kit, Thermo Scientific, Leusden, The Netherlands) and analyzed by qPCR (SensiMix SYBR No-ROX, Bioline, London, UK) on a CFX384 Real-Time System (C1000 Thermal Cycler, Biorad, Veenendaal, The Netherlands). qPCR data were normalized using ACTB and PPIA as housekeeping genes. A calibration curve was created from 1:10 serial dilutions of a pool of all samples. Using this calibration curve, relative starting quantities were determined for each gene in each sample. Expression of genes of interest was normalized by calculating log2-ratios of relative starting quantity between gene of interest and the housekeeping genes. Genes for qPCR were selected from the differentially expressed pathways in the microarray analysis using several criteria. Genes were required to have P-value < 0.05 in the comparison between the high MC-SFA+casein and the low MC-SFA+casein group, have a raw expression value above 200 (which leads to ct-values < 34), and have primers present in the microarray.
available in the PrimerBank database. Primer sequences are shown in Supplementary Table S1.

Statistical analysis
Differences in baseline characteristics were analyzed using independent t-tests using IBM SPSS Statistics, version 22.0.0.1. Body weight changes, body composition changes and qPCR data were analyzed using one sample t-tests and independent t-tests with GraphPad Prism version 5.04. For all analyses P-values < 0.05 were considered statistically significant.

RESULTS
Participant characteristics
In total, 52 subjects completed the original study. Baseline characteristics of the total population have been reported previously. In this study, we performed microarrays in adipose tissue samples of six subjects in the high MC-SFA+casein group and six subjects in the low MC-SFA+casein group. Baseline characteristics of these subgroups are shown in Supplementary Table S2.

Change in body weight and composition
Subjects consumed butter containing 63 g of milk fat (high or low in MC-SFAs) and 60 g of protein (whey or casein) daily for 12 weeks. Changes in body weight and composition for the full study population were reported in detail previously. Briefly, body weight was increased after intervention, though there was no difference between groups or difference depending on MC-SFA content and protein type. Total fat percentage and gynoid fat percentage increased when consuming the butter low in MC-SFA, whereas lean mass increased in the participants consuming the butter high in MC-SFA. The changes in body weight and composition in our microarray subgroups were similar to those in the full study population (Figure 1 and ). In our subgroups, body weight increased significantly in the low MC-SFA+casein group only (P = 0.024), though body weight changes were not different compared to the high MC-SFA+casein group. Furthermore, only the low MC-SFA+casein group showed an increased total body (P = 0.045) and android fat percentage (P = 0.028). Changes in total body and android fat percentage were also different between the two groups (P = 0.028 and P = 0.018, respectively).

Differentially expressed genes
We compared whole genome gene expression profiles after intervention to those before intervention in the high MC-SFA +casein and the low MC-SFA+casein groups. To examine the effects of MC-SFAs on adipose tissue, we compared the changes in gene expression between the high MC-SFA+casein and the low MC-SFA+casein group and found 986 genes to be differentially changed between these two groups (Figure 2). Principal component analysis on the changes in expression of these 986 genes revealed a clear separation between the low MC-SFA +casein and high MC-SFA+casein groups (Supplementary Figure S1).

Pathway analysis
To gain further insight into the biological processes affected by MC-SFAs, we performed GSEA for the difference in response between both groups and for the response within the groups. GSEA showed that 68 gene sets were significantly upregulated and 31 were significantly downregulated, when comparing the response on high MC-SFA to the response on low MC-SFA (Supplementary Table S3). These gene sets are clustered on overlapping genes and are visualized in Supplementary Figure S2 and summarized in Table 1. Gene sets related to citric acid cycle, oxidative phosphorylation and adipogenesis were significantly upregulated in the high MC-SFA+casein group and significantly downregulated in the low MC-SFA+casein group. Gene sets related to inflammation and complement cascade were significantly downregulated by intake of high MC-SFA+casein and significantly upregulated after intake of low MC-SFA+casein. For each of these gene set clusters, we selected the genes that were significantly differentially changed when comparing the high MC-SFA+casein group and the low MC-SFA+casein group. Individual expression changes upon both interventions of these genes are visualized in Figure 3.

Upstream regulator analysis
To examine potential regulators of the differentially expressed genes, we performed Ingenuity upstream regulator analysis

Two transcription factors regulating lipid metabolism and oxidative phosphorylation, PPARα and PPARGC1a, were predicted to be activated by MC-SFAs. Likewise, transcriptional regulators involved in inflammation STAT6, NFKB1, and IKBKB were predicted to be inhibited by MC-SFAs.

Table 1. Results of the gene set enrichment analysis

<table>
<thead>
<tr>
<th>Gene set cluster</th>
<th>High MC-SFA+casein</th>
<th>Low MC-SFA+casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid cycle</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Inflammation</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Complement system</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

*Gene sets differentially changed in the high MC-SFA+casein compared to the low MC-SFA+casein group were clustered and visualized using Enrichment Map in Cytoscape (Supplementary Figure S1). From this, gene set clusters were selected and changes in expression in each group are shown. Full GSEA results are shown in Supplementary Table S3. ↑, significantly upregulated; ↓, significantly downregulated.

QPCR validation of the microarray findings

To examine the expression changes of several selected genes of the microarray analysis in the total study population, we performed qPCR. Genes were selected from the inflammation, complement cascade, citric acid cycle, oxidative phosphorylation and adipogenesis pathways. Gene expression changes in the 4 intervention groups are shown in Figure 4. Expression changes of ACOX1, SLC25A10, SDHB, and IDH3B were significant for MC-SFA independent of protein type. Within the casein groups we observed changes in similar directions as the microarray results for both high and low MC-SFA groups for all genes, except CFH. An MC-SFA and protein interaction effect was observed for the SLC25A10, DLAT, and SDHB genes, indicating differences in response to the MC-SFAs between the casein and the whey groups.

DISCUSSION

In this study, we showed that 12 weeks consumption of butter high in MC-SFA+casein in subjects with central obesity had marked effects on subcutaneous adipose tissue gene expression profiles compared to consumption of butter low in MC-SFA.
+casein. MC-SFAs induced gene expression in energy metabolism-related pathways such as oxidative phosphorylation and citric acid cycle, as well as lipid metabolism-related pathways. Furthermore, MC-SFAs decreased pathways related to inflammation and complement system. These effects were observed with a simultaneous protection against body fat accumulation and provide valuable insight into the potential mechanism behind the beneficial effects of MC-SFAs on fat accumulation and adipose tissue metabolism.

The increase in gene expression in the citric acid cycle and oxidative phosphorylation pathways in the high MC-SFA group could be indicative of an increase in oxidative energy metabolism in the adipose tissue. Moreover, PPARGC1a, which is the key regulator of the oxidative phosphorylation pathway, was identified as a likely upstream regulator pointing to a potential activation of this pathway via this transcription factor. To our knowledge, these effects of MC-SFAs on energy metabolism pathways in the adipose tissue are novel findings. In the liver, dietary medium-chain fatty acids do not require carnitine to reach the mitochondria, but passively cross the mitochondrial membrane and are beta-oxidized rapidly. Subsequently, the produced acetyl-CoA enters the citric acid cycle and is oxidized, resulting in a post-prandial increase in energy expenditure after consumption of MC-SFAs. MC-SFAs have been shown to reach the adipose tissue, where they may be rapidly beta-oxidized. We observed an MC-SFA-induced increase in expression of ACOX1, which codes for an enzyme involved in fatty acid beta-oxidation.

**Table 2. Top 10 potential upstream regulators explaining the differences in gene expression changes between the high MC-SFA+casein and the low MC-SFA+casein groups**

<table>
<thead>
<tr>
<th>Upstream regulator</th>
<th>Molecule type</th>
<th>Predicted activation state</th>
<th>Activation z-score</th>
<th>P-value of overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARA</td>
<td>ligand-dependent nuclear receptor</td>
<td>Activated</td>
<td>2.049</td>
<td>4.28E-04</td>
</tr>
<tr>
<td>ESR1</td>
<td>ligand-dependent nuclear receptor</td>
<td>Inhibited</td>
<td>-3.285</td>
<td>9.53E-04</td>
</tr>
<tr>
<td>PDLIM2</td>
<td>other</td>
<td>Activated</td>
<td>2.111</td>
<td>1.27E-03</td>
</tr>
<tr>
<td>PPARGC1A</td>
<td>transcription regulator</td>
<td>Activated</td>
<td>2.24</td>
<td>3.47E-03</td>
</tr>
<tr>
<td>NFKB1</td>
<td>transcription regulator</td>
<td>Inhibited</td>
<td>-2.377</td>
<td>2.03E-03</td>
</tr>
<tr>
<td>STAT6</td>
<td>transcription regulator</td>
<td>Inhibited</td>
<td>-2.35</td>
<td>3.18E-03</td>
</tr>
<tr>
<td>MITF</td>
<td>transcription regulator</td>
<td>Inhibited</td>
<td>-3.727</td>
<td>3.78E-03</td>
</tr>
<tr>
<td>IKKB</td>
<td>kinase</td>
<td>Inhibited</td>
<td>-2.358</td>
<td>3.88E-03</td>
</tr>
<tr>
<td>BID</td>
<td>other</td>
<td>Inhibited</td>
<td>-2.000</td>
<td>5.29E-03</td>
</tr>
<tr>
<td>HRG</td>
<td>other</td>
<td>Inhibited</td>
<td>-2.000</td>
<td>7.85E-03</td>
</tr>
</tbody>
</table>

*The 10 regulators with the lowest P-value and a z-score > 2 or < -2 are shown for the difference in gene expression changes in response to a 12-week dietary intervention containing high MC-SFA+casein compared to low MC-SFA+casein. Z-scores predict the activation state of the regulator and are based on the gene expression of its downstream genes. Upstream regulators with z scores > 2 are considered activated and regulators with z-scores < -2 are considered inhibited. P-values of overlap measure the significance of the overlap between the differentially expressed genes and the genes reported to be regulated by the upstream regulator and are calculated using Fisher's exact test.

**Figure 4.** QPCR results of a selection of genes showing the changes in expression in all four groups. Results of two-way ANOVA are shown. *P*-value < 0.05 is considered significant.
of which the expression was upregulated upon high MC-SFA is APOC3. APOC3 inhibits LPL activity resulting in a decreased triglyceride hydrolysis and hence a reduced uptake of fatty acids in the adipose tissue. The potential MC-SFAs-induced increase in fatty acid oxidation, increase in energy metabolism and decrease in uptake of fatty acids in the adipose tissue may result in a reduced fat storage which may explain the observed preventive effect of MC-SFAs on body fat accumulation.

We did not observe a downregulation in expression of genes involved in adipogenesis upon MC-SFA, which is in contrast with findings from rat studies. Sinahara et al.\textsuperscript{1,2} reported a suppressing effect of triglycerides containing medium and long-chain fatty acids compared to triglycerides containing long-chain fatty acids on adipogenesis in adipose tissue in rats, as shown by lower fatty acid synthase (FAS) enzyme activity, though gene expression of FAS was not affected. Similarly, another study in rats showed a downregulation in expression of the adipogenic genes PPAR\(\gamma\) and C/EBP\(\alpha\) in adipose tissue in response to medium-chain triglycerides.\textsuperscript{8} We did not observe changes in adipogenesis-related gene expression, including FAS, PPAR\(\gamma\) and C/EBP\(\alpha\), though it is still possible that the enzyme activity may have been increased. Taken together, based on these findings, we hypothesize that the preventive effects of MC-SFAs on body fat accumulation were not caused by a decrease in adipogenesis.

The MC-SFA-induced downregulation in pathways related to inflammation, and especially in genes related to the complement system may point towards a decreased inflammatory state of the adipose tissue. Complement components are produced in adipose tissue and are involved in both adipose tissue inflammation as well as adipose tissue metabolism.\textsuperscript{2,3,4} The MC-SFAs-induced decrease in inflammation-related pathways may be beneficial considering the deleterious role of inflammatory pathways in the adipose tissue in initiating and sustaining the low-grade inflammatory state that is associated with obesity.\textsuperscript{25} To our knowledge, no previous studies have observed effects of MC-SFAs on inflammation-related gene expression in adipose tissue in humans. In mice, a high-fat diet supplemented with medium-chain triglycerides resulted in significantly lower serum IL-6 levels and higher IL-10 levels compared with an isocaloric high-fat diet.\textsuperscript{26} Our upstream regulator analysis identified NF-kB and p38 MAPK as potential inhibited upstream regulators in the adipose tissue, indicating that an increased MC-SFA intake may inhibit the NF-kB pathway.

The changes in inflammation-related gene expression were not paralleled by a reduction in plasma inflammatory markers as no changes in IL-6, IL-1RA, high-sensitive CRP, adiponectin and MCP-1 between the four groups were found in this study.\textsuperscript{27} It should be noted that gene expression of none of these markers were altered in our microarray analyses. However, gene expression changes in the adipose tissue may be one of the first changes induced by dietary interventions,\textsuperscript{28} and a reflection of this on circulating inflammation markers may only develop after a prolonged exposure.

The qPCR analyses confirmed the described effects of MC-SFAs on gene expression in the full casein groups. However, in the two whey groups, we did not observe the same effect of MC-SFA. Accordingly, the changes in body fat percentage also appear less pronounced in the whey groups.\textsuperscript{11} One study in mice showed that they reduced high-fat diet-induced body weight and body fat gain compared with casein.\textsuperscript{29} They used qPCR to measure gene expression of a limited number of genes in adipose tissue and, inconsistent with our findings, found no effects of whey protein on expression of genes related to energy metabolism, adipogenesis, or inflammation. It should be noted, that the genes reported in the mice study were different from the genes observed to be differentially expressed in our study. Furthermore, differences between species, length of the dietary intervention and dietary composition may also explain the differential findings between this mouse study and our study.

We have used a relatively small number of subjects for the microarray analysis with six subjects in the high MC-SFA+casein group and six subjects in the low MC-SFA+casein group. Because of this, we found no differentially expressed genes when using FDR q-value < 0.05 as significance cutoff. Instead, we used a P-value cut-off of 0.05 for selecting the differentially expressed genes. For this reason, we focused this discussion mostly on the GSEA analyses and less on individual genes. To increase the strength of our GSEA findings, we used a strict cut-off of FDR q < 0.1. Despite the small sample size and the use of stringent cut-off values, we still observed a robust gene expression pathway effect of 12 weeks of supplementation with butter high in MC-SFAs. Furthermore, we used qPCR to validate our findings for several genes in the total study population and found that our microarray findings in the high or low MC-SFA+casein groups were largely confirmed in the full groups.

In the Dietary Health study,\textsuperscript{10} subjects with abdominal obesity were protected from an increase in body fat accumulation by a 12 week high MC-SFA dietary intervention compared with low MC-SFA.\textsuperscript{11} We investigated the underlying mechanism behind this protective effect of MC-SFAs on adipose tissue using whole genome gene expression analyses. Our findings on gene expression point towards an MC-SFA-induced increase in energy metabolism, an increase in fatty acid oxidation and a decrease in fatty acid uptake in adipose tissue that is potentially responsible for the protective effect on body fat accumulation. In addition, a decrease in expression of genes involved in inflammation and complement system pathways may reflect a decreased inflammatory state of the adipose tissue. Taken together, these findings indicate that the beneficial effects of MC-SFAs are not only achieved in the liver, but suggest that the adipose tissue could play a key role in mediating the effects of MC-SFAs as well.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

MB, SG, KH, and LAA: designed the trial; MB: conducted the trial; JCM and LAA: designed the research; JCM: conducted the research, analyzed the data and wrote the manuscript; and MB, SG, KH and LAA: critically reviewed the manuscript.

**REFERENCES**

Supplementary Information accompanies this paper on International Journal of Obesity website (http://www.nature.com/ijo)