

## ORIGINAL ARTICLE

# Differential effects of saturated versus unsaturated dietary fatty acids on weight gain and myocellular lipid profiles in mice

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**Objective:** In conditions of continuous high-fat (HF) intake, the degree of saturation of the fatty acids (FAs) in the diet might have a crucial role in the onset of obesity and its metabolic complications. In particular, the FA composition of the diet might influence the storage form of lipids inside skeletal muscle. The aim of the present study was to examine whether the FA composition of HF diets differentially affects weight gain and accumulation of myocellular triacylglycerol (TAG) and diacylglycerol (DAG). Furthermore, we examined whether the FA composition of the diet was reflected in the composition of the myocellular lipid intermediates.

**Design:** C57Bl6 mice were fed HF diets (45% energy) mainly containing palm oil (PO), cocoa butter (CB), olive oil (OO) or safflower oil (SO;  $n = 6$  per group) for 8 weeks. A low-fat diet (10% energy, PO) was used as control. Body weight was monitored weekly. At the end of the dietary intervention, myocellular TAG and DAG content and profiles were measured.

**Results:** We here show that HF\_CB prevented weight gain after 8 weeks of HF feeding. Furthermore, the HF diet rich in SO prevented the accumulation of both myocellular TAG and DAG. Interestingly, the FA composition of DAG and TAG in skeletal muscle was a reflection of the dietary FA composition.

**Conclusion:** Already after a relatively short period, the dietary FA intake relates to the FA composition of the lipid metabolites in the muscle. A diet rich in polyunsaturated FAs seems to prevent myocellular lipid accumulation.

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## Introduction

Obesity is a serious health problem, predisposing individuals to a range of metabolic complications. Skeletal muscle and adipose tissue are important organs in regulating fatty acid (FA) metabolism, and disturbances in their function may lead to insulin resistance and dyslipidemia.<sup>1</sup> When the capacity of adipose tissue to store lipid becomes insufficient, which is most often associated with chronic excessive energy intake (in particular by means of dietary fat), this may lead to

an increased supply of triacylglycerols (TAGs) and free FAs to non-adipose tissues, such as skeletal muscle and liver.<sup>1</sup> Consequently, if the uptake of FAs exceeds the capacity or the need to oxidize fat in skeletal muscle or liver, the FAs are stored. Indeed, TAG content in human skeletal muscle has been reported to increase by 36–90% after high-fat (HF) feeding periods ranging from 24 h to 7 weeks,<sup>2–5</sup> and numerous studies have shown a negative association between fat accumulation in these tissues and insulin sensitivity.<sup>6–9</sup>

Next to total fat content, it has nowadays become apparent that dietary fat quality (that is, dietary FA composition) may also be important, as each individual FA has distinct chemical properties linked to its unique structure. Thus, it has been shown that the type of dietary fat can influence the onset of obesity by differentially

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affecting weight gain and body fatness. Saturated FAs (SFAs) have been shown to result in higher rates of weight gain,<sup>10,11</sup> whereas polyunsaturated FAs (PUFAs) did not induce obesity in mice and rats.<sup>12,13</sup> In humans, Doucet *et al.*<sup>14</sup> showed that waist circumference, as a measure of central adiposity, is increased in individuals with a higher total, saturated and monounsaturated fat content in their diets compared with PUFAs.

Furthermore, the degree of saturation of the diet has been suggested to affect the rate of storage of FA intermediates. Both in rats and humans, it was shown that unsaturated FAs are oxidized more rapidly than SFAs,<sup>15,16</sup> and SFAs, which are less readily oxidized, accumulate as diacylglycerols (DAGs) and ceramide *in vitro*,<sup>17</sup> whereas monounsaturated FAs (MUFAs) and PUFAs are efficiently stored as TAGs.<sup>17–19</sup>

Despite these suggestions that dietary fat quality can affect body weight gain and muscle fat storage, no studies are available that consistently tested the effect of well-controlled experimental HF diets varying in FA composition on myocellular lipid content. Moreover, whereas it has been shown for adipose tissue that TAG composition reflects habitual dietary FA intake over a considerably long time,<sup>20,21</sup> such information is lacking for skeletal muscle.

Therefore, we investigated whether the FA composition of HF diets, with different degree of saturation, differentially affects body weight gain and storage of dietary FAs into either myocellular TAG or DAG. Furthermore, we were interested whether, after a short period of change in dietary fat intake, the FA profiles of myocellular TAG and DAG represent the dietary FA composition.

## Materials and methods

### Animals and diets

Thirty, male C57Bl/6J mice, aged 4 weeks, were obtained from Harlan Laboratories (Horst, The Netherlands) and were acclimatized, two per cage, to the environmentally controlled laboratory environment (temperature  $22 \pm 1$  °C and relative humidity  $55 \pm 2\%$ ) with a 12:12 light-dark cycle. Three weeks before the onset of the experimental intervention, mice were allowed *ad libitum* access to a low-fat diet (LF\_PO, palm oil; 70%, 10% and 20% of calories as carbohydrate, fat and protein, respectively) that contained both SAT FAs and MUFAs derived from PO (Research Diet Services, Wijk bij Duurstede, The Netherlands).

After these 3 weeks, mice were randomly assigned to one of the six experimental diet groups for a period of 8 weeks. The first group was maintained on the LF\_PO ( $n=6$ ). The other mice switched to a HF diet (35% carbohydrate; 45% fat; and 20% protein) that consisted mainly of SFAs, derived from cocoa butter (HF\_CB,  $n=6$ ), a mixture of both SFAs and MUFAs, derived from PO (HF\_PO,  $n=6$ ), two mixtures primarily of MUFAs and PUFAs, derived from olive oil (HF\_OO,  $n=6$ ) and safflower oil (HF\_SO,  $n=6$ ) (Research

**Table 1** Macronutrient composition of the diets

	LF_PO D12450B	HF_PO D12451	HF_CB D12451	HF_OO D12451	HF_SO D12451
<i>Based on formula</i>					
Protein (kcal %)	20	20	20	20	20
Carbohydrate (kcal %)	70	35	35	35	35
Fat (kcal %)	10	45	45	45	45
Total	100	100	100	100	100
<i>Percentage of fatty acids</i>					
C16:0	43	43	25	12	6
C18:0	4	4	34	2	3
C18:1	41	41	33	73	12
C18:2	10	10	3	10	78

Abbreviations: HF\_CB, high-fat cocoa butter; HF\_OO, high-fat olive oil; HF\_PO, high-fat palm oil; HF\_SO, high-fat safflower oil; LF\_PO, low-fat palm oil.

Diet Services). The exact composition of the diets is given in Table 1. Animals were provided with food and water *ad libitum* throughout the experimental period. Body weight of the mice was monitored weekly during the dietary intervention period. To determine food intake, non-absorbable chromic oxide was supplemented to the diets for 1 week (week 5 of the diet intervention). At the end of this week, feces were quantitatively collected during 48 h and fecal chromic oxide levels were determined as previously described.<sup>22</sup> These fecal chromic oxide levels were then used to calculate the energy intake per mouse per day.

All experiments were approved by the Institutional Animal Care and Use committee of the Wageningen University and complied with the principles of laboratory animal care.

### Oral glucose tolerance test

To investigate the effect of dietary FA composition on whole-body glucose tolerance, mice underwent an oral glucose tolerance test after 7 weeks of the dietary intervention. After a 6-hour fast, glucose (20% wt/vol solution) was administered by oral gavage at a dose of 2.5 g/kg body weight, and glucose was measured in blood obtained from the tail vein, before glucose administration ( $t=0$ ) and at 15, 30, 45, 60 and 90 min thereafter using Accu-Chek blood glucose meters (Roche Diagnostics, Almere, The Netherlands). Incremental area under the curve of glucose concentration versus time curve was calculated using the trapezoidal method.

### Tissue sampling

After 8 weeks of dietary intervention, mice were anaesthetized in the postprandial phase at 1400 hours with a mixture of isoflurane, nitrogen oxide and oxygen, followed by cervical dislocation. Gastrocnemius muscle was rapidly dissected out, immediately frozen in liquid nitrogen-cooled isopentane and stored at  $-80$  °C until further analyses. Epididymal fat depots were isolated and weighed.

#### Measurement of skeletal muscle TAG and DAG content

Total lipids were extracted from frozen muscle using the method of Folch *et al.*<sup>23</sup> The extracts were filtered and lipids recovered in the chloroform phase. Total TAGs and DAGs were isolated using thin-layer chromatography on Silica Gel 60 A plates (Merck kGaA, Darmstadt, Germany) developed in petroleumbenzin-diethyl ether-acetic acid (120:25:1.5 by volume) and visualized by rhodamine 6G. The TAG and DAG bands were scraped from the plate and methylated using a mixture of toluene-methanol (BF<sub>3</sub>/methanol 14%; 20–55–25% by volume), as described by Morrison and Smith.<sup>24</sup> The methylated FAs were extracted with hexane and analyzed by capillary gas liquid chromatography using a 50 m × 0.25 mm CP-sil 88 silica column (Varian, Agilent, Santa Clara, CA, USA), with helium as carrier gas at a flow of 130 kPa. The column oven was maintained at 165 °C for 10 min and increased at a rate of 5 °C min<sup>-1</sup> to 190 °C. This temperature was maintained for 15 min. Then temperature was increased to 230 °C for 22 min, with a flow rate of 2 °C min<sup>-1</sup>. FA methyl esters were identified by comparing retention times to those of known standards. Inclusion of an internal standard, 17:0 (heptadecanoic acid, internal standard for TAG), 19:0 (dinonadecanoic acid, internal standard for DAG) and an average molecular mass for each FA methyl ester permits quantification of the amount of TAG and DAG in the sample, expressed as micromolar per gram of wet tissue weight. Contribution of the individual FAs as percentage of total DAG and TAG content was calculated.

#### Enzyme activity measurements

Muscle  $\beta$ -hydroxyacyl-CoA dehydrogenase and citrate synthase, and enzyme activities were determined spectrophotometrically. In short, 30 (20  $\mu$ m) sections of muscle were added to 100  $\mu$ l sucrose-EDTA-Tris buffer (250 mmol l<sup>-1</sup> sucrose, 2 mmol l<sup>-1</sup> EDTA and 10 mmol l<sup>-1</sup> Tris (pH 7.4)) and sonicated (Branson 2210; Branson Ultrasonics, Danbury, CT, USA) for 25 s. Samples were centrifuged (10 min, 10 000 *g*, 4 °C), and the supernatants were analyzed spectrophotometrically (Multiskan Spectrum; Thermo labsystems, Breda, The Netherlands) to determine  $\beta$ -hydroxyacyl-CoA dehydrogenase<sup>25</sup> and citrate synthase enzyme activities.<sup>26</sup> Activity of proteins is expressed as U g<sup>-1</sup> protein.

#### Measurement of liver fat content

Animals were killed and livers were rapidly excised, rinsed of blood and homogenized by polytron (Brinkman Instruments, Westbury, NY, USA) in homogenization buffer (containing 10 mM Tris, 2 mM EDTA and 0.25 M sucrose, pH 7.5, 4 °C). Triglyceride concentration was determined by the commercially available enzymatic kit (Instruchemie, Delfzijl, The Netherlands). Briefly, homogenates were centrifuged at 12 000 *g* for 10 min, and TAG amounts in the supernatants were determined by enzyme-linked immunosorbent assay. The TAG content was corrected for tissue weight.

#### Western blotting

Muscle samples were homogenized as described previously<sup>27</sup> and processed for standard SDS-polyacrylamide gel electrophoresis and western blotting. Protein concentration was assessed, and equal amounts of protein were loaded per lane. Actin was used as a loading control. Membranes were incubated with antibodies against oxidative phosphorylation proteins (OXPHOS) (MS601; MitoSciences, Eugene, OR, USA), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC1 $\alpha$ ) (516557; Calbiochem; VWR International BV, Amsterdam, The Netherlands) and adipose tissue triglyceride lipase (ATGL) (2138; Cell Signaling Technology; Bioké; Leiden, The Netherlands). Blots incubated with OXPHOS and PGC1 $\alpha$  were probed with IR-Dye800-conjugated or IRDye700-conjugated secondary antibodies (Rockland, Gilbertsville, PA, USA, and LICOR Biosciences, Westburg, Leusden, The Netherlands), and bands at a molecular weight corresponding to the control samples were quantified using the Odyssey infrared imaging system (LICOR Biosciences, Warrington, The Netherlands). Blots detecting ATGL were probed with the appropriate horse radish-conjugated antibodies, and after incubation, specific protein bands were visualized by chemiluminescence and analyzed by Chemidoc XRS system (Bio-Rad, Veenendaal, The Netherlands).

#### Statistical analysis

Results are presented as the mean  $\pm$  s.e. Differences between groups were analyzed by one-way analysis of variance, with least significant differences *post hoc* correction. The accepted level of statistical significance was  $P < 0.05$  for all analyses. All calculations were done using the Statistical Package for Social Sciences (SPSS 16.0 software, SPSS, Chicago, IL, USA).

## Results

#### Body weight, food intake, feces production and epididymal fat pads

Body weights gradually increased over the 8 weeks of intervention in all dietary groups (Figure 1). However, mice receiving HF\_PO gained the most weight, whereas LF\_PO and HF\_CB mice displayed the lowest weight gain over the entire feeding protocol. The delta body weight was significantly different between groups (+43% in HF\_PO versus LF\_PO,  $P = 0.001$ ; +41% in HF\_PO versus HF\_CB,  $P = 0.001$ ; +20% in HF\_PO versus HF\_OO,  $P > 0.05$ ; and +26% in HF\_PO versus HF\_SO,  $P = 0.030$ ). Food intake between the different dietary groups was not different (LF\_PO,  $12.4 \pm 1.4$ ; HF\_PO,  $12.1 \pm 1.4$ ; HF\_CB,  $10.0 \pm 0.4$ ; HF\_OO,  $12.9 \pm 1.1$ ; and HF\_SO,  $15.1 \pm 0.7$  kcal per day,  $P > 0.05$ ). Feces production was significantly higher for HF\_CB compared with the other dietary groups (LF\_PO,  $0.40 \pm 0.02$ ; HF\_PO,  $0.41 \pm 0.03$ ; HF\_CB,  $0.53 \pm 0.01$ ; HF\_OO,  $0.39 \pm 0.01$ ; and HF\_SO,  $0.42 \pm 0.02$  g per day,  $P < 0.05$ ). Epididymal fat pad weights were not different between the dietary groups (LF\_PO,

$1.31 \pm 0.15$ ; HF\_PO,  $2.14 \pm 0.13$ ; HF\_CB,  $1.74 \pm 0.15$ ; HF\_OO,  $1.72 \pm 0.28$ ; and HF\_SO,  $1.71 \pm 0.25$  g,  $P > 0.05$ ).

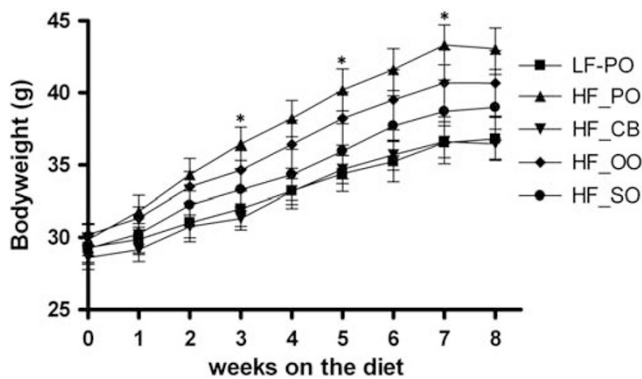
#### Muscle lipid content

After 8 weeks of dietary intervention, skeletal muscle TAG and DAG content were assessed.

Skeletal muscle TAG content increased to the same extent in all HF diets compared with LF\_PO ( $P < 0.05$ , Figure 2a), except for HF\_SO, which did not lead to accumulation of myocellular TAG.

HF\_PO, HF\_CB and HF\_OO also significantly increased myocellular DAG levels compared with LF\_PO ( $P < 0.05$ ), whereas HF\_SO did not significantly increase DAG content ( $P = 0.39$ , Figure 2b).

Dietary FA composition exerted a profound effect on the FA profile in skeletal muscle DAG and TAG (Figures 3a and b). Both LF\_PO and HF\_PO, which contain an almost equal amount of SFAs (49% of total FAs, Table 1) and MUFAs (41% of total FAs), increased the proportion of the SFA C16:0 and the MUFA C18:1 in both DAG and TAG. Also, HF\_OO and HF\_SO, two diets that are characterized by a high amount of MUFAs (73% of total FAs) and PUFAs (78% of total FAs), respectively, elevated the proportion of the unsaturated

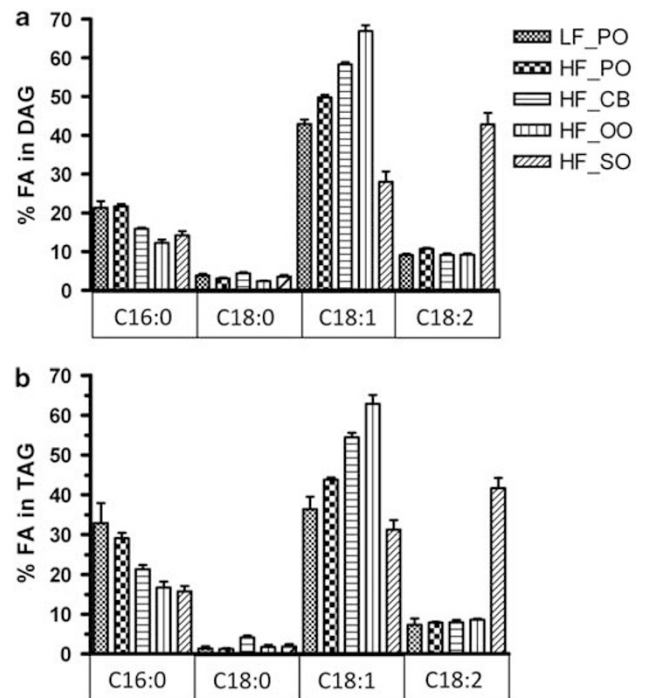


**Figure 1** Effect of dietary FA composition on body weight gain. Body weight gradually increased over the 8 weeks of dietary intervention. Data are expressed as mean  $\pm$  s.e.m. ( $n = 6$ ). \* $P < 0.05$  HF\_PO versus LF\_PO and HF\_CB.

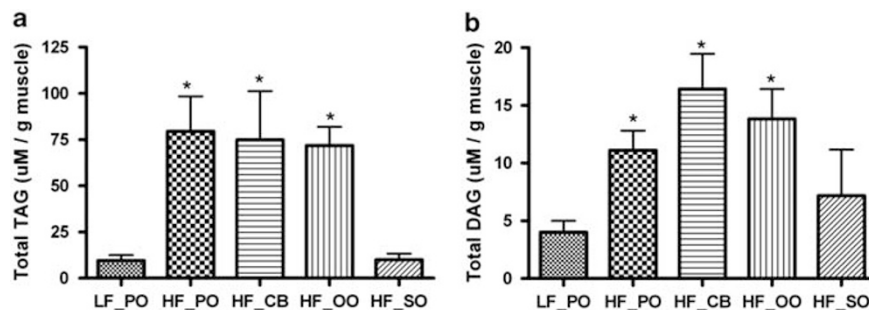
FAs in both skeletal muscle DAG and TAG content. For HF\_OO, the increased proportion of MUFAs resulted in an increase in C18:1, whereas for HF\_SO the elevated PUFAs in DAG and TAG content were due to an increase in C18:2. HF\_CB, a diet consisting of 63% of SFAs and of 37% of unsaturated FAs, elevated both the proportion of C18:0 and C18:1 in skeletal muscle DAG and TAG content.

#### Glucose tolerance

Fasting blood glucose values were not affected by the FA composition of the different diets (LF\_PO,  $10.18 \pm 0.40$ ;



**Figure 3** Effect of dietary FA composition on skeletal muscle DAG (a) and TAG (b) FAs. Representation of the FAs that account for the highest percentage in the diet and account, on average, for 80% of total DAG and TAG content. Data are expressed as mean  $\pm$  s.e.m. ( $n = 6$ ).



**Figure 2** Effect of dietary FA composition on TAG (a) and DAG (b) content in gastrocnemius muscle. Data are expressed as mean  $\pm$  s.e.m. ( $n = 6$ ). \* $P < 0.05$  LF\_PO versus HF\_PO, HF\_CB and HF\_OO.



HF\_PO,  $12.14 \pm 0.84$ ; HF\_CB,  $11.0 \pm 0.23$ ; HF\_OO,  $11.5 \pm 1.59$ ; and HF\_SO,  $10.88 \pm 0.65$  mM,  $P > 0.05$ ).

The degree of saturation of the different HF diets had no major influence on whole-body glucose tolerance, that is, all the HF diets reduced the clearance of glucose from the blood compared with the LF\_PO (Figures 4a and b), although this did not reach statistical significance for HF\_OO (+45.6% in HF\_PO versus LF\_PO,  $P < 0.005$ ; +38.5% in HF\_CB versus LF\_PO,  $P < 0.022$ ; +28.9% in HF\_OO versus LF\_PO,  $P = 0.16$ ; and +45.4% in HF\_SO versus LF\_PO,  $P < 0.003$ ). No significant differences between different HF groups were evident. We found a positive correlation between the incremental area under the blood glucose curve and

myocellular DAG and TAG content (Pearson correlation coefficient = 0.35,  $P = 0.10$ , Figure 5a; Pearson correlation coefficient = 0.43,  $P = 0.039$ , Figure 5b, respectively).

#### Skeletal muscle insulin signaling

Skeletal muscle IRS ser<sup>307</sup> phosphorylation, a marker of lipid-induced insulin resistance, was similar between all dietary groups (LF\_PO,  $0.81 \pm 0.04$ ; HF\_PO,  $1.25 \pm 0.19$ ; HF\_CB,  $0.96 \pm 0.14$ ; HF\_OO,  $0.82 \pm 0.09$ ; and HF\_SO,  $0.89 \pm 0.09$  AU,  $P > 0.05$ ).

GLUT4 protein content in sarcolemma was not affected by the composition of the HF diets (LF\_PO,  $0.94 \pm 0.14$ ; HF\_PO,  $1.05 \pm 0.09$ ; HF\_CB,  $0.98 \pm 0.14$ ; HF\_OO,  $1.08 \pm 0.20$ ; and HF\_SO,  $1.09 \pm 0.11$  AU,  $P > 0.05$ ).

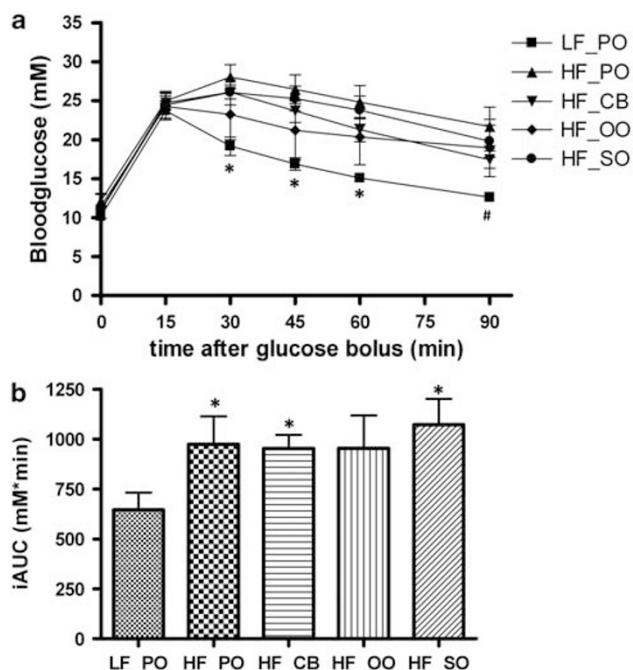
#### Liver lipid content

As liver is also an important organ involved in glucose tolerance, liver fat content was also measured. Liver TAG content was increased in mice receiving HF\_PO and HF\_OO compared with mice receiving LF\_PO, HF\_CB and HF\_SO after 8 weeks of HF feeding ( $P < 0.05$ , Figure 6).

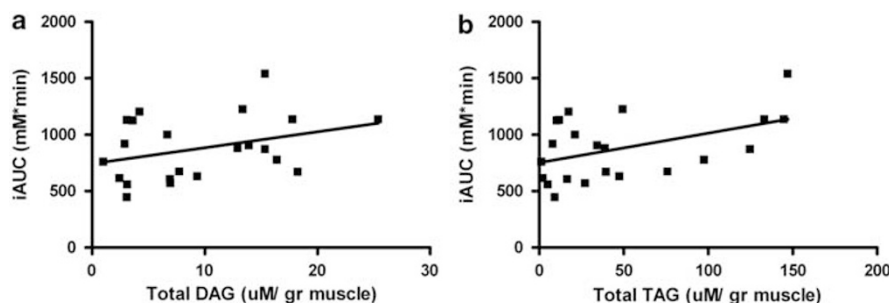
#### Muscle oxidative capacity

We also examined whether the composition of the HF diets lead to alterations in muscle oxidative capacity. Citrate synthase activity in whole-muscle homogenate was similar between the LF and the different HF groups (LF\_PO,  $21.88 \pm 1.59$ ; HF\_PO,  $21.33 \pm 1.12$ ; HF\_CB,  $19.10 \pm 0.89$ ; HF\_OO,  $22.93 \pm 2.23$ ; and HF\_SO,  $21.49 \pm 0.94$  U g<sup>-1</sup> protein,  $P > 0.05$ ). No differences in  $\beta$ -hydroxyacyl-CoA dehydrogenase activity were detected between the different dietary groups, indicating similar  $\beta$ -oxidative capacity (LF\_PO,  $34.70 \pm 4.79$ ; HF\_PO,  $37.95 \pm 3.10$ ; HF\_CB,  $37.17 \pm 4.50$ ; HF\_OO,  $36.98 \pm 6.75$ ; and HF\_SO,  $35.64 \pm 6.47$  U g<sup>-1</sup> protein,  $P > 0.05$ ).

Eight weeks of HF feeding did not decrease PGC1 $\alpha$  protein levels, a strong marker of mitochondrial biogenesis (Figure 7a). Moreover, mitochondrial density was not statistically different between diets as determined by western blotting of the structural complexes of the respiratory chain

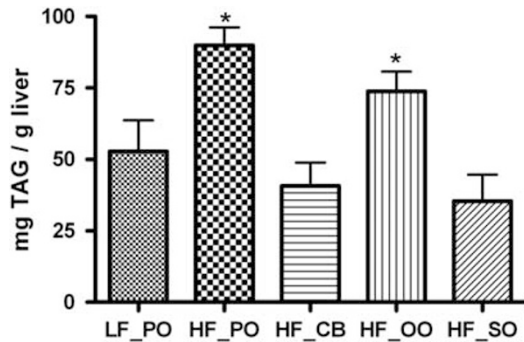


**Figure 4** Effect of dietary FA composition on whole-body glucose tolerance. Glucose clearance after oral glucose bolus ( $2.5 \text{ g kg}^{-1}$ ; a) and area under the curve (AUC) of the glucose tolerance test (b). Data are expressed as mean  $\pm$  s.e.m. ( $n = 6$ ). \* $P < 0.05$  LF\_PO versus HF\_PO, HF\_CB and HF\_SO. # $P < 0.05$  LF\_PO versus HF\_PO and HF\_SO.



**Figure 5** Relation between glucose intolerance and total muscular DAG (a) and TAG (b) content. Correlations ( $r = 0.35$  and  $P = 0.10$ ,  $r = 0.43$  and  $P = 0.039$  for DAG and TAG, respectively) are linear.

in whole-muscle homogenate (Figures 7b–e). Furthermore, protein levels of ATGL, the main lipase in the muscle responsible for the breakdown of TAG to DAG was

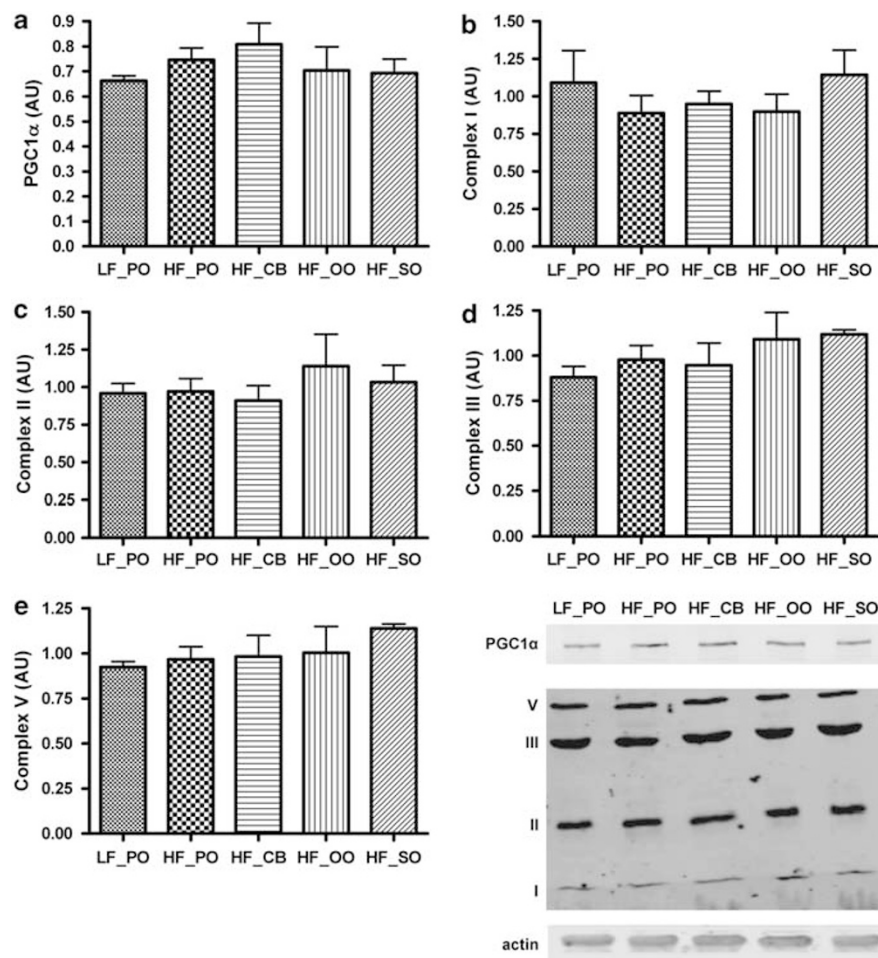


**Figure 6** Effect of dietary FA composition on liver TAG content. Data are expressed as mean  $\pm$  s.e.m. ( $n=6$ ). \* $P<0.05$  LF\_PO, HF\_CB and HF\_SO versus HF\_PO and HF\_OO.

unaffected by the composition of the different diets (LF\_PO,  $0.88 \pm 0.17$ ; HF\_PO,  $0.94 \pm 0.10$ ; HF\_CB,  $1.02 \pm 0.08$ ; HF\_OO,  $1.12 \pm 0.12$ ; and HF\_SO,  $1.08 \pm 0.12$  AU,  $P>0.05$ ).

## Discussion

From literature, it is known that in conditions of a continuous HF intake (that is, obesity), the degree of saturation of the FAs in the diet might have a crucial role in the development of obesity-related metabolic complications, as it might influence the storage form of FAs inside the muscle. Therefore, the present study examined whether diets varying in FA composition could differentially affect the rate of body weight gain and the accumulation of fat in skeletal muscle. Here, we show that after 8 weeks of HF feeding, only HF\_CB prevented body weight gain. Furthermore, we show that a high dietary fat intake in the form of SO prevented the accumulation of fat in skeletal muscle. Interestingly, the FA



**Figure 7** Effect of dietary FA composition on the expression of PGC1α (a) and the individual complexes I, II, III and V of the respiratory chain (b–e) in gastrocnemius muscle. Data are expressed as mean  $\pm$  s.e.m. ( $n=6$ ).

composition of DAG and TAG in skeletal muscle was a reflection of the dietary FA composition.

Eight weeks of HF feeding gradually increased body weight in all dietary groups. However, the change in body weight over the 8 weeks of dietary intervention varies between the different HF diets. This indicates that besides the high amount of fat, the type of FAs in the diet also influences the rate of weight gain in the development of obesity, as food intake was similar for all the dietary groups. Whereas HF\_PO, which is rich in palmitic acid (43% C16:0) and oleic acid (41% C18:1), induced the highest increase in body weight during the dietary intervention period, HF\_CB only led to a small increase in body weight, which was not significantly different from the LF\_PO diet. The lower body weight gain for the HF\_CB group can be explained by the fact that it is a vegetable fat that is rich in SFAs; palmitic acid (C16:0); and stearic acid (C18:0), thereby influencing the digestibility of the lipid. Animal studies have shown that 5–20% CB in the diet results in lower digestibility (60–70%), resulting in a higher fecal lipid elimination.<sup>28,29</sup> In accordance, mice receiving the HF\_CB diet also had a higher feces production. However, because food intake was only recorded during 1 week of the intervention, and feces was not analyzed for fat content, we cannot exclude the possibility that cumulative small, nonsignificant differences in food intake or a difference in fat partitioning may underlie the reduced body weight gain in these animals. Moreover, measurements of 24 h energy expenditure would have been helpful in interpreting energy and fat balance, but were not performed in the current study.

At the end of the dietary intervention, the storage of lipids inside the muscle for diets containing a high amount of SFAs (HF\_CB), MUFAs (HF\_OO) or a combination of both (HF\_PO) were significantly increased compared with the LF\_PO diet. Interestingly, for HF\_SO, myocellular TAG levels were comparable to the LF\_PO diet, indicating that a diet rich in PUFAs does not result in skeletal muscle lipid accumulation. For myocellular DAG accumulation, a similar pattern was observed, whereas the HF diets comprising mostly SFAs (HF\_CB), MUFAs (HF\_OO), or a combination of both types of FAs (HF\_PO) significantly elevated myocellular DAG content, HF\_SO, containing primarily PUFAs, did not increase DAG levels compared with the LF-fed animals.

These results indicate that diets rich in PUFAs may have beneficial effects on myocellular fat accumulation and are in line with reports showing a preferential oxidation of PUFAs, whereas SFAs appear to be predominantly stored in muscle rather than oxidized for energy.<sup>15,30</sup> The finding that the HF\_CB diet, which has by far the highest content of SFA and leads to a reduced fat absorbance and body weight increase, still increased DAG and TAG markedly, also suggests that SFAs that are taken up are redirected toward storage as TAGs and DAGs in skeletal muscle.

As it is well known that fat accumulation in muscle, especially also in the form of DAG, may lead to muscle insulin resistance, our data may also explain why dietary

MUFAs, and especially the PUFA linoleic acid (C18:2), have been linked to improved insulin sensitivity.<sup>31–33</sup> In literature, the beneficial effect of PUFAs on insulin sensitivity is generally accounted for by a direct effect on insulin action rather than lowering glucose values.<sup>31</sup> Unfortunately, we could not determine insulin sensitivity in our study, but we did find that whole-body glucose tolerance was positively correlated with myocellular DAG and TAG levels. We also measured makers of insulin sensitivity, such as IRS ser<sup>307</sup> phosphorylation—an indicator of lipid-induced insulin resistance<sup>34</sup>—and GLUT4 protein content of the sarcolemma but no changes were induced by the different dietary treatments. As muscle lipid content is not the only determinant of glucose tolerance, we also determined liver TAG content. Again, HF\_SO did not increase liver TAG after 8 weeks of HF feeding. Furthermore, HF\_CB also did not increase liver TAG. Further studies are necessary to examine whether changes in myocellular and hepatic lipid accumulation, induced by different types of dietary fat, will have functional consequences, which, however, was out of the scope of the present study.

We here also show that the dietary FA composition of the individual HF diets is reflected in the FA pattern of both myocellular TAG and DAG. Thus, the FA most represented in the individual HF diets also accounted for the highest percentage within both myocellular DAG and TAG content. These data are consistent with findings in adipose tissue for which it has also been shown that the FA composition of the TAG fraction reflects the dietary patterns over a considerable time.<sup>20,21</sup> It therefore seems that the dietary FA composition affects skeletal muscle FA composition of both DAG and TAG over a relatively short time. At this moment, too little information is available on the impact of changes in muscle FA composition on muscle function, although suggestions have been made that diet-induced modifications in membrane lipid composition are associated with changes in energy metabolism and may impact substantially on diseases such as the metabolic syndrome by influencing a range of glucose- and lipid-handling pathways.<sup>35</sup> Clearly, more information is needed on the impact of muscle FA composition on muscle metabolism, but our data are encouraging in that respect. On another note, analysis of muscle FA composition could help to test the adherence to diets in short-term nutritional intervention studies, although this would only be applicable in studies including invasive measurements.

An explanation for the differences in muscle fat accumulation between diets could be changes in fat oxidative and mitochondrial capacity. Indeed, in recent years, several studies have shown that a high intake of dietary fat affects mitochondrial oxidative capacity in skeletal muscle.<sup>36–41</sup> Although the functional analysis of mitochondrial function was out of scope of the present study, we did measure markers of mitochondrial metabolism. Measurement of citrate synthase activity revealed no such differences.  $\beta$ -Hydroxyacyl-CoA dehydrogenase activity, reflecting

$\beta$ -oxidation capacity, was also similar between the diets, with varying FA composition.

Mitochondrial function was also assessed by measurement of the OXPHOS complexes and PGC1 $\alpha$ . However, we did not observe a negative effect of HF feeding on these markers of mitochondrial metabolism. Although we did not directly measure the functional consequences of the different HF diets, our results are in accordance with some recent studies that also failed to show a negative effect of HF feeding on mitochondrial function. Thus, we showed that an 8-week HF dietary intervention in male Wistar rats did not affect the intrinsic functioning of rat skeletal muscle mitochondria as assessed by respirometry.<sup>42</sup> Also, Bonnard *et al.*<sup>43</sup> did not detect mitochondrial dysfunction after a 4-week HF diet intervention in C57BL/6J mice, whereas in an extended dietary intervention of 16 weeks, altered mitochondrial biogenesis, structure and function in muscle tissue were observed. According to Bonnard *et al.*,<sup>43</sup> mitochondrial alterations do not precede the onset of insulin resistance. Possibly, this also holds true for our data, but our data do not allow elaboration on insulin resistance.

As mitochondrial oxidative capacity could not explain differences in muscle fat accumulation, we also examined whether the different diets exert beneficial effects on muscle fat balance by stimulating lipolysis. However, the measurement of ATGL, the main lipase responsible for the breakdown of TAG into DAG, was unaffected by diet composition.

In summary, we show that after 8 weeks of HF feeding, the FA profile of both TAG and DAG lipid intermediates in the muscle mimicked the FA profile of the individual HF diets, indicating that after a relatively short period the dietary fat intake relates to the FA composition of the lipid metabolites in the muscle.

Furthermore, we show that a HF diet, rich in SO, is able to prevent accumulation of fat in skeletal muscle. Finally, we could not find evidence for a negative effect of HF diets on markers of mitochondrial metabolism.

## Conflict of interest

The authors declare no conflict of interest.

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