

# Skeletal muscle metabolic characteristics before and after energy restriction in human obesity: fibre type, enzymatic beta-oxidative capacity and fatty acid-binding protein content.

## Citation for published version (APA):

Kempen, K. P. G., Saris, W. H. M., Kuipers, H., Glatz, J. F., & van der Vusse, G. J. (1998). Skeletal muscle metabolic characteristics before and after energy restriction in human obesity: fibre type, enzymatic beta-oxidative capacity and fatty acid-binding protein content. *European Journal of Clinical Investigation*, 28(12), 1030-1037. <https://doi.org/10.1046/j.1365-2362.1998.00383.x>

## Document status and date:

Published: 01/01/1998

## DOI:

[10.1046/j.1365-2362.1998.00383.x](https://doi.org/10.1046/j.1365-2362.1998.00383.x)

## Document Version:

Publisher's PDF, also known as Version of record

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## Skeletal muscle metabolic characteristics before and after energy restriction in human obesity: fibre type, enzymatic $\beta$ -oxidative capacity and fatty acid-binding protein content

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### Abstract

**Background** Skeletal muscle has the ability to adapt as result of dietary, hormonal or pharmacological interventions affecting energy metabolism. The aim of the present study was to investigate the effects of energy restriction on skeletal muscle metabolic characteristics in obese women.

**Methods** The effects of 8 weeks' energy restriction on body composition, energy expenditure and skeletal muscle characteristics were investigated in 28 healthy obese women. Subjects were aged  $37.9 \pm 1.5$  years and had a body mass index of  $32.0 \pm 0.8 \text{ kg m}^{-2}$ .

**Results** Energy restriction ( $2800 \text{ kJ day}^{-1}$ ) resulted in a  $10.8 \pm 0.5 \text{ kg}$  weight loss consisting of  $8.6 \pm 0.5 \text{ kg}$  of fat mass and  $2.2 \pm 0.3 \text{ kg}$  of fat-free mass. Basal respiratory exchange ratio, sleeping metabolic rate and exercise-induced thermogenesis significantly declined in response to the diet. These changes were accompanied by an increase ( $P = 0.038$ ) in the skeletal muscle content of cytosolic fatty acid-binding protein (H-FABP), whereas no changes occurred in fibre type distribution or activities of enzymes reflecting  $\beta$ -oxidation and mitochondrial density (3-hydroxyacyl-CoA dehydrogenase and citrate synthase respectively).

**Conclusion** The results suggest that increased capacity of intracellular fatty acid transport in skeletal muscle cells is involved in the physiological adaptations of fat metabolism to energy restriction in obese female subjects.

**Keywords** Cytosolic fatty acid-binding protein, energy metabolism, substrate utilization, obesity, oxidative enzymes.

*Eur J Clin Invest* 1998; 28 (12): 1030–1037

### Introduction

Cytoplasmic fatty acid-binding proteins (FABPs) have been convincingly demonstrated to be present in cells of tissues that are actively involved in the uptake or utilization of fatty acids [1–4]. There is substantial evidence to suggest that FABPs are involved in transcytoplasmic transport and metabolism of the poorly soluble fatty acids [2]. Dietary, hormonal and pharmacological manipulations have been shown to change the FABP content of specific tissues in experimental animals [1,5,6]. Diets rich in fat

lead to modest increases in FABPs in heart, liver and intestine [1]. Furthermore, insulin and thyroid hormones are suggested to be involved in the expression of liver FABP [1], whereas muscle FABP is increased in experimental diabetes [5] and with testosterone treatment [6]. These animal data indicate that specific interventions affecting fat metabolism may be involved in the regulation of FABPs. In humans, hardly any data on the effects of such interventions are available. Therefore, the physiological importance of FABPs for human energy metabolism remains to be established.

For reasons of health, it is recommended that obese subjects reduce their weight, and the most effective methods to attain this goal are energy-restrictive dietary interventions. Energy restriction causes a shift in substrate metabolism associated with an enhanced fatty acid utilization by skeletal muscle [7–9]. As muscular metabolic characteristics have been shown to have the ability to adapt to 6–8 weeks of moderate endurance training [9,10], similar adaptive mechanisms might contribute to

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Received 29 July 1997; accepted 19 June 1998

the changes in fatty acid metabolism observed with energy restriction, as previously suggested, such as changes in fibre type composition and activities of oxidative enzymes [10–12].

Because a quantitative relationship has been demonstrated between the skeletal muscle content of cytoplasmic FABP, which has been shown to be identical to heart-type FABP (H-FABP) [13], and mitochondrial fatty acid oxidation capacity [2,3,9], it may be speculated that adaptations of muscle H-FABP are involved in adaptations of fuel metabolism to energy restriction. Furthermore, as fatty acid uptake and transport are the first events in cellular fatty acid oxidation, H-FABP may be an early indicator of metabolic adaptations in skeletal muscle.

Therefore, the aims of the present study were to investigate the effects of an 8-week energy restriction regimen on skeletal muscle H-FABP content, fibre type distribution and some marker enzymes of  $\beta$ -oxidative capacity. It was also examined whether these skeletal muscle characteristics were related to the contribution of lipids and carbohydrates to overall substrate oxidation during moderate exercise, as represented by the respiratory exchange ratio (RER).

## Subjects and methods

### Subjects

The study population was a subsample from a group of 63 obese female subjects who were recruited for a weight loss programme by advertisements in local newspapers. The subsample ( $n = 28$ ) was created on the basis of their willingness to undergo muscle biopsies. The study population consisted of premenopausal women aged between 20 and 51 years (mean  $\pm$  SEM;  $37.9 \pm 1.5$  years). Selection criteria were a body mass index between 28 and  $38 \text{ kg m}^{-2}$  and being apparently healthy according to a medical examination. Before treatment all subjects gave their written informed consent. The study was approved by the university's ethics committee.

The subsample did not differ significantly in baseline characteristics from the other subjects in the study, nor did they differ in changes in body composition and energy expenditure due to weight loss treatment (unpaired Student's *t*-test). Baseline characteristics of the present subsample ( $n = 28$ ) and the whole study population ( $n = 63$ ) are given in Table 1.

### Experimental design

Measurements of maximal aerobic capacity, body composition, and sleeping metabolic rate (SMR) and an exercise test were performed immediately before the start of an 8-week energy-restrictive dietary intervention period. Measurements of body composition and sleeping and exercise-induced energy expenditure (EE) were repeated at the end of the energy restriction period while subjects were still on

the energy-restrictive diet. Needle muscle biopsies were taken percutaneously before and at the end of energy restriction for measurement of skeletal muscle H-FABP content, and activities of 3-hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase (CS). Muscle fibre type composition was determined in 18 out of 28 biopsies.

### Diet

The energy-restrictive diet took place over an 8-week period that was divided into two parts. The first period consisted of a low-energy formula diet (Modifast, Sandoz, Switzerland) containing  $2000 \text{ kJ day}^{-1}$  for 4 weeks. It provided 50 g of carbohydrates, 52 g of protein, 7 g of fat and a micronutrient content that met with the Dutch recommended daily allowance. In the second part of the energy restriction period, from week 5 until week 9, a mixed diet of  $3500 \text{ kJ day}^{-1}$  was followed. This diet contained  $1400 \text{ kJ day}^{-1}$  of the formula diet and was supplemented to  $3500 \text{ kJ day}^{-1}$  by a free choice of foodstuffs. During the whole period subjects were instructed to keep a record of food intake. Subjects were asked to maintain their habitual activity pattern. During the energy restriction period the subjects came to the laboratory once a week to have their weight measured and food records checked.

### Measurements

For determination of the maximal aerobic capacity, each subject's maximal oxygen uptake ( $\text{VO}_{2\text{max}}$ ) and maximal mechanical power output ( $W_{\text{max}}$ ) were measured at least 3 and at most 7 days before the other measurements at the beginning of the study, using a progressive continuous cycling test on an ergometer (Lode, Groningen, The Netherlands) as previously described [8]. During the test, ventilatory and gas exchange responses were measured continuously, using a computerized open system (Oxycon Beta, Mijnhardt, Bunnik, The Netherlands). Criteria for maximal exercise were forced ventilation, levelling off of oxygen uptake and a RER exceeding 1.1. The highest oxygen uptake achieved for at least 30 s was taken as  $\text{VO}_{2\text{max}}$ .

SMR was measured during a 12-h overnight stay (19.00 h to 07.00 h) in a respiration chamber as described previously [8]. Briefly, SMR and RER were calculated from  $\text{O}_2$  consumption and  $\text{CO}_2$  production [14] during the sleeping period between 03.00 and 06.00 h, controlled for extra physical activity by a Doppler radar system. After the overnight fast in the respiration chamber and body density measurement, an exercise test was performed. The exercise protocol consisted of 45 min of exercise on a bicycle ergometer at a workload of 45% of the previously determined  $W_{\text{max}}$ . Because this type of dietary restriction has been shown to affect neither  $W_{\text{max}}$  nor  $\text{VO}_{2\text{max}}$  [7,15], the same absolute workloads were used for each individual subject before and at the end of the diet. The exercise was preceded by a 30-min resting period (baseline measurement) with the subject in the

supine position. Respiratory exchange measurements were carried out continuously by means of a computerized open-circuit ventilated hood system during the baseline period. During exercise, the respiratory exchange responses were measured periodically for a total of 20 min in sequential 5-min blocks of time, using a computerized open system (Oxycon Beta, Mijnhardt, Bunnik, The Netherlands). The subject was monitored continuously using an electrocardiograph during the experiment.

Before and at the end of the study, body weight was measured on a digital balance accurate to 0.1 kg (Sauter, D-7470, Ebingen, Germany) and height to the nearest 0.1 cm was measured using a wall-mounted stadiometer. Body mass index was calculated from weight and height ( $\text{kg m}^{-2}$ ). Body composition was measured using two different techniques: isotope dilution and underwater weighing with simultaneous lung volume measurement (helium dilution). The percentage of body fat was calculated from body density and total body water (TBW) using the method proposed by Siri [16]. The deuterium dilution technique was used to measure TBW. Before going to bed at night during the stay in the respiration chamber a  $^2\text{H}_2\text{O}$  dilution was drunk after emptying the bladder (baseline urine sample). The dosage calculation of  $^2\text{H}_2\text{O}$  was based on body weight in order to create a  $^2\text{H}$  excess of 100 ppm. From the second voiding between 08.00 and 10.00 h on the next morning a second urine sample was collected. Deuterium was measured in urine samples with an isotope ratio mass spectrometer (VG Aqua Sira). TBW was calculated as the measured deuterium dilution space divided by 1.04 [17]. On the same morning, whole-body density was determined by hydrostatic weighing with the subject in the fasted state. Underwater weight was measured to the nearest 0.1 kg (Sauter, D-7470, Ebingen, Germany). The residual lung volume was measured using a spirometer (Volugraph, 2000, Mijnhardt, Bunnik, The Netherlands) using helium dilution during submersion at the moment of underwater weighing. Residual lung volume and the density of the water at the temperature at the time of the measurement were used to correct body volume. The measurements were performed in triplicate and the average was used to calculate body density.

Skeletal muscle biopsies were taken at rest before the diet and at the end of 8 weeks' energy restriction, after at least a 6-h fast and low physical activity. After local anaesthesia, a Bergström biopsy needle (diameter 5 mm, Stöpler, Utrecht, The Netherlands) with simultaneous suction [18] was used to obtain 100–150 mg of skeletal muscle from the vastus lateralis muscle. Blood and connective tissue were immediately removed from excised muscle samples and the tissue was divided into two pieces. One piece was frozen immediately in liquid nitrogen and stored at  $-80^\circ\text{C}$  until the analyses of metabolic parameters. The other part of the biopsy was trimmed, mounted in an embedding medium (Tissue Tek, Miles Laboratory, Elkhart, USA) and quickly frozen in isopentane, cooled by liquid nitrogen to its freezing point, and stored at  $-80^\circ\text{C}$  until histological determination of fibre type composition was performed.

## Biochemical analyses

Fibre type composition of the vastus lateralis muscle was analysed according to Dubowitz [19]. For this, transverse sections were cut at  $10\ \mu\text{m}$  thickness by use of a cryostat maintained at  $-22^\circ\text{C}$ . On the basis of their staining reactions for myofibrillar ATPase after alkaline preincubation, muscle fibres were classified as either type I or type II. Type II fibres were further subclassified into IIA and IIB types by preincubation at pH 4.6 and 4.4. About 400–500 fibres were counted in each muscle sample. The other part of the muscle specimens was homogenized in ice-cold EDTA ( $0.002\ \text{mol L}^{-1}$ )/Tris ( $0.01\ \text{mol L}^{-1}$ ) buffer at pH 7.4. The homogenates were subsequently sonicated for  $4 \times 15\ \text{s}$  and centrifuged at  $10\ 000 \times g$  for 2 min at  $4^\circ\text{C}$  to remove cell debris. Citrate synthase (CS) activity was analysed at  $37^\circ\text{C}$  according to the method of Shepherd & Garland [20], whereas 3-hydroxyacyl-CoA dehydrogenase (HAD) activity was assayed at  $37^\circ\text{C}$  according to Bergmeyer [21]. Tissue content of muscle-type fatty acid-binding protein (H-FABP) in skeletal muscle was measured by a newly developed enzyme-linked immunosorbent assay (ELISA) that has a lower detection limit of  $0.2\ \mu\text{g L}^{-1}$  [22]. H-FABP content, and CS and HAD activities are expressed per g wet weight (ww) skeletal muscle. This suggests the possibility of overestimation at the end of the diet owing to glycogen depletion as a result of the energy restriction. However, glycogen depletion due to the diet used in the present study is not likely [7,23], and, even if a decrease in muscle glycogen concentration is assumed, from 1.5 to 1.0 g per 100 g wet weight (ww) [23], the data would still be within the detection limits of the used analytical methods.

## Statistical analyses

All data are given as means  $\pm$  SEM. Physiological responses to exercise were analysed by a repeated measurement analysis of variance (ANOVA) and post hoc tested using paired Student's *t*-tests corrected according to Bonferroni's inequalities. Total exercise-induced response curves of EE and heart rate (HR) were calculated as the total integrated changes over baseline values ( $\text{AUC}_{0-45}$ ). Two-sided paired *t*-tests were used to analyse differences between before and after diet. For the evaluation of effects on skeletal muscle H-FABP content and enzyme activities a one-sided *t*-test was used, because under the high oxidative condition of energy restriction an increase in the content is to be expected rather than a decrease.  $P < 0.05$  was considered as statistically significant. Correlations are Pearson product-moment correlations.

## Results

### Maximal aerobic capacity, body composition and energy expenditure

The energy restriction resulted in a decrease in body weight of  $10.8 \pm 0.5\ \text{kg}$  (Table 1). Subjects lost  $8.6 \pm 0.5\ \text{kg}$  of fat

**Table 1** Descriptive data of the subset of 28 subjects before and at the end of 8 weeks' energy restriction.

Variables	Baseline	8 weeks of energy restriction
Weight (kg)	88.5 ± 2.5	77.7 ± 2.4*
Body mass index (kg m <sup>-2</sup> )	32.0 ± 0.8	27.9 ± 0.7*
Percentage body fat (%)	41.3 ± 0.8	35.8 ± 1.0*
Fat mass (kg)	36.9 ± 1.6	28.3 ± 1.6*
Fat-free mass (kg)	51.6 ± 1.0	49.4 ± 1.0*
SMR (kJ min <sup>-1</sup> )	4.82 ± 0.18	4.30 ± 0.13*
SMR kg <sup>-1</sup> FFM (kJ h <sup>-1</sup> kg <sup>-1</sup> )	5.59 ± 0.10	5.23 ± 0.09*
Sleeping RER	0.81 ± 0.01	0.78 ± 0.01*

Values are means ± SEM for 28 subjects. Diet is 2800 kJ day<sup>-1</sup>. SMR, sleeping metabolic rate; RER, respiratory exchange ratio. Baseline characteristics of the subset were not significantly different from the whole study population (*n* = 63, age 36.1 ± 1.2 years, weight 87.1 ± 1.6 kg, body mass index 31.6 ± 0.5 kg m<sup>-2</sup>, fat mass 41.9 ± 0.6%, SMR 4.70 ± 0.09 kJ min<sup>-1</sup>, sleeping RER 0.81 ± 0.01).

\**P* < 0.001 (paired *t*-test) with respect to baseline values.

mass and 2.2 ± 0.3 kg of fat-free mass. Both SMR, SMR kg<sup>-1</sup> fat-free mass and sleeping RER significantly declined in response to the diet. Pre-diet *W*<sub>max</sub> and *VO*<sub>2max</sub> amounted to 172 ± 2 W and 2.3 ± 0.7 l min<sup>-1</sup>, respectively, whereas during maximal exercise HR was 178 ± 2 beats min<sup>-1</sup>.

Table 2 shows values of EE, RER and HR during the exercise test at 45% of the individual pre-diet *W*<sub>max</sub> (mean workload 77 ± 2 W). Exercise resulted in significant increases in EE, RER and HR (ANOVA; *P* < 0.001). Energy restriction resulted in decreased values of EE during exercise. Also, the total thermogenic response to exercise was significantly lower during the diet in comparison with pre-diet value (AUC<sub>0-45</sub> 1079 ± 30 vs. 947 ± 37 kJ per 45 min, before vs. during diet; *P* < 0.005), whereas total integrated response of HR did not change significantly (AUC<sub>0-45</sub> 3380 ± 134 vs. 3501 ± 127 beats per 45 min, before vs. during diet; NS). Baseline and recovery values of RER declined in response to the diet, but during exercise values were not statistically different.

### Fibre type composition

Figure 1 shows the percentage distribution of muscle fibres of the vastus lateralis. Before the diet, the proportion of type

I fibres was 43% ± 3%, whereas the percentages of type IIA and IIB were 27% ± 2% and 31% ± 2% respectively. There was no significant change in muscle fibre type distribution with energy restriction. In addition, no significant correlations could be observed between the proportion of type I muscle fibres and the body fat percentage either at week 0 (*r* = 0.27; *P* = 0.28) or at week 8 (*r* = 0.17; *P* = 0.50). There was also no significant relationship between muscle fibre type and RER during exercise before the diet treatment commenced (Fig. 2a and b, mean RER during exercise: type I, *r* = 0.03; *P* = 0.94; and type IIB, *r* = -0.06; *P* = 0.82). However, at the end of the diet a significant relationship was found between muscle fibre type and mean RER during the exercise period. Percentages of type I or type IIB were significantly correlated with mean RER value during exercise (Fig. 2c and d: *r* = 0.44, *P* < 0.05, and *r* = -0.51, *P* < 0.01, respectively). The RER values of the separately measured time periods during exercise were also significantly correlated with muscle fibre type (data not shown).

### Muscle H-FABP content and enzyme activities

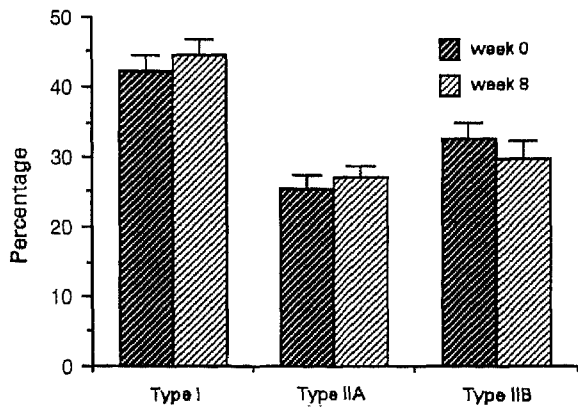
H-FABP content and activities of HAD and CS of the vastus lateralis muscle before and after the energy

**Table 2** Metabolic and heart rate responses to 45 min of exercise (45% pre-diet *W*<sub>max</sub>) before and at the end of 8-week diet in obese women.

	Week	Rest	Duration of exercise (min)				Recovery
			0-5	15-20	30-35	40-45	
EE (kJ min <sup>-1</sup> )	0	5.1 ± 0.1	25.8 ± 0.7	31.0 ± 0.7	31.7 ± 0.9	32.2 ± 0.8	5.4 ± 0.1
	8	4.8 ± 0.1†	23.3 ± 0.6†	28.1 ± 0.7*	28.7 ± 0.8†	29.1 ± 0.8†	5.1 ± 0.1†
RER	0	0.85 ± 0.01	0.89 ± 0.01	0.92 ± 0.01	0.88 ± 0.01	0.87 ± 0.01	0.78 ± 0.01
	8	0.79 ± 0.01†	0.87 ± 0.01	0.91 ± 0.02	0.87 ± 0.01	0.85 ± 0.01	0.74 ± 0.01†
Heart rate (beats min <sup>-1</sup> )	0	68 ± 2	139 ± 3	147 ± 3	151 ± 3	152 ± 3	77 ± 2
	8	68 ± 2	138 ± 3	147 ± 3	153 ± 3	155 ± 3	74 ± 2

Values are means ± SEM (*n* = 28). Diet is 2800 kJ day<sup>-1</sup>. EE, energy expenditure; RER, respiratory exchange ratio.

\*Significantly different from week 0, *P* < 0.005 paired *t*-test, †*P* < 0.001.



**Figure 1** Percentage distribution of muscle fibres (type I, IIA and IIB) in vastus lateralis muscle from obese women before and at the end of an 8-week energy-restrictive diet. Mean  $\pm$  SEM ( $n=18$ ); no significant change in muscle fibre type distribution as result of diet treatment.

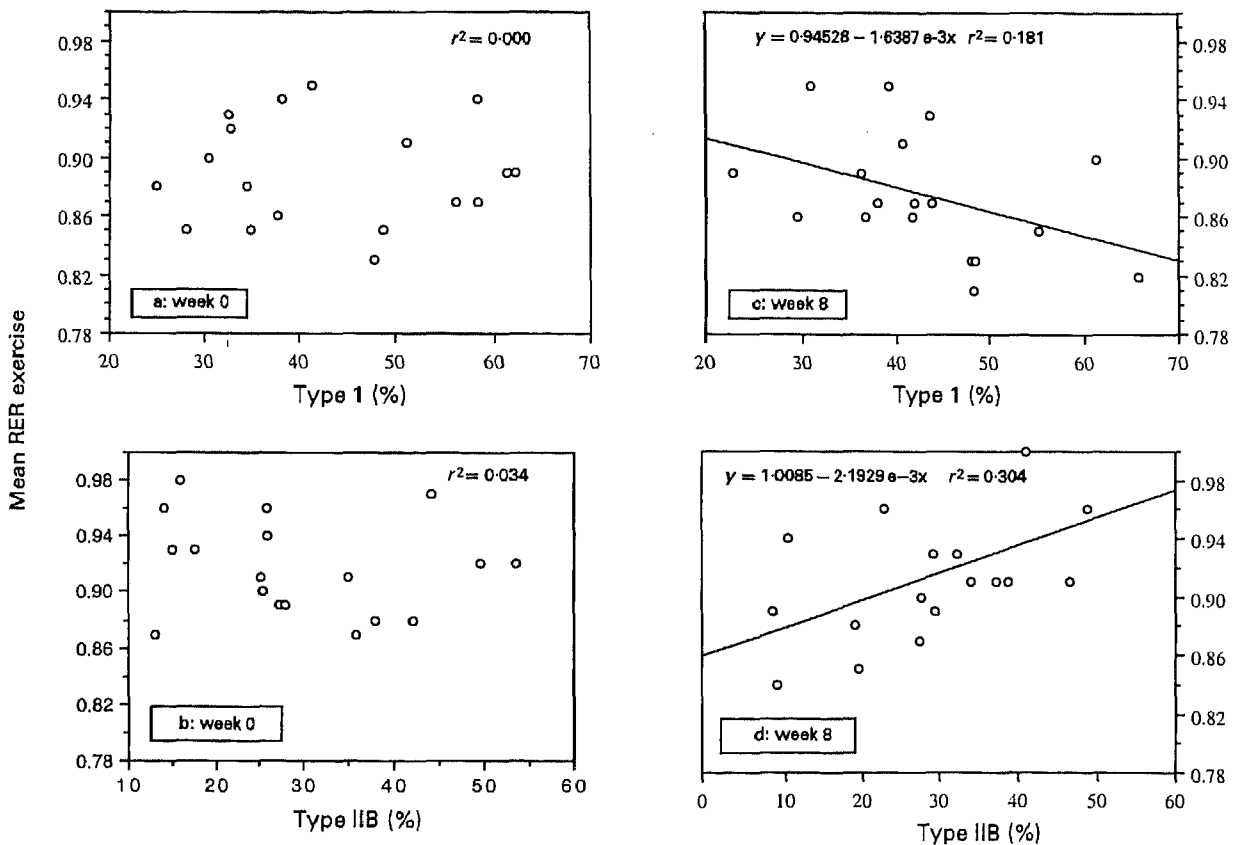
restriction are shown in Table 3. The H-FABP content of skeletal muscle increased in response to the diet ( $n=28$ ;  $P=0.038$ ), whereas activities of HAD and CS did not significantly change.

**Table 3** Skeletal muscle H-FABP content, and activities of HAD and CS in 28 obese women before and after an 8-week energy-restrictive diet

	P-value		
	Week 0	Week 8	Week 0 vs. week 8.
H-FABP	173 $\pm$ 12	216 $\pm$ 21	0.038*
HAD	12.4 $\pm$ 0.7	12.9 $\pm$ 1.0	0.65
CS	10.6 $\pm$ 0.8	11.3 $\pm$ 0.8	0.45

Values are means  $\pm$  SEM. Diet is 2.8 MJ day<sup>-1</sup>. H-FABP, heart-type fatty acid-binding protein ( $\mu\text{g g}^{-1}$  ww); HAD, 3-hydroxyacyl-CoA dehydrogenase ( $\text{U g}^{-1}$  ww); CS, citrate synthase ( $\text{U g}^{-1}$  ww). \* $P < 0.05$  one-tailed Student's paired *t*-test.

Before as well as at the end of the diet, H-FABP content was significantly correlated with HAD and CS activities (week 0,  $r=0.69$  and  $r=0.65$  respectively,  $P < 0.001$ , and week 8,  $r=0.79$  and  $r=0.78$  respectively,  $P < 0.001$ ), whereas activities of HAD and CS were also related to each other (week 0,  $r=0.91$ , and week 8,  $r=0.94$ ;  $P < 0.001$ , data not shown). H-FABP values also showed a significant relationship with the percentage of type I fibres ( $n=18$ ,  $r=0.53$ ;  $P < 0.05$ ). However, no statistically significant relationships could be demonstrated between skeletal muscle H-FABP content and relative substrate



**Figure 2** Relationship between muscle fibre type composition and mean respiratory exchange ratio (RER) during exercise in obese women ( $n=18$ ) before (a + b) and at the end of an 8-week energy-restrictive diet (c + d). Significant relationship between muscle fibre type and mean RER during exercise (for type I  $r=0.44$ ,  $P < 0.05$ ; for type IIB  $r=-0.51$ ,  $P < 0.01$ ).

utilization during rest or exercise, as estimated from the RER value.

## Discussion

Energy-restrictive diets led to a decrease in the whole-body RER, suggesting that a greater proportion of the energy is supplied by oxidation of lipids. As skeletal muscle represents the largest tissue of the body (>30% of body mass) and shows the ability to adapt, for example, to 6–8 weeks of moderate endurance training [10], it has been suggested that adaptations of skeletal muscle contribute to the increased fat utilization observed with energy restriction [9,10,24]. However, so far no significant changes in fibre type composition or enzyme activities involved in fat oxidation have been detected as a result of energy restriction [10,12]. On the other hand, a decrease in fasting skeletal muscle lipoprotein lipase activity has been demonstrated with weight reduction in obese women that was correlated with the resultant change in per cent body fat [25]. In the present study, we demonstrate that skeletal muscle cytoplasmic fatty acid-binding protein (H-FABP) increases as a result of energy restriction in obesity, indicating that skeletal muscle indeed adapts to this new situation.

The physiological significance of the skeletal muscle cytoplasmic fatty acid carrier H-FABP in human energy metabolism and during energy restriction remains to be established. As far as we know, this is the first study demonstrating an increase in the H-FABP content of skeletal muscle in response to hypocaloric dieting leading to substantial weight loss in obese subjects. This finding suggests that an increased capacity to transport fatty acids in the cytoplasmic space is related to substantial weight loss.

Circumstantial evidence is available that the ability to utilize fatty acids by skeletal muscle is related to its H-FABP content [3,9,26]. Current knowledge indicates that H-FABP functions as a carrier of fatty acids between the plasma membrane and the outer mitochondrial membrane [27], although the precise mechanism of action remains to be elucidated [2,3]. Dietary, hormonal and pharmacological interventions have been shown to alter the H-FABP content in experimental animals [1,2,5,6]. The results of the present study suggest that the H-FABP content of muscle in obese women adapts to hypocaloric dieting, and that this mechanism may play a role in increased fat oxidation under basal conditions.

The physiological significance of the enhanced H-FABP content of skeletal muscle with energy restriction remains, however, to be established, because in general FABP appears to be present in excess and it has been calculated that only a minor part (<2%) of the total FABP content is complexed with fatty acids [26]. The observed positive relationship between H-FABP content and proportion of type I fibres, found in the present study for humans, has been found previously by Vork *et al.* [28] for rat muscles. Type IIB muscle fibres, which demonstrate minimal fatty

acid utilization, have been shown to have the lowest cellular amount of H-FABP [1,6,9,13]. The significant correlations between skeletal muscle H-FABP content and activities of HAD and CS support the previously described existence of a quantitative relationship between mitochondrial fatty acid oxidation capacity and FABP content [2,3]. Therefore, the present study supports the notion that H-FABP is involved in intracellular fatty acid utilization in human skeletal muscle.

The mechanism by which FABP levels respond to changes in dietary intake has not yet been elucidated. Recently, however, it has been demonstrated *in vitro* that exogenous long-chain fatty acids induce gene expression of the adipocyte-type FABP at a transcriptional level in 3T3 adipocytes [29,30]. It may be speculated that such a mechanism also applies to the *in vivo* skeletal muscle in the present study, as energy restriction has been shown to be associated with increased basal plasma fatty acid levels [8]. However, the importance of the fatty acid concentrations in plasma, and possibly even more in muscle cells, as triggers for muscular H-FABP expression *in vivo* remains to be determined.

The results of the present study also show that an 8-week-long energy-restricted diet, resulting in a weight loss of  $10.8 \pm 0.5$  kg, did not significantly affect skeletal muscle fibre type distribution and activities of measured oxidative enzymes (HAD and CS), in accordance with findings by others [10,11,31] that no enzymatic adaptations of mitochondrial  $\beta$ -oxidation and subsequent oxidation of the products derived from  $\beta$ -oxidation occur. On the other hand, possible effects of hypocaloric dieting on skeletal muscle have been suggested to occur at the level of fibre type area instead of fibre type transformation [10,12,31]. Decreased cross-sectional area of type II fibres due to decreases in glycogen content or activities of glycolytic enzymes as a result of energy restriction, together with unchanged capillarization, may lead to a relative increase in capillary density in muscle tissue, enhancing insulin sensitivity [32] and most likely resulting in increased oxidative capacity.

Theoretically, it is possible that effects of hypocaloric dieting in the present study might have been affected by changes in physical activity that occurred during the diet. It has been shown that physical training and reduction in energy intake have synergistic effects on skeletal muscle metabolic adaptations [31]. However, as measured by heart rate monitoring and actometer recording [15] or doubly labelled water [33], no compensatory decrease in physical activity has been shown in obese women after a diet treatment. Therefore, the skeletal muscle adaptations detected in the present study are most likely to be largely attributable to the effects of energy restriction, consisting of the combined influences of weight loss and hypocaloric state.

Our results demonstrate that the energy costs of exercise decrease in response to energy restriction, probably mainly as a result of loss of some lean body tissue. In the present study, a significant relationship between skeletal muscle fibre type distribution and substrate oxidation during exercise could be observed at the end of the diet. This finding

appears to be in accordance with a study by Wade *et al.* [34], demonstrating that muscle fibre type proportion is related to the oxidation of fatty acids during exercise. We have, however, no clear explanation for the fact that a relationship between substrate utilization during exercise and muscle fibre type could not be demonstrated before the start of the study. It might be speculated that less confounding arousal in measuring RER values at the end of the 8-week treatment than before treatment may be involved, owing to lower interindividual variation in antecedent diet.

The present results, however, suggest a role for muscle fibre type profile in overall substrate utilization during exercise, whereas skeletal muscle H-FABP content is not likely to be a limiting factor in enhanced muscle lipid utilization. The notion that the transcytoplasmic fatty acid transport capacity is most likely not rate limiting in overall intramuscular fatty acid utilization has also been suggested previously by Vork *et al.* [27], because of the relatively abundant presence of this protein in oxidative muscle cells.

In summary, the results of the present study demonstrate an adaptation of skeletal muscle cytosolic fatty acid-binding protein to energy restriction in obese females.

This suggests that adequate transcytoplasmic transport of fatty acids might be involved in the energy restriction-induced modification of skeletal muscle fatty acid utilization. Further research should elucidate the physiological importance of skeletal muscle H-FABP in human energy metabolism and obesity.

## Acknowledgements

The authors thank J. H. C. H. Stegen, P. Geurten, G. van Kranenburg and Y. F. de Jong for expert technical assistance. This work was supported by The Netherlands Organization for Scientific Research, grant 900-562-090.

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