Normal insulin sensitivity and IMCL content in overweight humans are associated with higher fasting lipid oxidation

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Perseghin, Gianluca, Paola Scifo, Massimo Danza, Alberto Battezzati, Stefano Benedini, Elena Meneghini, Alessandro Del Maschio, and Livio Luzi. Normal insulin sensitivity and IMCL content in overweight humans are associated with higher fasting lipid oxidation. Am J Physiol Endocrinol Metab 283: E556–E564, 2002; 10.1152/ajpendo.00127.2002.—Intramyocellular lipid (IMCL) storage was considered a local marker of whole body insulin resistance; because increments of body weight are supposed to impair insulin sensitivity, this study was designed to assess IMCL content, lipid oxidation, and insulin action in individuals with a moderate increment of body fat mass and no family history of diabetes. We studied 14 young, nonobese women with body fat <30% (n = 7) or >30% (n = 7) and 14 young, nonobese men with body fat <25% (n = 7) or >25% (n = 7) by means of the euglycemic-insulin clamp to assess whole body glucose metabolism, with indirect calorimetry to assess lipid oxidation, by localized 1H NMR spectroscopy of the calf muscles to assess IMCL content, and with dual-energy X-ray absorptiometry to assess body composition. Subjects with higher body fat had normal insulin-stimulated glucose disposal (P = 0.80), IMCL content in both soleus (P = 0.22) and tibialis anterior (P = 0.75) muscles, and plasma free fatty acid levels (P = 0.075) compared with leaner subjects in association with increased lipid oxidation (P < 0.05), resting energy expenditure (P = 0.046), resting oxygen consumption (P = 0.049), and plasma leptin levels (P < 0.01) in the postabsorptive condition. In conclusion, in overweight subjects, preservation of insulin sensitivity was combined with increased lipid oxidation and maintenance of normal IMCL content, suggesting that abnormalities of these factors may mutually determine the development of insulin resistance associated with weight gain.

intramyocellular lipid content; lipid oxidation; insulin resistance; leptin; tumor necrosis factor-α; nuclear magnetic resonance spectroscopy

HIGHER RISK OF TYPE 2 DIABETES is associated with moderate overweight (58), and a detrimental lipid profile is frequently combined with obesity. The abnormalities of lipid metabolism may be primarily involved in the pathogenesis of obesity and type 2 diabetes (32); intramyocellular lipid (triglyceride) (IMCL) storage was proposed to modulate the development of insulin resistance (32). 1H NMR spectroscopy showed the feasibility of identifying and measuring lipids within the fat cells and within the muscle cells separately (8, 45), and this measurement was shown to be comparable to biochemical assay (53). With use of this approach, a relationship between IMCL content and whole body insulin sensitivity was shown in normal humans (28, 50), offspring of type 2 diabetic parents (22, 41), and obese patients with type 2 diabetes mellitus (51). Even if the metabolic effects of IMCL accumulation are intensively investigated, little is known about the rate-limiting factors involved in the regulation of the IMCL content. Increments of IMCL might be due to increased fatty acid flux from the adipose tissue or to a reduced oxidative disposal. Obesity and type 2 diabetes mellitus were proposed to be associated with decreased muscle lipid oxidation (6, 9, 24), but its relationship with insulin sensitivity and the effects on IMCL content are controversial. Increased fat oxidation, because of the increased fat flux, has been considered responsible for insulin resistance (25); recently, insulin resistance was more precisely associated with a metabolic muscle inflexibility characterized by a reduced ability to utilize fat in the postabsorptive state, but at the same time by reduced ability to suppress lipid oxidation in insulin-stimulated states (25). This study aimed to assess the effect of moderate overweight in young healthy subjects on whole body insulin action, IMCL content, lipid oxidation, and plasma free fatty acid (FFA) concentrations in a cross-sectional fashion. To avoid the confounding effect of genetic factors, we carefully selected young, healthy, normal-weight [body mass index (BMI) <27 kg/m²], and nonexercising subjects who had no family history of diabetes or additional metabolic diseases and who were not taking any medications.

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Methods

Subjects

Twenty-eight healthy men and women were recruited at the Istituto Scientifico H San Raffaele. The criteria for inclusion in the study were 1) no family history of diabetes, obesity, and hypertension traced through their grandparents, 2) age (24–40 yr), 3) white race, 4) BMI <27 kg/m², 5) sedentary life style, and 6) no history of hypertension, endocrine/metabolic disease, or cigarette smoking. Habitual physical activity was assessed using a questionnaire (1). Body weight was stable for ≥6–12 mo, and women had not taken oral steroidal contraception for ≥12 mo. The anthropometric characteristics of the subjects are summarized in Table 1. To compare the effects of moderate increased body fat on fatty acid metabolism and insulin action, we studied women with a fat content >30% (n = 7) and men with that >25% (n = 7) (overweight subjects) and compared them one by one with subjects matched for age, gender, and physical activity but with fat content <30% in women and <25% in men (normal subjects). We set the threshold at 30% fat content in women, because higher body fat content was shown to be associated with higher risk to develop the metabolic syndrome in normal-weight women (12), and at 25% in men because 30%, as in women, would probably have selected several nonobese men with BMI closer to 26–27 than to 22–24 kg/m². All subjects were in good health as assessed by medical history, physical examination, hematology, and urinalysis. Informed consent was obtained from all subjects after explanation of purposes, nature, and potential risks of the study. The protocol was approved by the Ethical Committee of the Istituto Scientifico H San Raffaele.

Experimental Protocol

Subjects were asked to consume an isocaloric diet containing 250 g/day of carbohydrates and to abstain from exercise activity for 3 wk before the studies. Women were studied between day 3 and day 8 of the menstrual cycle. Subjects were studied by means of the euglycemic-hyperinsulinemic clamp and indirect calorimetry to assess whole body insulin sensitivity, resting energy expenditure (REE), and glucose and lipid oxidation after a 10-h overnight fast period and during the insulin clamp. Within 2–3 days they were studied by means of 1H NMR spectroscopy to assess IMCL content and by means of dual-energy X-ray absorptiometry (DEXA) to assess body composition. The NMR session was performed in the Division of Diagnostic Radiology of the Istituto Scientifico H San Raffaele.

Euglycemic-hyperinsulinemic clamp. Subjects were admitted to the Metabolic Unit of the Division of Internal Medicine I of the Istituto Scientifico H San Raffaele at 7:00 AM after a 10-h overnight fast. A Teflon catheter was inserted into an antecubital vein for infusions, and an additional one was inserted retrogradely into a wrist vein for blood sampling. The hand was kept in a heated box (50°C) throughout the experiment to allow sampling of arterialized venous blood. A bolus (5 mg/kg body wt), followed by a 300-min period of continuous infusion (0.05 mg/kg body wt−1·min−1) of [6,6-2H2]glucose obtained from MassTrace (Woburn, MA), was administered. Basal blood samples for glucose and tracer enrichment were collected on four occasions before insulin infusion during the last 45 min of the 150-min tracer equilibration period; samples for FFA, the lipid profile, insulin, C-peptide, tumor necrosis factor receptor-2 (TNFR-2), and leptin were drawn twice in the same 45-min interval (at −30 and time 0). After the 150-min tracer equilibration period, a

Table 1. Anthropometric parameters of study groups

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Overweight Subjects</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men 7</td>
<td>Women 7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>80 ± 1</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 1</td>
<td>167 ± 2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.2 ± 0.5</td>
<td>21.9 ± 1.1</td>
</tr>
<tr>
<td>Total body fat, kg</td>
<td>21.4 ± 0.5</td>
<td>21.7 ± 2.3</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>27.8 ± 0.5</td>
<td>36.0 ± 1.5</td>
</tr>
</tbody>
</table>

Fat content, %

| Arms | 23.0 ± 1.0 | 31.9 ± 2.9 | 27.5 ± 1.9 | 10.9 ± 2.3 | 18.0 ± 1.5 | 14.9 ± 1.8 |
| Trunk | 28.9 ± 0.8 | 29.2 ± 2.2 | 29.4 ± 1.1 | 13.3 ± 2.1 | 18.4 ± 1.6 | 16.1 ± 1.5 |
| Legs | 28.6 ± 0.7 | 45.5 ± 1.8 | 37.1 ± 2.5 | 15.8 ± 2.3 | 31.6 ± 1.8 | 24.1 ± 2.8 |
| Abdominal | 30.9 ± 1.8 | 35.7 ± 2.1 | 31.4 ± 1.3 | 14.2 ± 2.2 | 22.4 ± 1.9 | 17.8 ± 1.7 |

% Total fat

| Arms | 10.7 ± 0.3 | 9.3 ± 1.1 | 10.0 ± 0.6 | 9.2 ± 0.5 | 8.3 ± 0.5 | 8.8 ± 0.4 |
| Trunk | 55.4 ± 0.9 | 43.0 ± 1.2 | 48.2 ± 1.6 | 49.1 ± 1.3 | 46.4 ± 2.1 | 47.7 ± 1.2 |
| Legs | 31.0 ± 1.0 | 43.6 ± 2.0 | 37.3 ± 2.0 | 36.2 ± 1.6 | 41.0 ± 2.4 | 38.6 ± 1.5 |
| Abdominal | 19.6 ± 0.8 | 18.3 ± 1.4 | 18.9 ± 0.8 | 16.1 ± 0.6 | 18.5 ± 0.8 | 17.3 ± 0.6 |
| LBM, kg | 55.6 ± 0.9 | 37.7 ± 2.2 | 46.6 ± 2.7 | 54.8 ± 1.9 | 41.4 ± 1.3 | 48.1 ± 2.2 |
| Physical activity index | 9.2 ± 0.3 | 8.7 ± 0.8 | 8.9 ± 0.6 | 9.0 ± 0.5 | 9.0 ± 0.9 | 9.0 ± 0.6 |

Statistical analysis was performed to compare overweight and normal subjects. BMI, body mass index; LBM, lean body mass. The range of possible scores for the physical activity index is 3–15; the lowest value corresponds to the level of physical activity of a clerical worker who plays a light sport (energy expended is <0.76 MJ/h; e.g., bowling) and who participates in sedentary activities during leisure time. The highest value corresponds to the level of physical activity of a person who is very physically active at work (e.g., a construction worker), who plays heavy sports (energy expended is >1.76 MJ/h; e.g., boxing, basketball, football or rugby), and who is very physically active during leisure time (e.g., walking >1 h/day or biking >45 min/day).

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euglycemic-hyperinsulinemic clamp was performed as previously described (10). Insulin was infused at 40 mU·m⁻²·min⁻¹ to reach a plasma insulin concentration of ~350 pmol/l, and plasma glucose concentration was kept at ~5 mmol/l for an additional 150 min by means of a variable infusion of 20% dextrose. Blood samples for plasma hormones, substrates, and tracer enrichment were drawn every 15 min throughout the study.

**Indirect calorimetry.** While subjects lay quietly for 45 min during the basal equilibration period and at the end of the euglycemic-hyperinsulinemic clamp, indirect calorimetry was performed continuously with a ventilated hood system (Sensor Medics 2900, Metabolic Measurement Cart) to measure oxygen consumption (V˙O₂) and carbon dioxide production (V˙CO₂) for calculations of glucose and lipid oxidation. The mean coefficient of variation within the session for both O₂ and CO₂ measurements was below 2%. In our metabolic unit, the daily variability of the REE assessed in 25 subjects in the 1999–2001 period was 3.5 ± 0.3% for V˙O₂ and 4.1 ± 0.5% for V˙CO₂.

**1H NMR spectroscopy.** 1H NMR spectroscopy was performed on a GE Sigma 1.5 Tesla scanner (General Electric Medical Systems, Milwaukee, WI) with a conventional linear extremity coil, as previously described (41). High-resolution T1-weighted images of the right calf were obtained before the spectroscopic acquisitions to localize the voxel of interest for the 1H spectroscopy study. The voxel shimming was executed to optimize the homogeneity of the magnetic field within the specific volume of interest. Two 1H spectra were collected from a 15 × 15 × 15-mm³ volume within the soleus and tibialis anterior muscles. A PRESS pulse sequence (repetition time = 2,000 ms and echo time = 60 ms) was used, and 128 averages were accumulated for each spectrum, with a final acquisition time of 4.5 min. The water signal was suppressed during the acquisition, because it would dominate the other metabolites’ peak signals of interest. A third 1H spectrum of a triglyceride solution inside a glass sphere, positioned within the extremity coil next to the calf, was also obtained during the same session to have an external standard for nonwater correction.

Postprocessing, executed with the Sage/IDL software (GE Medical Systems, Milwaukee, WI) with a conventional linear extremity coil, consisted of highpass filtering, spectral apodization, zero-filling, Fourier transformation, and the phasing of the spectra. The integral of the methylene signal (CH2) at 1.35 ppm was used to calculate IMCL content expressed in arbitrary units as a ratio with the methylene signal (CH2) at 1.35 ppm. Daily variability of the soleus IMCL content assessed in seven subjects in the 1999–2001 period was 11 ± 3%.

**Body composition.** DEXA was performed with a Lunar-DPX-IQ scanner (Lunar, Madison, WI). A different scan mode was chosen with respect to each subject's body size, as suggested by the manufacturer's operator manual. For regional analysis, three-compartment processing was performed in the arms, trunk, and legs (34). Fat content is expressed as kilograms of fat mass and as a percentage of tissues. Svendsen et al. (52) showed that abdominal fat content assessed by means of DEXA explained ~80% of the variation in the abdominal fat assessed by computed tomography scan in postmenopausal women. We also assessed the abdominal fat content with a procedure available in the software by manually selecting the region of interest (ROI). The ROI was selected by the same operator, as previously described (52), comprising the abdominal tissue between the 1st and 4th lumbar intervertebral disks, and the lines of the rib box were adjusted (standard software option).

**Analytical Procedures**

Plasma glucose was measured with a Beckman glucose analyzer (41). Plasma FFAs and plasma total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides were measured as previously described (41). Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula. Serum urea nitrogen was measured in the postabsorptive and hyperinsulinemic conditions by use of an enzymatic method on a Hitachi 747. Plasma insulin was measured with microparticle enzyme immunoassay technology (35) with no cross-reactions with proinsulin, C-peptide, and glucagon (IMx Insulin assay, Abbott Laboratories, Rome, Italy), and C-peptide was measured with a double-antibody RIA (41). Plasma leptin concentrations were determined by RIA (Linco Research, St. Charles, MO) as previously described (41). TNFR-2 was measured with an enzyme immunoassay (Immunotech, Beckman Coulter, Marseille, France) recommendations. The [3H]glucose enrichment was measured by gas chromatography-mass spectrometry, as previously described (2).

**Calculations**

Glucose turnover was calculated in the basal state by dividing the [6,6-2H₂]glucose infusion rate by the steady-state plateau of plasma [6,6-2H₂]glucose enrichment achieved during the last 45 min of the basal period. Glucose kinetics during the insulin clamp were calculated by using Steele’s equations for the nonsteady state (49). Steady state of plasma enrichments was reached in the study groups during the last 30 min of the insulin clamp. Endogenous glucose production (EGP) was calculated by subtracting the glucose infusion rate (GIR) from the rate of glucose appearance measured with the isotopic tracer technique. Total body glucose uptake (Rg) was determined during the clamp by adding the rate of residual EGP to the GIR. Insulin sensitivity, SIclamp, was obtained as follows: Rg/(ΔI × G), where ΔI is the increment of total glucose uptake [normalized to kg lean body mass (LBM)], G is the increment of plasma insulin concentration (calculated at both basal and clamp steady-state conditions), and is the plasma glucose concentration during the clamp (3). REE was calculated by Weir’s standard equation (57) from the V˙O₂ and the V˙CO₂ measured by means of indirect calorimetry (excluding the first 10 min of data acquisition) and from the urinary nitrogen excretion. Glucose, lipid, and protein oxidation were estimated, as previously described (14), in the postabsorptive state. During the insulin clamp, protein oxidation rates were corrected for changes in pool size (54); postabsorptive (9.41 ± 0.63 vs. 9.61 ± 0.37 mmol/l; P = 0.79) and clamp (8.26 ± 0.67 vs. 8.49 ± 0.35 mmol/l; P = 0.78) serum urea nitrogen concentrations were similar and similarly suppressed during hyperinsulinemia (13 ± 2% vs. 12 ± 2%; P = 0.69) in the study groups. Nonoxidative glucose disposal was calculated by subtracting the glucose oxidation rate from the tissue glucose disposal. Protein oxidation was estimated from urinary nitrogen excretion (18). Glucose and lipid oxidation was expressed as milligrams per kilogram LBM per minute for two reasons: first, DEXA assessment of lean mass does not exclude the nonfat and metabolically active components of the adipose tissue; second, in the postabsorptive condition, adipose tissue is responsible for 5% of REE in adult humans (13).
Statistical Analysis

All data are presented as means ± SE. The steady state for plasma [6,6-2H2]glucose enrichment was defined as a nonsignificant correlation with time (P > 0.05) by use of standard linear regression. Comparisons among groups were performed by use of ANOVA, with Scheffé’s post hoc testing when appropriate. Simple regression analysis was performed to assess relationships between variables.

RESULTS

Anthropometric Characteristics

Anthropometric parameters of study subjects are summarized in Table 1. Overweight and normal subjects were comparable for age, gender, and physical activity index; in addition, LBM and height were not different. BMI was higher in the overweight than in the normal subjects (23.5 ± 0.8 vs. 21.1 ± 0.6 kg/m²; P = 0.05), even if within the normal range. Total body fat content, regional distribution of fat in the appendicular (arms and legs), trunk, and abdominal areas, and fat content in each area normalized to the total fat content are summarized in Table 1.

Postabsorptive Energy Homeostasis

\( V_{O2} \) and \( V_{CO2} \) were comparable in the study groups, as well as the respiratory quotient (RQ; Table 2). When normalized to kilograms of LBM, which consists of the metabolically active tissue of the human body, the \( V_{O2} \) was significantly increased in the overweight compared with normal subjects (Table 2; P = 0.049). Resting carbohydrate, lipid, and protein consumption was comparable in either energetic (kcal/day) or percentage terms in the two study groups (Table 2). The REE was comparable between the two groups (Table 2), but when it was expressed as kilograms of LBM, it was found to be increased in the overweight subjects (P = 0.046).

<table>
<thead>
<tr>
<th>Subject (n)</th>
<th>Overweight Subjects</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>( V_{O2}, \text{ml/min} )</td>
<td>275 ± 18</td>
<td>206 ± 16</td>
</tr>
<tr>
<td>( V_{CO2}, \text{ml/min} )</td>
<td>221 ± 11</td>
<td>166 ± 12</td>
</tr>
<tr>
<td>RQ</td>
<td>0.80 ± 0.01</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td>NPRQ</td>
<td>0.80 ± 0.01</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>( V_{O2}, \text{ml-kg LBM}^{-1} \cdot \text{min}^{-1} )</td>
<td>4.86 ± 0.17</td>
<td>5.42 ± 0.23</td>
</tr>
<tr>
<td>Carbohydrates, kcal/day</td>
<td>557 ± 81</td>
<td>359 ± 56</td>
</tr>
<tr>
<td>Lipids, kcal/day</td>
<td>1064 ± 74</td>
<td>752 ± 72</td>
</tr>
<tr>
<td>Proteins, kcal/day</td>
<td>295 ± 38</td>
<td>318 ± 35</td>
</tr>
<tr>
<td>Carbohydrates, %</td>
<td>29 ± 3</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>Lipids, %</td>
<td>55 ± 4</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>Proteins, %</td>
<td>16 ± 2</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>REE, kcal/day</td>
<td>1915 ± 85</td>
<td>1429 ± 95</td>
</tr>
<tr>
<td>REE/kg body wt, kcal-kg⁻¹-day⁻¹</td>
<td>23.9 ± 0.9</td>
<td>23.4 ± 1.1</td>
</tr>
<tr>
<td>REE/kg LBM, kcal-kg⁻¹-day⁻¹</td>
<td>34.4 ± 1.1</td>
<td>37.9 ± 1.5</td>
</tr>
</tbody>
</table>

\( V_{O2} \), oxygen consumption; \( V_{CO2} \), carbon dioxide production; NPRQ, nonprotein respiratory quotient; REE, resting energy expenditure.

Glucose Metabolism in the Postabsorptive State and During the Insulin Clamp

Postabsorptive plasma glucose levels and EGP rates were similar in overweight and normal subjects (Table 3). Insulin-stimulated glucose disposal was comparable in overweight and normal subjects (Fig. 1), even if the overweight subjects showed higher glucose oxidative disposal (Table 3; P < 0.05) and a nonsignificantly lower nonoxidative glucose disposal (Table 3; P = 0.19) compared with normal subjects.

Plasma Lipid Profile in the Postabsorptive State and During the Insulin Clamp

In the postabsorptive state, the plasma lipid profile was comparable between the groups (Table 3). Plasma FFA concentrations showed a trend to be increased in overweight compared with normal subjects (P = 0.075); on the contrary, circulating glycerol and \( \beta \)-hydroxybutyrate concentrations were similar in the two groups (P = 0.46 and P = 0.11, respectively). During the insulin clamp, levels of plasma FFA, glycerol, and \( \beta \)-hydroxybutyrate concentrations similarly dropped in the study groups (Table 3).

Lipid Oxidation

Postabsorptive lipid oxidation was similar between the overweight and normal subjects when corrected as kilograms of body weight (0.898 ± 0.058 vs. 0.916 ± 0.059 mg·kg⁻¹·min⁻¹; P = 0.82) but significantly increased in overweight compared with normal subjects when corrected as kilograms of LBM, which represents the metabolically active tissues (Table 3 and see Fig. 2; P < 0.05). The higher lipid oxidative disposal in the overweight subjects in relation to normal subjects was found to be significant in women (1.41 ± 0.15 vs. 1.11 ± 0.07 mg·kg LBM⁻¹·min⁻¹; P < 0.04) and showed only a trend in men (1.32 ± 0.09 vs. 1.24 ± 0.10 mg·kg LBM⁻¹·min⁻¹; P = 0.41). Lipid oxidation during the
Glucose metabolism

<table>
<thead>
<tr>
<th></th>
<th>Overweight Subjects</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.07 ± 0.07</td>
<td>5.12 ± 0.07</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>35 ± 3</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>Endogenous glucose production mg·kg⁻¹·min⁻¹</td>
<td>2.10 ± 0.09</td>
<td>2.19 ± 0.12</td>
</tr>
<tr>
<td>Glucose disposal, mg·kg LBM⁻¹·min⁻¹</td>
<td>1.79 ± 0.15</td>
<td>1.79 ± 0.12</td>
</tr>
<tr>
<td>Nonoxidative glucose disposal, mg·kg LBM⁻¹·min⁻¹</td>
<td>5.35 ± 0.39</td>
<td>6.24 ± 0.48</td>
</tr>
<tr>
<td>SIPclamp, l·min⁻¹·kg LBM⁻¹ per pmol/l × 10³</td>
<td>19.0 ± 1.4</td>
<td>20.0 ± 2.0</td>
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</tbody>
</table>

Fatty acid metabolism

<table>
<thead>
<tr>
<th></th>
<th>Overweight Subjects</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total cholesterol, mmol/l</td>
<td>4.36 ± 0.17</td>
<td>4.49 ± 0.20</td>
</tr>
<tr>
<td>Plasma HDL-cholesterol, mmol/l</td>
<td>1.27 ± 0.11</td>
<td>1.36 ± 0.07</td>
</tr>
<tr>
<td>Plasma LDL-cholesterol, mmol/l</td>
<td>2.74 ± 0.16</td>
<td>2.74 ± 0.15</td>
</tr>
<tr>
<td>Plasma triglyceride, mmol/l</td>
<td>0.77 ± 0.11</td>
<td>0.83 ± 0.11</td>
</tr>
<tr>
<td>Plasma free fatty acids, μmol/l</td>
<td>547 ± 56</td>
<td>38 ± 14</td>
</tr>
<tr>
<td>Glycerol, μmol/l</td>
<td>42 ± 7</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>Lipid oxidation, mg·kg LBM⁻¹·min⁻¹</td>
<td>1.37 ± 0.08*</td>
<td>0.47 ± 0.11</td>
</tr>
<tr>
<td>Suppression of lipid oxidation, %</td>
<td>66 ± 1</td>
<td>58 ± 7</td>
</tr>
</tbody>
</table>

IMCL Content

1H NMR spectroscopy of the calf muscle showed that IMCL content in the soleus (Fig. 3A: 62 ± 7 vs. 51 ± 6 AU; P = 0.22) and tibialis anterior (Fig. 3B: 13 ± 3 vs. 12 ± 2 AU; P = 0.67) muscles was similar in the overweight and normal subjects. Normal men showed slightly lower (P = 0.17) IMCL content in the soleus muscle compared with women, and a nonsignificantly lower value compared with overweight men (P = 0.14), in whom the soleus IMCL content showed a threefold larger variability.

Plasma Insulin, C-Peptide, and Cortisol Concentrations

Postabsorptive plasma insulin concentrations were comparable in the study groups and similarly increased during the insulin clamp (Table 3), with no significant gender difference. Plasma postabsorptive C-peptide concentrations were also comparable between the groups (0.39 ± 0.03 vs. 0.40 ± 0.05 nmol/l; P = 0.84, respectively, in overweight and normal subjects), and the concentration levels dropped similarly during the clamp (55 ± 5 vs. 58 ± 5%; P = 0.71). Postabsorptive serum cortisol concentration was also similar in the study groups, with no gender-related difference (73 ± 12 vs. 58 ± 9 ng/ml; P = 0.20).

Plasma Leptin Concentration

Postabsorptive plasma leptin concentration was reduced in men compared with women in both normal (Fig. 4: 2.43 ± 0.61 vs. 5.18 ± 0.85 ng/ml; P = 0.02) and overweight subjects (Fig. 4: 5.12 ± 0.63 vs. 11.0 ± 2.01 ng/ml, P = 0.05). Overweight subjects had higher circulating plasma leptin than normal subjects (Fig. 4: 8.04 ± 1.30 vs. 3.81 ± 0.63 ng/ml; P < 0.01); nevertheless, when normalized to kilogram of body fat, it was comparable to that of the normal subjects (Fig. 4: 0.37 ± 0.05 vs. 0.34 ± 0.05; P = 0.71).
Plasma Soluble H9251-TNFR-2 Concentration

Postabsorptive plasma H9251-TNFR-2 concentration was comparable in overweight and normal subjects (1.5 ± 0.1 vs. 1.3 ± 0.0 ng/ml; P = 0.23) and slightly higher in women than in men (1.6 ± 0.0 vs. 1.3 ± 0.0 ng/ml; P = 0.03) when analysis was performed for gender.

Regression Analysis

A stepwise regression analysis was performed, with insulin sensitivity selected as the dependent variable, and age, BMI, percent fat content, LBM, IMCL muscle content, lipid oxidation and its insulin-dependent suppression, REE (corrected for kg body wt or lean mass), postabsorptive plasma FFA concentration, plasma total cholesterol (or HDL/LDL fractions) and triglycerides, postabsorptive plasma leptin (or leptin levels corrected to kg of fat mass), and α-TNFR-2 concentrations selected as the independent variables. Insulin sensitivity was inversely related to the IMCL soleus content ($R^2 = 0.38, P < 0.01$), plasma FFA levels ($R^2 = 0.41, P < 0.01$), and to a lesser extent IMCL tibialis anterior content ($R^2 = 0.36, P < 0.02$) in both groups. To assess whether the increment of leptin levels was paralleled by the maintenance of normal insulin sensitivity in the overweight subjects, a relationship between insulin-stimulated glucose disposal and leptin levels was tested and found to be nonsignificant ($R^2 = 0.21, P = 0.10$), as well as the relationship with lipid oxidative disposal ($R^2 = 0.20, P = 0.16$). Nevertheless, leptin was strongly related to both postabsorptive $V_O_2$ ($V_O_2 \cdot kg \cdot LBM^{-1} \cdot min^{-1}; R^2 = 0.36, P < 0.001; \text{Fig. 5a}$) and REE (REE $\cdot kg \cdot LBM^{-1} \cdot day^{-1}; R^2 = 0.35, P = 0.001; \text{Fig. 5B}$) when data regarding both groups were pooled together.

DISCUSSION

The results of this work demonstrate that, in healthy young sedentary subjects with no genetic background of diabetes or other diseases known to impair insulin sensitivity, the lack of deleterious effects of moderately higher body fat content (>25% in men and >30% in women) on insulin sensitivity and fatty acid metabolism was associated with increased lipid oxidation in the postabsorptive condition. To our knowledge, this is the first report in which increased postabsorptive lipid oxidation is associated with a beneficial effect on insulin action in healthy sedentary humans.

Fatty acids may induce insulin resistance by means of inhibition of insulin signaling (11), and their accumulation in the myocytes has been associated with insulin resistance (22, 28, 41, 48, 50, 51). The findings of the present study would support the hypothesis that IMCL accretion in a condition of insulin resistance may...
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The present study represents the reverse experiment, in which subjects able to trigger the oxidative disposal of fatty acids developed an efficient compensatory mechanism to fight muscle fatty acid accumulation (driven by the higher fat flux) and consequently to avoid insulin resistance. Even in sedentary subjects, the fatty acid oxidation would represent a crucial compensatory mechanism to fight insulin resistance and additional body weight gain in the early stages of the pathogenesis of obesity and type 2 diabetes (5, 7). In agreement with these findings, we recently observed that, in human immunodeficiency virus (HIV)-infected patients with lipodystrophy, insulin resistance and abnormal accumulation of IMCL content were associated with a marked impairment of postabsorptive lipid oxidation (39), probably related in part to the infection per se and in part to the effects of the anti-retroviral drugs.

It was interesting to notice that our overweight subjects were normally sensitive to insulin stimulation, which might be in contrast to previous reports (44). In a recent study (12), metabolically obese but normal-weight women, a subset of the normal-weight general population with increased body fat content similar to the women of our study, showed along with higher body fat content a cluster of phenotypic characteristics that may predispose them to the insulin-resistant syndrome (12). We believe that our subjects showed a different behavior because their increased body fat mass was not located in the abdominal area and, most importantly, they were carefully screened to have no personal or family history of insulin-resistant states, which allowed us the selection of subjects with lower risk to develop the metabolic syndrome and to inherit abnormalities of fatty acid metabolism (fat oxidation).

We (42) and others (15) recently reported that, in nonobese subjects, women showed some degree of protection from fatty acid-induced muscle insulin resistance compared with men; in the present study, we confirm the observation of higher IMCL soleus content in the normal women compared with men, regardless of the fact that insulin sensitivity was similar. Very recently it was shown (4) that fatty acid transport protein-1 mRNA expression in skeletal muscle in lean women was higher than in lean men, and that this might contribute to the higher IMCL content in women. In addition, in the present study we observed that the overweight women compared with men showed a stronger aptitude to increase lipid oxidation (Fig. 3) and therefore to keep IMCL content (Fig. 2) within levels similar to those of normal subjects, suggesting also that, in conditions of moderate increment of body fat mass, women seem better able to handle muscle fatty acid-induced insulin resistance. We think that the protection is due to the effects of estrogens (19, 42) and to a greater ability to store fat in the adipose tissue.

In our study lipid oxidation was assessed using indirect calorimetry, but this method is not a direct measure of whole-body lipid oxidation (24, 25) or of the metabolic oxidative capacity of the muscle (17, 24, 48); therefore, these data must be taken with caution. Nevertheless, we believe that these limitations have been partially circumvented by correcting the lipid oxidative disposal for LBM. In fact, in the postabsorptive condition, the skeletal muscle and the liver are the tissues contributing most to fat oxidation; meanwhile, the adipose tissue is minimally involved. In addition, female athletes demonstrated higher postabsorptive lipid oxidation than sedentary women, and exercise training is known to be associated with increased insulin sensitivity (40); also in that work (30), differences in lipid oxidation rates were detected by means of indirect calorimetry.

In animal models, leptin was shown to prevent lipotoxicity (55) and insulin resistance (36, 46), repositioning fatty acids toward oxidation and away from storage (56) via peroxisome proliferator-activated receptor-α (PPARα) stimulation (59). Leptin levels, as expected, were increased in the overweight compared with normal subjects, and because the ratio of leptin to kilogram of fat mass was unchanged (Fig. 4), the higher levels were proportional to the increased body fat mass. Because these overweight subjects accumulated the higher fat mass homogeneously in the body, with no predilection for trunk or abdominal accretion (Table 1), and because leptin is known to be predominantly secreted by subcutaneous adipose tissue rather than visceral tissue (33, 34), it is possible that these subjects were counteracting body weight gain with appropriate leptin secretion. We failed to find a relationship between leptin levels and insulin sensitivity ($R^2 = 0.21, P = 0.10$) or postabsorptive lipid oxidation...
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\( R^2 = 0.20, P = 0.16 \). Nevertheless, because the higher lipid oxidative rates were paralleled by higher postabsorptive \( \text{VO}_2 \) and higher \( \text{REE} \) (Table 2), we also tested whether leptin levels were associated with these parameters and found that the relationships were significant (Fig. 5). Therefore, even if the fact that leptin was involved in this compensatory mechanism was not statistically proved, in our opinion this possibility may not be excluded. Obesity-related insulin resistance may be also mediated by increased expression of TNF-\( \alpha \) (21), and soluble, circulating \( \alpha\)-TNFR-2 has been suggested to be involved in human obesity in women, modulating the action of TNF-\( \alpha \) (20). The postabsorptive levels of the soluble receptor were similar between the two groups, suggesting that a major role of the \( \alpha\)-TNF system activity in obesity-induced insulin resistance may become evident in more severe degrees of obesity.

The higher lipid oxidation rates found in our overweight subjects may represent a factor that counteracts IMCL accumulation and avoids fatty acid-induced insulin resistance (38, 43); we must also emphasize that the overweight subjects had a significant increment of body fat mass, and this may reflect a successful storing capacity of the excessive dietary intake in the adipose tissue, sparing its deposition in the skeletal muscle, the liver, and the \( \beta \)-cell. In lipotoxic adipocytes, a lack of adipose tissue is associated with severe insulin resistance in humans (23, 31); also, in the A-ZIP/F-1, a transgenic mouse with no adipose tissue (16), insulin resistance is mediated by lipotoxicity in the liver and skeletal muscle (47). The protective role of the adipose tissue is further reflected by the fact that its surgical implantation in this animal model reverses diabetes (16), requesting fatty acids from the other peripheral tissues. Moreover, in obese and diabetic patients, the expression of adipogenic genes was found to be decreased (37), further supporting the importance of the normal function of the adipose tissue in controlling energy and glucose homeostasis (47). It is possible that, in the overweight subjects, a proper adipose tissue storing capacity may have contributed to the counteraction against fatty acid-induced insulin resistance in the other peripheral tissues.

In conclusion, this work demonstrated that moderately overweight subjects may maintain normal IMCL content and insulin sensitivity in association with increased fasting lipid oxidation; whether this compensatory mechanism is mediated by the increment of the leptin levels is uncertain. We believe that this study suggests that 1) maintenance of normal IMCL content is crucial for preserving insulin sensitivity, and 2) inherited or acquired alterations in the ability of muscle (and possibly liver) to oxidize fat represent predisposing factors for development of abnormal IMCL accretion and, in turn, insulin resistance and obesity.

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