Human obesity is characterized by defective fat storage and enhanced muscle fatty acid oxidation, and trimetazidine gradually counteracts these abnormalities

Marco Bucci,1,2 Ronald Borra,1,3 Kjell Någren,1 Romina Maggio,1 Helena Tuunanen,1,4 Vesa Oikonen,1 Silvia Del Ry,2 Tapio Viljanen,1 Markku Taittonen,5 Sara Rigazio,1 Daniela Giannessi,2 Riitta Parkkola,3 Juhani Knuti,1 Pirjo Nuutila,1,4 and Patricia Iozzo1,2

1Turku PET Centre, University of Turku, Turku, Finland; 2Institute of Clinical Physiology, National Research Council, Pisa, Italy; Departments of 3Radiology, 4Medicine, and 5Anesthesiology, University of Turku and Turku University Hospital, Turku, Finland

Submitted 14 December 2010; accepted in final form 13 April 2011

Bucci M, Borra R, Någren K, Maggio R, Tuunanen H, Oikonen V, Del Ry S, Viljanen T, Taittonen M, Rigazio S, Giannessi D, Parkkola R, Knuti J, Nuutila P, Iozzo P. Human obesity is characterized by defective fat storage and enhanced muscle fatty acid oxidation, and trimetazidine gradually counteracts these abnormalities. Am J Physiol Endocrinol Metab 301: E105–E112, 2011. First published April 19, 2011; doi:10.1152/ajpendo.00680.2010.—An impaired ability to store fatty acids (FA) in subcutaneous adipose tissue (SAT) may be implicated in the pathogenesis of obesity-related diseases via overexposure of lean tissues and production of free radicals from FA oxidation (FAO). We studied regional FA metabolism in skeletal muscle and adipose tissue in humans and investigated the long-term effects of the FAO inhibitor trimetazidine on glucose and FA metabolism. Positron emission tomography (PET) and [11C]palmitate were used to compare FA metabolism in SAT and skeletal muscle between eight obese and eight nonobese subjects (BMI ≥30 kg/m2). A subgroup of nine subjects underwent a 1-mo trimetazidine administration. PET with [11C]palmitate and [18F]fluorodeoxyglucose, indirect calorimetry, and MRI before and after this period were performed to characterize glucose and FA metabolism, fat masses, skeletal muscle triglyceride, and creatine contents. Obesity was characterized by a 100% elevation in FAO and a deficit in the FA esterification rate constant (P < 0.05) in skeletal muscle. FA esterification was reduced by ~70% in SAT (P < 0.001) in obese vs. control subjects. The degrees of obesity and insulin resistance were both negatively associated with esterification-related parameters and positively with FAO (P < 0.05). Trimetazidine increased skeletal muscle FAO esterification (P < 0.01) and mildly upregulated glucose phosphorylation (P = 0.066). Our data suggest that human obesity is characterized by a deficit in tissue FA storage capacity, which is accompanied by a (potentially compensatory) elevation in skeletal muscle FAO; trimetazidine diverted FA from oxidative to nonoxidative pathways and provoked an initial activation of glucose metabolism in skeletal muscle. One potential explanation is that FAO may initially increase to compensate for an augmented FA release from adipose tissue (12, 27), thus preventing the accumulation of intramyocellular lipids (32). Once this pathway becomes saturated, the build-up of intramyocellular triglycerides (12) and free radicals (34) may compromise mitochondrial function (6), resulting in a decline in FAO. A limited capacity of adipose tissue to act as a sink for FA might provoke a compensatory elevation in skeletal muscle FAO, thus initiating the above cascade of events.

A metabolic modulator able to relieve the oxidative pressure caused by FA overload in the early phase of the disease might be an effective preventive measure. Trimetazidine has been shown to partially inhibit FAO, thus promoting a shift toward glucose utilization in the heart (29). In cardiomyopathic patients with type 2 diabetes, trimetazidine has been shown to improve systemic glucose metabolism, as reflected by a reduction in fasting glycemia and an increase in forearm and whole body glucose uptake during hyperinsulminemic euglycemia (8). The effects on forearm lipid metabolism were investigated only in the short term (15 days), demonstrating a downregulation in lipid oxidation during insulin stimulation (25). The effects of trimetazidine on adipose tissue metabolism and adipokine secretion have not been investigated despite the dominant role played by this organ in the regulation of systemic FA disposal, and production of a variety of hormones and proinflammatory cytokines affecting insulin action, lipolysis, and oxidative stress (45).

The primary aim of the current study was to examine whether adipose tissue FAO esterification and skeletal muscle FAO differ between and are inversely related in obese and nonobese individuals, thus supporting a link between adipose tissue FAO storage capacity and compensatory skeletal muscle FAO. Our secondary aim was to study the modulation of FAO and glucose metabolism induced by trimetazidine treatment at the whole body and regional (skeletal muscle and adipose tissue) levels in a subgroup of subjects.

RESEARCH DESIGN AND METHODS

Study participants were included if they were 20–75 yr of age, had no diabetes mellitus, no chronic disease, normal physical examination, and stable weight and diet for the previous 3 mo. Written informed consent was obtained from all subjects after providing detailed information on the nature, purpose, and potential risks of the study. The
study protocol was approved by the Ethics Committee of the Hospital District of Southwest-Finland.

Cross-sectional study: effects of obesity. This evaluation included the comparison between eight obese and eight nonobese subjects (BMI ≥1.3 30 kg/m²), undergoing metabolic-anthropometric characterization; one patient with full-blown metabolic syndrome but a BMI < 30 kg/m² was included in the obese group. Fat, fat-free, and skeletal muscle masses were estimated by published formulas (2, 16). Insulin resistance was evaluated through the homeostasis model assessment (HOMA) index (26). The adipose tissue insulin resistance index (AIRI) was calculated as product of fasting FFA and plasma insulin concentrations (5). PET studies were conducted to characterize FA metabolism in skeletal muscle and subcutaneous abdominal adipose tissue (SAT).

PET studies in lean and obese subjects. Each study was performed after an 8- to 10-h overnight fast. PET images were obtained by the GE PET-CT (Discovery VCT; GE Medical Systems, Milwaukee, WI), or ECAT 931/08-12 tomographs (CTI, Knoxville, TN). [11C]Palmitate was produced as previously described (30). Two catheters were inserted, one in an antecubital vein for injection of the radiolabeled tracer, another in the opposite antecubital vein for blood sampling. Dynamic imaging of the upper abdomen was performed after intra-

tracer, another in the opposite antecubital vein for blood sampling. Inserted, one in an antecubital vein for injection of the radiolabeled

was produced as previously described (30). Two catheters were

inserted, one in an antecubital vein for injection of the radiolabeled

or ECAT 931/08-12 tomographs (CTI, Knoxville, TN). [11C]palmitate

after an 8- to 10-h overnight fast. PET images were obtained by the

PET studies before and after treatment. PET studies with [11C]palmitate or [18F]fluorodeoxyglucose ([18F]FDG) were done on separate days (5 ± 1 day interval for the 18 studies) according to the acquisition and processing protocol described in the cross-sectional study. PET images and tissue TACs were analyzed with the same approach described above. Because CT images, providing anatomic information, were available in the obese subjects, in whom visceral fat mass is large and more easily identifiable than in lean individuals, in this group we were able to obtain TACs from the visceral adipose tissue (VAT) region (Supplemental Fig. S1). VAT TACs were analyzed with the same 4k model of SAT. An example of VAT TAC and the respective model fit is shown in Supplemental Fig. S3. The FA metabolic rates were calculated according to the equations described in SUPPLEMENTAL MATERIALS AND METHODS. Since visceral and retroperitoneal fat masses were quantified in this study group, the metabolic rates for the entire SAT were computed by discounting the whole body fat mass from the two intra-abdominal fat compartments. [18F]FDG was produced with a modified method of Hamacher et al. (13). A three-K, three-compartment model was used to describe [18F]FDG transport and phosphorylation rate constants (28, 40) in skeletal muscle, SAT, and VAT. The fitting was performed with the same optimization algorithms as in the palmitate model. The organ-specific fractional extraction rate constant (1/min) was calculated by the following equation:

$$Ki = K_1 \times k_3 / (k_2 + k_3) \quad (Eq.7)$$

The glucose uptake rate (GU) was obtained by:

$$GU = \frac{\text{plasma glucose}}{Ki/LC} \quad (Eq.8)$$

where LC indicates the lumped constant. LC values of 1.2 for skeletal muscle (31) and 1.14 for adipose tissue (44) were used.

MRI and 1H-MRS studies. A Philips Gyroscan Intera 1.5 T Nova Dual MR scanner (Philips Medical Systems, Best, The Netherlands) with a flexible surface coil and body coil was used for MRI and MRS.
A single T1W FFE image was obtained at the level of the intervertebral disc L2-L3 for visceral and retroperitoneal mass assessment, as previously described (1). Fat volumes were converted in weight, by using 0.9196 g/ml as density of adipose tissue (7).

A single voxel with a volume of 1.8 cm³ was positioned in the tibialis anterior muscle to quantify the intramyocellular and extramyocellular lipid contents (IMCL, EMCL) and creatine-to-water ratios. To ensure identical voxel placement before and after trimetazidine treatment, voxel location was graphically recorded in each patient. A PRESS ¹H-MRS sequence was used with the following parameters: TR = 3,000 ms, TE = 27 ms. All spectra were analyzed using LCMR (33). The lipid and water amplitudes were corrected due to different T-2 decay and molar concentrations of ¹H nuclei in fat and water (35). ¹H-MRS findings of IMCL content have been validated in both animal and human studies (41).

Other measurements: glucose and FA systemic disposal rates. Circulating glucose and FFA plasma levels were determined three times during each PET scan to document steady concentrations. Plasma concentrations of [¹³C]palmitate and [¹⁸F]FDG over time were used to calculate the whole body FFA and glucose clearances as ratios of the injected doses to the areas under the tracers concentration curves (0 → ∞); the latter was calculated by the trapezoid rule. Clearance rates were multiplied by plasma FFA and glucose levels to obtain the rates of FA and glucose appearance (numerically equaling disappearance) in the circulation (μmol/min) (18). The clearance of palmitate was assumed to be representative of the overall FFA clearance, as supported by others (23).

Biochemical analysis. Please see SUPPLEMENTAL MATERIALS AND METHODS.

Statistics. All calculations were performed with the SPSS/Win statistical program (v. 15.0 for Windows; SPSS, Chicago, IL). All data are presented as means ± SE. Cross-sectional differences were evaluated using ANOVA or a Mann-Whitney nonparametric test according to the distribution of the variables. Differences in paired data were tested with a nonparametric Wilcoxon signed rank test. Regression analyses were carried out according to standard techniques, using Pearson or Spearman correlation coefficients, as appropriate. A P value of <0.05 was considered statistically significant.

RESULTS

Cross-sectional study: effects of obesity. Anthropometric and metabolic characteristics of study subjects are reported in Table 1. By definition, obese subjects had higher BMI and fat mass than the control group. Fasting FFA levels were not different between the two groups, whereas fasting insulin and triglyceride levels and insulin resistance indexes (HOMA-IR and AIRI) were significantly greater in the obese group.

Results from PET modeling showed that obese subjects were characterized by a decreased skeletal muscle FA esterification rate constant (k₅; P = 0.002, Supplemental Table S1) and an increased FAO (Fig. 1, A and C) compared with the control group. The esterification rate in SAT was markedly lower at the unit-of-tissue level, i.e., nearly halved in obese subjects with respect to that of leaner controls (Fig. 1B), although the difference was compensated for by an increased whole body fat mass (Fig. 1D). Systemic lipolysis did not differ significantly between the groups (control vs. obese group, 352 ± 34 vs. 291 ± 39 μmol/min), although if expressed per kilogram of fat mass, it was lower in obese than in control subjects (9 ± 2 vs. 16 ± 2 μmol·min⁻¹·kg fat mass⁻¹, P < 0.03).

In the whole study population, skeletal muscle esterification rate constant, FAO, and SAT esterification rate were not only different by groups but also individually correlated with the degree of obesity, as a higher BMI was related to lower esterification parameters in both tissues (R = −0.79, R = −0.66) and higher skeletal muscle FAO (R = 0.53). The SAT esterification rate was associated with the fractional contribution of FA esterification and oxidation to the FU in skeletal muscle (R = 0.50, R = −0.50, P < 0.05); thus, the higher the SAT esterification capacity was, the lower was the FAO contribution to skeletal muscle FA uptake. Insulin and HOMA-IR were both negatively associated with the skeletal muscle FA esterification rate constant (R = −0.50, P < 0.05) and positively with FAO (R = 0.50, P < 0.03). AIRI was positively associated with skeletal muscle FU and FAO (R = 0.60, P < 0.02).

Effects of trimetazidine. All patients completed the study, and their general characteristics before and after treatment are reported in Table 2. The fat mass distribution, insulin resistance indexes, blood metabolic profiles, and adipokines were reported in Table 2. The fat mass distribution, insulin resistance indexes, blood metabolic profiles, and adipokines were not affected by the treatment. There was a tendency toward a decrease in Hb A₁c and LDL cholesterol. Whole body substrate oxidation, assessed by indirect calorimetry, did not change after treatment, as reported in Table 2. Whole body and regional utilization rates of glucose and FA in skeletal muscle, SAT, and VAT, assessed by PET, are reported in Table 3 and in Fig. 2, A and B, and they did not change. The baseline comparison between VAT and SAT FU showed significantly greater values in the former (Fig. 2B). FAO from indirect calorimetry measurements was positively correlated with skeletal muscle FAO as derived by PET, supporting the validity of the current methodology (R = 0.8, P < 0.05).

The only changes observed after treatment included an elevation in the esterification rate constant of FA (P = 0.008) and a tendency toward an increment in the glucose phosphorylation rate constant (P = 0.066) in skeletal muscle (Supplemental Table S2). No significant changes were observed in the rate constants of SAT. Skeletal muscle EMCL-to-water and IMCL+EMCL-to-water ratios were significantly increased (Table 2) in the six patients with available measurements before and after treatment. Trimetazidine did not change significantly the IMCL-to-water and IMCL-to-EMCL ratios, but positive correlations were found between changes in these variables and those in plasma triglycerides (R = 0.8, P = 0.035; R = 0.9, P = 0.011, respectively). The Creatine-to-water ratio was associated with the PET-derived skeletal mus-
cle oxidative pool (positively, $R = 0.8, P < 0.05$) and with the esterification rate constant (negatively, $R = -0.8, P < 0.05$).

**DISCUSSION**

To the best of our knowledge, this is the first study to address noninvasively the relationship between FA metabolism in adipose tissue and skeletal muscle in obese and nonobese humans. The main finding of the study is the evidence of an impairment in FA esterification capability in obese subjects in both adipose tissue and skeletal muscle and a possibly compensatory increase of FAO in skeletal muscle during the fasting state. Furthermore, the treatment of subjects with these abnormalities with a partial inhibitor of mitochondrial β-oxidation resulted in a diversion of FA from the oxidative toward the nonoxidative pathway in skeletal muscle, reverting these processes toward the values observed in the control group. The hypothesized shift toward glucose metabolism operated by the drug was observed as a tendency of the glucose phosphorylation pathway to increase after treatment.

*Cross-sectional study: effects of obesity.* The fasting state was chosen as the target condition, in which FAs are the main fuel for skeletal muscle, and adipose tissue lipolysis is not inhibited by postprandial insulin levels. Our obese subjects had similar systemic lipolytic rates and FFA levels to controls. This is likely explained by fasting hyperinsulinemia, compensating for adipose tissue insulin resistance, as found in these subjects. It is in agreement with previous evidence of an inverse relation between lipolysis and insulin levels in uncomplicated obesity (3, 10) and with larger population studies showing that obesity is accompanied by a reduction in lipolysis relative to the increase in adiposity (24), although the release of FA with respect to the fat-free mass remains greater, indicating an overload of FA to lean tissues. Our findings corroborate and extend these observations by suggesting that the FA overload...
There is a growing belief that the progression of lipotoxicity relies on the balance between adipose tissue function and FAO, that this relationship changes dynamically across different severities of obesity and its complications, and that changes in FAO represent an adaptive or maladaptive response rather than a primary event (6, 22, 27). We observed a severe impairment in the capability of adipose tissue to incorporate FA in triglyceride depots at the level of the unit mass (i.e., per 100 g of tissue). This defect was compensated for by an increased adipose tissue mass in obese individuals. This may lead to speculation that the defect in storage fosters the development of “compensatory” obesity. In turn, the growth of adipose tissue in obese individuals may limit (or delay) the propagation of the intrinsic tissue defect (and the consequent release of substrates) to the rest of the body. It is of note that the ability for reesterification was also impaired in skeletal muscle despite the FA overload. The correlations found in this study document that, as insulin resistance grows, the esterification rate declines and FAO increases and that these two metabolic processes occur in a reciprocally related fashion. Although associations do not establish cause-effect relationships, these observations rule in favor of the primacy of an FA storing defect leading to the activation of FAO.

Overall, our data suggest that uncomplicated obesity is characterized by adipose tissue and whole body insulin resistance, which are accompanied (or may be preceded) by a hyperactivation of skeletal muscle FAO in obese individuals, an effect that was evident at both tissue (per unit mass) and whole body levels (per whole organ). This observation is in line with some, but not all, previous evidence. By use of indirect calorimetry, Perseghin et al. (32) reported an elevation in fasting lipid oxidation in individuals with a greater, compared with those with a smaller, fat mass. In line with our interpretation, they suggested a compensatory role of muscle FAO against the increasing flux of FA to lean tissues. Others have used in vitro gas exchange measurements to show that only extremely obese subjects have a defective FAO in skeletal muscle, again confirming that overweight/obese individuals show a trend toward higher FAO rates compared with lean subjects (15). Our data are also compatible with the lack of suppression in leg FAO during insulin infusion shown by Kelley et al. (20), but those authors observed a defect in oxidation during fasting conditions that is at variance with our findings. Different methodologies may explain the discrepancy, since those authors used the organ balance technique, which cannot discount the contribution of adipose tissue to the resulting FAO; since the amount of fat in the leg differs between subjects, it varies affects the results in terms of both blood flow and gas exchange, i.e., the variables used in FAO calculations. The use of PET is more specifically targeted to the individual contribution of skeletal muscle and adipose tissue to the process of interest. In support of the current interpretation of the compartmental configuration of skeletal muscle FA metabolism, we observed a significant association between the creatine/water ratio and the intracellular oxidative pool, in accord with the notion that the creatine content relates to the oxidative potential of skeletal muscle (4), and FA oxidation, as measured independently with indirect calorimetry, was positively related to the PET-derived FA oxidative rate and to the rate constants regulating the transfer of activity in and out of the oxidative compartment.

Table 2. Effects of trimetazidine on metabolic and anthropometric variables

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After One Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>89 ± 4</td>
<td>87 ± 3‡</td>
</tr>
<tr>
<td>BMI, kg m²</td>
<td>31 ± 1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>34 ± 2</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td>55 ± 3</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Visceral fat mass, kg</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Retropertitoneal fat mass, kg</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>IMCL, % water resonance</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>EMCL, % water resonance</td>
<td>1.5 ± 0.7</td>
<td>2.2 ± 0.9*</td>
</tr>
<tr>
<td>IMCL + EMCL, % water resonance</td>
<td>2.0 ± 0.8</td>
<td>2.8 ± 1.0*</td>
</tr>
<tr>
<td>IMCL/EMCL ratio</td>
<td>0.9 ± 0.7</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>AIRI</td>
<td>39 ± 9</td>
<td>40 ± 8</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.2 ± 0.1</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>58 ± 10</td>
<td>57 ± 10</td>
</tr>
<tr>
<td>Fasting FFA, mmol/l</td>
<td>0.62 ± 0.05</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td>Hb A1c, ‰</td>
<td>5.6 ± 0.1</td>
<td>5.5 ± 0.1†</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.8 ± 0.3</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>5.6 ± 0.3</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
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<tr>
<td>LDL, mmol/l</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Apelin-12, pg/l</td>
<td>340 ± 42</td>
<td>271 ± 26</td>
</tr>
<tr>
<td>Adiponectin, μg/l</td>
<td>22 ± 3</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Resistin, ng/l</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>PAI-1 total, ng/l</td>
<td>55 ± 4</td>
<td>47 ± 3</td>
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<tr>
<td>IL-6, pg/l</td>
<td>5.8 ± 3.1</td>
<td>6.4 ± 3.1</td>
</tr>
<tr>
<td>IL-8, pg/l</td>
<td>5.9 ± 1.0</td>
<td>6.1 ± 1.0</td>
</tr>
<tr>
<td>Leptin, ng/l</td>
<td>27 ± 9</td>
<td>32 ± 11</td>
</tr>
<tr>
<td>TNE, pg/l</td>
<td>5.8 ± 0.6</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>MCP-1, pg/l</td>
<td>329 ± 31</td>
<td>365 ± 42</td>
</tr>
<tr>
<td>HGF, pg/l</td>
<td>543 ± 98</td>
<td>553 ± 96</td>
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</table>

Values are means ± SE. IMCL and EMCL, intramyocellular and extramyocellular lipid contents. *P < 0.05, ‡P < 0.08.

translates in a hyperactivation of skeletal muscle FAO in obese individuals, an effect that was evident at both tissue (per unit mass) and whole body levels (per whole organ). This observation is in line with some, but not all, previous evidence. By use of indirect calorimetry, Perseghin et al. (32) reported an elevation in fasting lipid oxidation in individuals with a greater, compared with those with a smaller, fat mass. In line with our interpretation, they suggested a compensatory role of muscle FAO against the increasing flux of FA to lean tissues. Others have used in vitro gas exchange measurements to show that only extremely obese subjects have a defective FAO in skeletal muscle, again confirming that overweight/obese individuals show a trend toward higher FAO rates compared with lean subjects (15). Our data are also compatible with the lack of suppression in leg FAO during insulin infusion shown by Kelley et al. (20), but those authors observed a defect in oxidation during fasting conditions that is at variance with our findings. Different methodologies may explain the discrepancy, since those authors used the organ balance technique, which cannot discount the contribution of adipose tissue to the resulting FAO; since the amount of fat in the leg differs between subjects, it varies affects the results in terms of both blood flow and gas exchange, i.e., the variables used in FAO calculations. The use of PET is more specifically targeted to the individual contribution of skeletal muscle and adipose tissue to the process of interest. In support of the current interpretation of the compartmental configuration of skeletal muscle FA metabolism, we observed a significant association between the creatine/water ratio and the intracellular oxidative pool, in accord with the notion that the creatine content relates to the oxidative potential of skeletal muscle (4), and FA oxidation, as measured independently with indirect calorimetry, was positively related to the PET-derived FA oxidative rate and to the rate constants regulating the transfer of activity in and out of the oxidative compartment.
proportional inability to store FA in adipose tissue and skeletal muscle fat depots. Under this line, hyperinsulinemia and FAO may act against the overflow of FA into the circulation, the former by inhibiting the breakdown of existing tissue fat depots and the latter by augmenting the washout of FA from the circulation via oxidation.

Effects of trimetazidine. The long-term metabolic effects of the partial inhibitor of β-oxidation trimetazidine were studied in a drug-naïve human model of moderately obese subjects in whom the still flexible stage of the disease is characterized by an increased FAO, and we performed all measurements during fasting to avoid the confounding suppressive effects of insulin on FA metabolism. One previous study, in which the modulation of FA and glucose metabolism by trimetazidine was simultaneously investigated in humans in an organ different from the myocardium (25) in diabetic patients with cardiomyopathy, showed that forearm lipid and glucose oxidation were reciprocally modulated during the insulin-stimulated state, but no changes occurred during the fasting state despite the higher FA turnover typical of fasting vs. insulin-stimulated conditions. Our present study extends previous findings in several regards, since 1) our human model discounts for the confounding suppressive effects of insulin on FA metabolism, thus targeting skeletal muscle and adipose tissue separately; and 2) by combining plasma tracer kinetics and indirect calorimetry, we estimated endogenous glucose production, hepatic insulin sensitivity, and whole body FA release and oxidation.

In agreement with our previous findings in idiopathic dilated cardiomyopathic patients, in whom an improvement in glycemic and cholesterol profiles was reported following 3 mo of treatment (43), our data indicate an immediate tendency toward a decline in Hb A1c and LDL cholesterol despite a shorter study duration. Neither endogenous glucose production nor systemic FA release were changed; thus, the modifications in glucose control and insulin sensitivity observed here and/or in previous investigations seem to be mediated by substrate consuming, rather than producing organs.

Although trimetazidine did not affect skeletal muscle FAO, in line with results from indirect calorimetry at the whole body level, it accelerated significantly the movement rate of FA toward the nonoxidative, i.e., esterification, pathway. This change was accompanied by a borderline increase in the glucose phosphorylation rate constant in this target tissue. The rate constants are the fraction (or percentage) of tracer that goes in a pathway, representing the intrinsic system response discounted from the mass action or systemic contribution of the tracee. Conversely, the flux rate incorporates the combined effect of intrinsic tissue response and extra-tissue substrate delivery. In synthesis, the rate constant identifies tissue-related defects, whereas the flux includes the changes in tissue metabolism due to extrinsic, in addition to intrinsic, causes. Early changes in the rate constants confirm the reciprocal substrate regulation by trimetazidine and are likely to precede a net diversion of FA from oxidation toward esterification in complex lipids, in line with the reported observation that trimetazidine stimulates phospholipid synthesis (38) with potential protective effects on cellular membranes (37, 38).

It was interesting to observe a higher baseline visceral compared with subcutaneous FA uptake. Previous studies have used tissue biopsies to compare dietary FA uptake in SAT and VAT in normal-weight humans, showing that VAT accumulates more dietary fat (milligram meal fat per gram of adipose tissue lipid) than either upper body subcutaneous or lower body subcutaneous fat (19). We have previously described similar results with PET and a different long-chain FA tracer in lean subjects (14), and our investigation extends this finding to obese humans. The current results show that fasting FA uptake was approximately tripled in visceral compared with subcutaneous fat. Notably, glucose uptake was 12 times greater in the whole body SAT organ than in the entire VAT mass, as opposed to FA uptake, which was only four times greater in SAT than in VAT. Thus, VAT is proportionally more FA avid and much less glucose avid than SAT. Ad hoc studies are required to explain the mechanisms underlying this finding and to clarify whether SAT is relatively more effective in preventing fasting hyperglycemia by serving as a glucose sink.

Adipokines did not seem to play a role as mediators of trimetazidine action within the time frame of the present study. Similarly, adipose tissue topography and absolute masses of either visceral or subcutaneous fat or IMCL content were not changed. The only exception was the expansion in the extramyocellular lipid content, suggesting that a preferential
diversion of fat stores in adipose tissue adjacent to skeletal muscle may prevent IMCL accumulation.

The limitation of this study was in the lack of a placebo control group, a decision that was guided by the demanding nature of the studies and the radiation exposure, which (though limited, i.e., in the clinical diagnostic routine range) we did not find to be justified in a pilot phase. Although the few changes observed may have benefited from the comparison with a placebo group, they seem sufficient to clarify that trimetazidine actions in moderate obesity are relatively mild and may require a long time before translating into systemic effects. The most conclusive value of this investigation was in the large number of measured variables that did not change, which allows one to exclude with confidence a series of potential mechanisms from being mediators of trimetazidine action.

In summary, obesity is associated with an impairment in the esterification of FA in adipose tissue and skeletal muscle, which is accompanied by the upregulation in skeletal muscle FAO. The inability to store may initiate a cascade of events leading to FA oversupply to lean tissue, overload of the oxidative pathway, and accumulation of toxic lipid species and triglycerides, and it was paralleled by a proportional growth in insulin resistance. Trimetazidine increased the FA esterification and glucose phosphorylation rate constants in skeletal muscle, and the EMCL in one month, supporting the hypothesis of an indirect, albeit very gradual, diversion of FA from the oxidative to the nonoxidative pathway. A longer time may be required before the known systemic benefits of this compound become appreciable in moderately obese, otherwise healthy individuals.

GRANTS

The study was conducted within the Finnish Centre of Excellence in Molecular Imaging in Cardiovascular and Metabolic Research, supported by the Academy of Finland, University of Turku, Turku University Hospital, and Åbo Academy University. Support was also received from the European Foundation for the Study of Diabetes and the Finnish Diabetes Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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