Muscle acylcarnitines during short-term fasting in lean healthy men

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ABSTRACT

The transition from the fed to the fasted resting state is characterized by, among other things, changes in lipid metabolism and peripheral insulin resistance. Acylcarnitines have been suggested to play a role in insulin resistance, as well as other long-chain fatty acid metabolites. Plasma levels of long-chain acylcarnitines increase during fasting, but this is unknown for muscle long-chain acylcarnitines. In the present study we investigated whether muscle long-chain acylcarnitines increase during fasting and we investigated their relationship with glucose/fat oxidation and insulin sensitivity in lean healthy humans. After 14 h and 62 h of fasting, glucose fluxes, substrate oxidation, and plasma and muscle acylcarnitines were measured before and during a hyperinsulinaemic–euglycaemic clamp. Hyperinsulinaemia decreased long-chain muscle acylcarnitines after 14 h of fasting, but not after 62 h of fasting. In both the basal state and during the clamp, glucose oxidation was lower and fatty acid oxidation was higher after 62 h compared with 14 h of fasting. Absolute changes in glucose and fatty acid oxidation in the basal compared with hyperinsulinaemic state were not different. Muscle long-chain acylcarnitines did not correlate with glucose oxidation, fatty acid oxidation or insulin-mediated peripheral glucose uptake. After 62 h of fasting, the suppression of muscle long-chain acylcarnitines by insulin was attenuated compared with 14 h of fasting. Muscle long-chain acylcarnitines do not unconditionally reflect fatty acid oxidation. The higher fatty acid oxidation during hyperinsulinaemia after 62 h compared with 14 h of fasting, although the absolute decrease in fatty acid oxidation was not different, suggests a different set point.

INTRODUCTION

Short-term fasting can be defined as the first 72 h of starvation in which progressive alterations in lipid and glucose metabolism occur [1,2]. The adaptation to short-term fasting is characterized by, among other things, an increase in lipolysis with concomitant increases in plasma NEFAs (non-esterified fatty acids; ‘free fatty acids’) and FAO (fatty acid oxidation) [1], and a decrease in peripheral insulin sensitivity and CHO (carbohydrate oxidation) [2–4].

To be oxidized, activated long-chain fatty acids can only cross the mitochondrial membranes as ACs (acylcarnitines) [5]. The coupling of an activated long-chain fatty acid to carnitine (3-hydroxy-4-N,N,N-trimethylammonium) is catalysed by CPT1 (carnitine palmitoyltransferase 1) on the outer mitochondrial leaflet [6]. CPT1 is considered to be the

Key words: acylcarnitine, glucose oxidation, lipid oxidation, insulin resistance.

Abbreviations: AC, acylcarnitine; BMI, body mass index; CHO, carbohydrate oxidation; CPT, carnitine palmitoyltransferase; EGP, endogenous glucose production; FAO, fatty acid oxidation; FC, free carnitine; NEFA, non-esterified fatty acid (‘free fatty acid’); Rd, rate of glucose disposal; REE, resting energy expenditure; VCO₂, carbon dioxide production; VO₂, oxygen consumption.

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rate-limiting enzyme for long-chain fatty acid entry into the mitochondria and subsequent oxidation [5,7]. Furthermore CPT1 activity increases during fasting in animal studies [8]. Inside the mitochondrion, the AC is activated to acyl-CoA again by CPT2. The released carnitine is exchanged for a new incoming AC by the mitochondrial membrane protein CACT (carnitine-AC translocase) [6].

Plasma ACs are thought to reflect the mitochondrial acyl-CoA pool, and AC profile analysis is the current standard for the diagnosis of FAO disorders at the metabolite level [9,10]. Previously, muscle ACs have been implicated in insulin resistance via a currently unknown mechanism [8,11,12]. These studies suggested that increased β-oxidation outpaces the TCA (tricarboxylic acid) cycle with subsequent inhibition of complete FAO via a high-energy redox state (rising NADH/NAD+ and acetyl-CoA/CoA ratios). The accumulation of metabolic by-products (e.g. ACs) would then activate stress kinases or other signals, interfering with insulin action [11]; however, how ACs directly affect insulin-mediated glucose uptake is currently unexplained.

Fasting increases plasma long-chain ACs [13–15], but it is unknown whether muscle long-chain ACs increase during short-term fasting in humans. Animal studies have shown increased muscle long-chain AC levels during fasting [16,17]. Such an increase in ACs during fasting would match increased lipid oxidation and decreased peripheral insulin sensitivity [1,3,4].

In the present study, we examined the association of muscle ACs during short-term fasting with glucose and fat metabolism. Healthy lean subjects were studied before and after short-term fasting in both the basal state and during an hyperinsulinaemic–euglycaemic clamp. We hypothesized that fasting induced an increase of whole-body FAO resulting in an increase in muscle ACs which would explain the expected peripheral insulin resistance.

**MATERIALS AND METHODS**

**Subjects**

We studied healthy lean male volunteers who participated in a study on fasting-induced insulin resistance [4]. Subjects were in self-reported good health, confirmed by medical history and physical examination. Criteria for inclusion were (i) the absence of a family history of diabetes; (ii) age 18–35 years; (iii) Caucasian race; (iv) BMI (body mass index) 20–25 kg/m²; (v) normal oral glucose tolerance test according to ADA (American Diabetes Association) criteria [18]; (vi) normal routine blood examination; (vii) no excessive sport activities, i.e. <3 times per week; and (viii) no medication. Written informed consent was obtained from all subjects after explanation of the purpose, nature and potential risks of the study. The study was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

**Experimental protocol**

Subjects were studied twice, after 14 h and 62 h of fasting. Study days were separated by at least a week. Subjects were fasting from 20.00 hours the evening before the first study day and from 20.00 hours 3 days before the second study day until the end of the study days. They were allowed to drink water only.

After admission to the metabolic unit at 07.30 hours, a catheter was inserted into an antecubital vein for infusion of stable isotope tracers, insulin and glucose. Another catheter was inserted retrogradely into a contralateral hand vein and kept in a thermoregulated (60 °C) plexiglas box for sampling of arterialized venous blood. Saline was infused as 0.9 % NaCl at a rate of 50 ml/h to keep the catheters patent. [6,6-2H2]Glucose and [1,1,2,3,3,5-2H5]glycerol were used as tracers (>99 % enriched; Cambridge Isotopes) to study glucose kinetics and lipolysis [total triacylglycerol (triglyceride) hydrolysis] respectively.

At t = 0 h (08.00 hours), blood samples were drawn for determination of background enrichments and a primed continuous infusion of both isotopes was started: [6,6-2H2]glucose (prime, 8.8 μmol/kg; continuous, 0.11 μmol·kg⁻¹·min⁻¹) and [1,1,2,3,3,5-2H5]glycerol (prime, 1.6 μmol/kg; continuous, 0.11 μmol·kg⁻¹·min⁻¹) and continued until the end of the study. After an equilibration period of 2 h (14 h of fasting), three blood samples were drawn for measurement of glucose and glycerol enrichments and one blood sample for glucoregulatory hormone, NEFA and plasma AC levels. Thereafter (t = 3 h), infusions of insulin (60 milliunits · m⁻² · min⁻¹) (Actrapid, 100 international units/ml; Novo Nordisk Farma) and 20 % glucose (to maintain a plasma glucose level of 5 mmol/l) were started. [6,6-2H2]Glucose was added to the 20 % glucose solution to achieve glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimizing changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose [19]. Plasma glucose levels were measured every 5 min at the bedside. At t = 8 h, five blood samples were drawn at 5 min intervals for determination of glucose and glycerol enrichments. Another blood sample was drawn for determination of glucoregulatory hormone, NEFA and plasma AC levels.

Subjects were studied under the same conditions after 62 h of fasting. Volunteers were allowed to drink water ad libitum. To prevent sodium and potassium depletion during fasting, subjects were supplied with 80 mmol of oral NaCl/day (Tablets, In-House Pharmacy, Amsterdam Medical Center) and 40 mmol of oral KCl/day (Slow-K; Novartis).
**Indirect calorimetry and muscle biopsies**

\( V_{O2} \) (oxygen consumption) and \( V_{CO2} \) (CO2 production) were measured continuously during the final 20 min of both the basal state and the clamp by indirect calorimetry using a ventilated hood system (model 2900; Sensormedics).

Muscle biopsies were performed to assess muscle AC concentrations at the end of both the basal state and the clamp. Biopsies were performed under local anaesthesia (Lidocaine 20 mg/ml; Fresenius) using a Pro-Mag I biopsy needle (MDTECH). Biopsy specimens were quickly washed in buffer (0.9% NaCl and 28.3 g/l Hepes) to remove blood, were inspected for fat or fascia content, dried on gauze swabs, and subsequently stored in liquid nitrogen until analysis.

**Glucose and lipid metabolism measurements**

Plasma glucose and NEFA concentrations were measured as described previously [4]. \( [{\text{6,6-}}^{2}\text{H}_2] \) Glucose enrichment was measured as described previously [20]. For \( [{\text{6,6-}}^{2}\text{H}_2] \) glucose enrichment (tracer/tracee ratio) the intra-assay variation was 0.5–1%, the inter-assay variation was 1%, and the detection limit was 0.04%. \( [{\text{1,1,2,3,3-}}^{2}\text{H}_5] \) Glycerol enrichment was determined as described previously [21]. For glycerol enrichment the intra-assay variation was 1–3% and the inter-assay variation was 2–3%. For \( [{\text{1,1,2,3,3-}}^{2}\text{H}_5] \) glycerol enrichment the intra-assay variation was 4%, and the inter-assay variation was 7%.

**Glucoregulatory hormones**

Insulin, glucagon, cortisol, noradrenaline (norepinephrine) and adrenaline (epinephrine) were measured as described previously [22].

**Plasma and muscle carnitine measurements**

AC plasma concentrations were analysed as described previously [23]. Muscle biopsies were freeze-dried and analysed as described previously [24]. Total long-chain ACs represent the sum of \( \text{C}_{12:1-1}^-, \text{C}_{12:2-1}^-, \text{C}_{14:2-1}^-, \text{C}_{16:1-1}^-, \text{C}_{14:0}^-, \text{C}_{16:1-2}^-, \text{C}_{16:0}^-, \text{C}_{18:2}^-, \text{C}_{18:1-1}^- \) and \( \text{C}_{18:0}^- \)-carnitine.

**Calculations and statistics**

EGP (endogenous glucose production) and Rd (rate of glucose disposal) were calculated using the modified forms of the Steele equations as described previously [4,19,25]. EGP and Rd were expressed as \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). Rd was also expressed in relation to the plasma insulin levels (Rd/insulin ratio). Lipolysis (glycerol turnover) was calculated by using formulas for steady-state kinetics and expressed as \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) and as \( \mu \text{mol} \cdot \text{cal} \) (where 1 kcal \( \approx 4.184 \) kJ) as described previously [22].

REE (resting energy expenditure) was expressed as kcal/day, FAO and CHO rates were calculated from \( V_{O2} \) and \( V_{CO2} \) [26].

Statistical comparisons and correlation analyses were performed using the Wilcoxon signed rank test and Spearman’s rank correlation coefficient (\( \rho \)) respectively. The SPSS statistical software program version 12.0.2 was used for statistical analysis. Values are presented as the median (minimum–maximum).

**RESULTS**

**Anthropometric characteristics**

The subject characteristics have been reported previously [4]. In brief, subject characteristics were: age, 23 (20–26) years; weight, 70.1 (62.5–75.5) kg after 14 h and 69.0 (60.0–72.8) kg after 62 h of fasting \( (P = 0.012, 14 \text{ h compared with } 62 \text{ h of fasting}) \).

**Indirect calorimetry**

REE (kcal/day) in the basal state was significantly higher after 62 h of fasting compared with 14 h of fasting: 1682 (1518–1820) kcal/day compared with 1578 (1308–1783) kcal/day respectively, \( P = 0.017 \) (Figure 1). During the clamp, however, REE was significantly lower after 62 h of fasting compared with 14 h of fasting: 1604 (1470–1734) kcal/day compared with 1815 (1494–1933) kcal/day respectively, \( P = 0.012 \).

FAO \( (\mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \) in the basal state was significantly higher after 62 h of fasting compared with 14 h of fasting (Figure 1). During the clamp, FAO remained significantly higher after 62 h of fasting compared with 14 h of fasting: 1604 (1470–1734) kcal/day compared with 1815 (1494–1933) kcal/day respectively, \( P = 0.012 \).

If FAO and CHO were expressed as \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) the basal state was significantly lower after 62 h of fasting compared with 14 h of fasting: 12.8 (10.8–14.8) kg/m2 after 14 h and 20.3 (18.3–22.6) kg/m2 after 62 h of fasting \( (P = 0.011, 14 \text{ h compared with } 62 \text{ h of fasting}) \).

During the clamp, CHO was significantly lower after 62 h of fasting compared with 14 h of fasting. The absolute change between the basal state and the clamp in CHO was significantly lower after 62 h of fasting compared with 14 h of fasting: 12.8 (5.9–17.1) \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in the basal state was significantly higher after 62 h of fasting compared with 14 h of fasting: 1604 (1470–1734) kcal/day compared with 1815 (1494–1933) kcal/day respectively, \( P = 0.012 \).

If FAO and CHO were expressed as \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) of LBM \( \cdot \min^{-1} \) (where LBM is lean body mass) comparable results were obtained (results not shown).

**Glucose and lipid metabolism measurements**

Plasma glucose concentrations and EGP (Table 1) were significantly lower after 62 h of fasting compared with 14 h of fasting [4]. No differences were found in plasma glucose concentrations after 62 h compared with 14 h of fasting during the clamp. Rd during the clamp was significantly lower after 62 h of fasting compared with
Table 1  Glucose and lipid metabolism measurements
Values are presented as medians (minimum–maximum).

<table>
<thead>
<tr>
<th></th>
<th>Basal state</th>
<th>Hyperinsulinaemic–euglycaemic clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 h (n = 8)</td>
<td>62 h (n = 8)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.0 (4.5–5.6)</td>
<td>3.7 (3.3–4.1)</td>
</tr>
<tr>
<td>EGP (μmol·kg(^{-1})·min(^{-1}))</td>
<td>11.8 (8.9–19.7)</td>
<td>8.3 (7.0–8.7)</td>
</tr>
<tr>
<td>Rd (μmol·kg(^{-1})·min(^{-1}))</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0.33 (0.19–0.95)</td>
<td>1.10 (0.85–1.24)</td>
</tr>
<tr>
<td>Lipolysis (μmol·kg(^{-1})·min(^{-1}))</td>
<td>1.5 (1.1–4.4)</td>
<td>3.8 (2.3–4.2)</td>
</tr>
<tr>
<td>Lipolysis (μmol/kgcal)</td>
<td>101 (68–261)</td>
<td>214 (135–250)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>30 (18–58)</td>
<td>15 (15–20)</td>
</tr>
</tbody>
</table>

* During the clamps, EGP and NEFAs were completely suppressed.

14 h of fasting (Table 1). The Rd/insulin ratio was significantly lower after 62 h of fasting compared with 14 h of fasting: 0.079 (0.069–0.116) compared with 0.090 (0.072–0.18) respectively, P = 0.036.

Basal plasma NEFAs and the rate of lipolysis were significantly higher after 62 h of fasting (Table 1). Plasma NEFAs were suppressed during the hyperinsulinaemic–euglycaemic clamps after 62 h and 14 h of fasting (Table 1). Lipolysis was not different after 62 h of fasting compared with 14 h of fasting during the clamp (Table 1).

**Glucoregulatory hormones**
Insulin was significantly lower after 62 h of fasting in the basal state as well as during the clamp (Table 1). Other data on glucoregulatory hormones have been presented elsewhere [4].

**Muscle and plasma carnitines**
After 62 h of fasting, muscle FC (free carnitine) was higher compared with 14 h of fasting: 9503 (5326–14360) pmol/mg of dry weight compared with 5299 (3331–7051) pmol/mg of dry weight respectively, P = 0.028. During the clamp, muscle FC was not different after 62 h of fasting compared with 14 h of fasting: 7095 (2559–12278) pmol/mg of dry weight compared with 6408 (4189–8621) pmol/mg of dry weight, P = 0.16. After 14 h of fasting, hyperinsulinaemia during the clamp tended to increase muscle FC levels compared with the basal state, but this was not statistically significant (P = 0.06). Muscle FC did not change between the basal state and the clamp after 62 h of fasting (P = 0.22).

Basal muscle acetylcaritnine was significantly higher after 62 h of fasting compared with 14 h of fasting: 1175
Table 2  AC concentrations in muscle (pmol/mg of dry weight) in the basal state after 14 h and 62 h of fasting
Values are presented as medians (minimum–maximum).

<table>
<thead>
<tr>
<th>AC</th>
<th>Basal state 14 h</th>
<th>62 h</th>
<th>P*</th>
<th>Hyperinsulinaemic–euglycaemic clamp 14 h</th>
<th>62 h</th>
<th>P*</th>
<th>P†</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:1</td>
<td>1.21 (0.13–4.27)</td>
<td>1.17 (0.14–2.97)</td>
<td>0.78</td>
<td>0.15 (0–1.25)</td>
<td>0.71 (0.21–2.63)</td>
<td>0.012</td>
<td>0.025</td>
<td>0.26</td>
</tr>
<tr>
<td>C12:0</td>
<td>4.15 (0.13–14.69)</td>
<td>2.42 (0.42–7.97)</td>
<td>0.48</td>
<td>0.26 (0.18–6.41)</td>
<td>1.45 (0.21–5.38)</td>
<td>0.16</td>
<td>0.069</td>
<td>0.48</td>
</tr>
<tr>
<td>C14:2</td>
<td>2.05 (0.13–9.24)</td>
<td>1.19 (0.31–5.51)</td>
<td>0.58</td>
<td>0.15 (0–3.59)</td>
<td>0.97 (0.11–4.00)</td>
<td>0.036</td>
<td>0.050</td>
<td>0.40</td>
</tr>
<tr>
<td>C14:1</td>
<td>7.6 (0.25–37.68)</td>
<td>4.68 (0.56–20.00)</td>
<td>0.89</td>
<td>0.17 (0.12–10.94)</td>
<td>3.41 (0.32–9.00)</td>
<td>0.036</td>
<td>0.036</td>
<td>0.33</td>
</tr>
<tr>
<td>C14:0</td>
<td>6.12 (0.25–35.31)</td>
<td>4.15 (0.51–16.61)</td>
<td>0.67</td>
<td>0.22 (0.12–13.44)</td>
<td>2.77 (0.32–9.75)</td>
<td>0.16</td>
<td>0.12</td>
<td>0.40</td>
</tr>
<tr>
<td>C16:1</td>
<td>6.18 (0.13–46.45)</td>
<td>4.59 (0.61–22.80)</td>
<td>1.00</td>
<td>0.30 (0.13–14.92)</td>
<td>2.48 (0.21–14.00)</td>
<td>0.069</td>
<td>0.093</td>
<td>0.67</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.07 (0.88–72.80)</td>
<td>6.46 (2.50–29.41)</td>
<td>0.67</td>
<td>1.38 (0.42–20.15)</td>
<td>9.41 (1.60–33.13)</td>
<td>0.012</td>
<td>0.025</td>
<td>0.67</td>
</tr>
<tr>
<td>C18:1</td>
<td>3.42 (0.13–46.45)</td>
<td>1.84 (0.56–6.44)</td>
<td>0.58</td>
<td>0.71 (0.23–5.39)</td>
<td>2.40 (0.32–12.63)</td>
<td>0.012</td>
<td>0.036</td>
<td>0.78</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.10 (0.25–88.86)</td>
<td>7.42 (2.36–37.12)</td>
<td>0.67</td>
<td>1.56 (0.58–20.00)</td>
<td>6.18 (0.96–32.63)</td>
<td>0.012</td>
<td>0.036</td>
<td>0.48</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.46 (0.25–25.36)</td>
<td>2.32 (0.97–7.29)</td>
<td>0.40</td>
<td>1.18 (0.47–5.39)</td>
<td>3.04 (0.85–9.50)</td>
<td>0.012</td>
<td>0.017</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* Represent differences between basal states and clamps after 14 h and 62 h of fasting respectively.
† Represent differences within clamps after 14 h and 62 h of fasting respectively.

Figure 2  Total long-chain ACs in plasma and muscle
(A) Total long-chain plasma ACs in the basal state and during the clamp after 14 h of fasting (open boxplots) and 62 h of fasting (grey boxplots). *P = 0.012 (14 h of fasting compared with 62 h of fasting). ** P = 0.012 for the decrease in ACs during both clamps after 62 h of fasting and 14 h of fasting. (B) Total long-chain muscle ACs in the basal state and during the clamp after 14 h of fasting (open boxplots) and 62 h of fasting (grey boxplots) * P = 0.012 (14 h of fasting compared with 62 h of fasting during the clamp). *** P = 0.05 for the decrease in ACs between the basal state and clamp after 14 h of fasting.

(411–3380) pmol/mg of dry weight compared with 697 (269–1149) pmol/mg of dry weight respectively, P = 0.05. Clamp muscle acetylcarnitine was significantly lower after 62 h of fasting compared with 14 h of fasting: 316 (170–632) pmol/mg of dry weight compared with 513 (335–959) pmol/mg of dry weight respectively, P = 0.021. After 14 h of fasting, hyperinsulinaemia during the clamp did not change acetylcarnitine levels compared with the basal state (P = 0.25), whereas the clamp decreased muscle acetylcarnitine levels compared with the basal state after 62 h of fasting (P = 0.017).

In the basal state, muscle long-chain ACs were not significantly different after 62 h compared with 14 h of fasting (Figure 2). Total muscle long-chain ACs were significantly higher during the clamp after 62 h of fasting compared with 14 h of fasting (Figure 2). Individual muscle long-chain ACs showed a similar pattern (Table 2).

Total plasma long-chain ACs were significantly higher after 62 h of fasting compared with 14 h of fasting in the basal state and during the clamp. The same was true for individual plasma long-chain ACs (results not shown).

Muscle long-chain ACs did not correlate with lipolysis, Rd, FAO or plasma long-chain ACs (results not shown).
DISCUSSION

In the present study, muscle long-chain ACs after 14 h and 62 h of fasting were studied and correlated with glucose oxidation and FAO rates, as well as peripheral insulin sensitivity. In the basal state, there was no significant difference in muscle long-chain ACs between 14 h and 62 h of fasting. In contrast, during hyperinsulinaemia we found a decrease in muscle long-chain ACs after 14 h of fasting compared with no changes in muscle long-chain ACs after 62 h of fasting. The latter finding was accompanied by higher whole-body FAO.

REE increased approx. 7.5 % during 62 h of fasting, which is in agreement with previous observations [3,27–29], although the increase in REE has not fully been accounted for. It was proposed that increased energy requirements of gluconeogenesis and ketogenesis are reflected in increased REE [27,28]. On the other hand it was suggested that the noradrenaline-induced thermogenic response results in a slight increase in REE during short-term fasting [27,29], but we did not detect differences in plasma noradrenaline [4].

The results of the present study confirmed previous reports on increased plasma NEFAs and lipolysis [1]. Higher plasma NEFAs are also found in different models of insulin resistance and are thought to be one of the main mediators of obesity-induced insulin resistance [30]. Lipid mediators that induce insulin resistance are mainly derived from long-chain fatty acids [31]. Moreover, muscle long-chain ACs have been suggested to induce peripheral insulin resistance in animal studies [8,11,12]. Since fasting induces insulin resistance [2,4], we hypothesized that this may be related to an increase in muscle long-chain ACs. Human studies on muscle ACs during fasting are lacking, but previous animal studies showed an increase of muscle long-chain ACs in rodents after fasting [8,17].

The lack of an increase in muscle long-chain ACs between 14 h and 62 h of fasting is unexpected since we demonstrated an increase in whole-body FAO. The CPT1-dependent rate of long-chain fatty acid entrance into the mitochondria is thought to determine the rate of FAO [5,7]. The results of our present study imply that the muscle concentration of ACs during fasting in humans does not reflect whole-body FAO.

Hyperinsulinaemia resulted in a decreased concentration of long-chain muscle ACs after 14 h of fasting. Other studies have not found a decrease in ACs during hyperinsulinaemia [32]. This may be explained by differences in the methods to analyse muscle ACs. Another possibility includes that we measured muscle ACs of chain-length 12–18 in contrast with all muscle ACs.

After 62 h of fasting, the suppressive effect of insulin on muscle long-chain ACs was not found. Increased muscle long-chain ACs during the clamp after 62 h of fasting are unlikely to reflect accumulation of non-utilizable long-chain ACs or ongoing FAO since we could not demonstrate such a relationship in the basal state after 62 h of fasting. Although indirect calorimetry does not reflect muscle FAO only, ongoing FAO is likely to occur since clamp values of whole-body FAO were higher after 62 h of fasting compared with 14 h of fasting. This suggests that, despite 5 h of hyperinsulinaemia, peripheral glucose uptake is still attenuated and activated fatty acids are continued to be transported to the mitochondrion in order to be oxidized [5,7]. Ongoing FAO may be needed since peripheral glucose uptake is attenuated.

Our results on increased plasma long-chain ACs after 62 h of fasting confirm previous studies [13–15]. In contrast with our finding in muscle, plasma long-chain ACs were higher after 62 h of fasting compared with 14 h of fasting, but decreased equally during hyperinsulinaemia. This and the absence of a correlation of plasma ACs with muscle ACs, negates that plasma ACs reflect muscle ACs. It may support the notion that the liver is the most likely source of plasma long-chain ACs during short-term fasting [7,33,34].

No human studies are available on muscle FC during fasting, however it has been shown in animal studies that muscle FC increases during fasting [17,35]. Moreover, the plasma membrane transporter of carnitine (OCTN2) is up-regulated by fasting in a PPARα (peroxisome-proliferator-activated receptor α)-dependent mechanism [36]. Indeed, in the subjects used in the present study, muscle FC increased during 62 h of fasting compared with 14 of fasting. This may reflect the dependence of FAO on FC for transport of long-chain acyl-CoAs across the mitochondrial membranes. However, the exact mechanism remains elusive.

We found lower insulin levels during the clamp after 62 h of fasting, which are unlikely to interfere with our results. Since insulin infusions were almost identical this may be due to increased renal clearance of infused insulin in contrast with the first-pass effect of endogenous insulin by the liver [37]. Fasting is generally known to induce insulin resistance [3]. In the present study, infusing insulin at a rate of 60 milliunits·m⁻²·min⁻¹ does not augment glucose disposal significantly compared with 40 milliunits·m⁻²·min⁻¹: both infusion rates yield similar disposal rates after an overnight fast, despite very different plasma insulin levels [38]. This may be explained by the shape of the dose-response curve for insulin. Moreover, when standardized to the plasma insulin levels, Rd was lower after 62 h of fasting.

In conclusion, in the present study we show that fasting for 62 h results in higher rates of lipolysis and FAO, together with lower CHO rates and lower peripheral insulinaemia, the suppression of the concentration of muscle long-chain ACs was less compared with after 14 h of fasting. In addition,
whole-body FAO rates remained higher during the clamp after 62 h of fasting. Despite previous reports on possible interference of the muscle long-chain ACs with peripheral insulin sensitivity, the present study does not support such a role for muscle long-chain ACs during fasting. To clarify whether muscle ACs are just innocent bystanders or active players in insulin resistance, further studies in different models of insulin resistance are needed.

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