Changes in insulin sensitivity precede changes in body composition during 14 days of step reduction combined with overfeeding in healthy young men

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Knudsen SH, Hansen LS, Pedersen M, Dejgaard T, Hansen J, Van Hall G, Thomsen C, Solomon TP, Pedersen BK, Krogh-Madsen R. Changes in insulin sensitivity precede changes in body composition during 14 days of step reduction combined with overfeeding in healthy young men. J Appl Physiol 113: 7–15, 2012. First published May 3, 2012; doi:10.1152/japplphysiol.00189.2011.—A lifestyle characterized by inactivity and a high-calorie diet is a known risk factor for impaired insulin sensitivity and development of Type 2 diabetes mellitus. To investigate possible links, nine young healthy men (24 ± 3 y; body mass index of 21.6 ± 2.5 kg/m²) completed 14 days of step reduction (10,000 to 1,500 steps/day) and overfeeding (+50% kcal). Body composition (dual X-ray absorptiometry, MRI), aerobic fitness (maximal O₂ consumption, VO₂ max), systemic inflammation and insulin sensitivity (oral glucose tolerance test, OGTT), hyperinsulinemic euglycemic clamp were assessed before (day 0), during (days 3 and 7), and immediately after the intervention (day 14), with follow-up tests (day 30). Body weight had increased at days 7 and 14 (P < 0.05). The amount of visceral fat had increased at day 14 compared with day 0 (P < 0.05). The insulin response to the OGTT had increased at days 7 and 14 (P < 0.05). Insulin sensitivity, estimated using the Matsuda index, had decreased at days 3 and 7 (P < 0.01). At day 14, glucose infusion rates had decreased by ~44% during the euglycemic clamps (P < 0.05). Also, plasma levels of leptin and adiponectin had increased (P < 0.05), whereas no changes were seen in inflammatory markers. At day 30, body weight and whole body adiposity were still elevated compared with day 0 (P < 0.05), whereas the insulin sensitivity as well as the insulin response to the OGTT did not differ from baseline. The glucose response to the OGTT was only affected at day 30, with a decrease compared with day 0. Our data show that insulin sensitivity was impaired after 3 days of inactivity and overfeeding. Impairments in insulin sensitivity occurred before changes in body composition, supporting the notion that the initial steps in impairment of insulin sensitivity may be linked directly to the effects of inactivity and a high calorie intake. Type 2 diabetes mellitus; insulin resistance; physical activity; high caloric intake; glucose tolerance

A lifestyle characterized by inactivity and a high-calorie diet is a known risk factor for impaired glucose tolerance and insulin resistance, which may in turn lead to Type 2 diabetes mellitus (T2DM) (5, 24, 40). However, the mechanisms underlying these impairments are still unknown. By studying effects on metabolism when shifting from a high to a low level of activity, this problem may be addressed. Prior studies have shown that even a few days of inactivity reduce insulin sensi-

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http://www.jap.org
ducted during the 14-day intervention period at returning to an uncontrolled, free living environment for the clamp protocol. The first day (Fig. 1A) included a hyperinsulinemic euglycemic clamp (H-E clamp), and the second day (Fig. 1B) included an oral glucose tolerance test (OGTT), abdominal magnetic resonance (MRI) scan, whole-body dual-energy X-ray absorptiometry (DXA) scan, and a VO₂max-test. OGTTs, MRI scans, and DXA scans were also conducted during the 14-day intervention period at days 3 and 7. After returning to an uncontrolled, free living environment for 16 days, seven subjects returned for follow-up testing (day 30) including OGTT, DXA scan, and a VO₂max test.

**Measurements and determinations.** OGTTs were performed after an overnight fast of ~10 h. Blood was sampled from a catheter placed in an antecubital vein. Baseline blood samples were obtained to measure plasma and serum levels of glucose, insulin, C-peptide, lipids, inflammation markers [tumor necrosis factor-α (TNF-α) and interleukin (IL)-6], leptin, and adiponectin. After the oral intake of glucose (glucose: body weight × 1 g of glucose dissolved in water; body weight/0.3 ml water), blood samples were drawn at 30, 60, 120, and 180 min. The analyses of serum levels of glucose, insulin, and C-peptide were performed at the Department of Clinical Biochemistry, Rigshospitalet [colorimetric hexokinase assay used for glucose and electrochemiluminescent immunoassay (ELISA) for C-peptide and insulin]. TNF-α (K15025C-1), IL-6 (K15025C-1), adiponectin (K151BXC-1), and leptin (K151BYC-1) were measured by ELISA (Meso Scale Discovery, Gaithersburg, MD). Free fatty acid (FFA) levels were determined using a colorimetric assay (Cobas Fara, Roche, Basel, Switzerland).

Subjects underwent H-E clamps combined with stable isotopes ([6,6-²H₂] glucose, Cambridge Isotopes Laboratories) after an overnight fast of ~10 h (Fig. 2). H-E clamps were performed as previously described (31). In brief, peripheral catheters were placed for blood sampling and infusion of glucose, insulin, and stable isotopes. Initially, the glucose pool was primed by administrating a bolus of 17.5 μmol/kg of [6,6-²H₂] glucose followed by a continuous infusion (rate 0.4 μmol·kg⁻¹·min⁻¹) for 120 min before initiation of the clamp. Insulin (Actrapid, Novo Nordisk Insulin, 100 IE/ml) was infused continuously at a rate of 40.0 μU·min⁻¹·m⁻², and plasma glucose concentrations were kept at 5.0 mM by a co-infusion of glucose (200 g/1,000 ml, enriched with [6,6-²H₂] glucose to 2.5%) at a variable rate [glucose infusion rate (GIR)]. Adjustments were made based on continuous analysis of plasma glucose levels at intervals of 5 min during the first hour, and every 10 min during the last 2 h of the clamp. Baseline blood samples were obtained before initiation of the stable isotope infusion (time point –150) (Fig. 2). The samples were collected in heparin-containing tubes and immediately centrifuged for
The plasma glucose enrichment was measured as previously described (43). The glucose turnover rate of appearance (Ra) and rate of disappearance (Rd) were calculated assuming steady state:

\[
\text{Baseline: } R_a = \frac{F_{\text{infusion}}}{E_{\text{glucose}}},
\]

\[
\text{Clamp: } R_d(\text{endogenous}) = \frac{F_{\text{total}}}{E_{\text{glucose}}} - \text{GIR}, \quad R_d = \frac{F_{\text{total}}}{E_{\text{glucose}}},
\]

where \( F_{\text{infusion}} \) is the infusion rate of glucose tracer (\( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) in terms of fat-free body mass (FFM), \( E_{\text{glucose}} \) is the enrichment of glucose in plasma, \( F_{\text{cal}} \) is the sum of \( F_{\text{infusion}} \) and the [6,6-\(^2\text{H}_2\]glucose infused by the clamp, and GIR is the glucose infusion rate. Calculations were also adjusted for clamp levels of insulin.

All DXA images and MRI quantifications were adjusted and analyzed by the same person. Body composition, including the amount and distribution of fat and muscle, was calculated by the enCORE 2004 software (GE Medical Systems Lunar Prodigy). Amount of visceral fat was determined from the intervertebral space between L4 and L5 using the single-slide model (Windows Synyo Platform, software version V17).

**Statistics.** Data from the included nine subjects were tested for normal distribution by the Kolmogorov-Smirnov analysis. All data (except body composition data from DXA and MRI scans and \( V_{O_2} \text{max} \) data) were logarithmical transformed before analyses. Changes from day 0 to day 14 were analyzed with paired t-tests. Furthermore, a mixed model with Dunnett’s corrections were used to compare results from day 0 with day 3, day 7, day 14, and day 30 (body composition, fasting and OGTT levels of blood metabolites, and \( V_{O_2} \text{max} \)). All reported values are presented as means ± SE or as geometric means with 95% confidence interval with regard to the log-transformed data. \( P < 0.05 \) was considered statistically significant. Analyses were performed in SAS (statistical software package, version 9.1.3 SAS Institute).

**RESULTS**

**Energy balance and fitness level.** The subjects reduced their daily number of steps during the inactivity and overfeeding period, resulting in reduction in TEE (\( P < 0.005 \)) (Table 2). Subjects increased their TEI by 57 ± 6.7%, and, combined with the reduction in TEE, this resulted in a positive energy balance of 1,978 ± 146 kcal (Table 2). Furthermore, aerobic fitness (\( V_{O_2} \text{max} \) expressed by ml·kg·min\(^{-1} \)) had reduced after 14 days of inactivity and overfeeding (\( P < 0.05 \)) (Table 3).

**Body composition.** As shown in Table 3, body composition was affected by the intervention. At days 7 and 14, the subjects demonstrated an increase in total body mass (TBM) of 1.7 ± 1.1 and 1.8 ± 3.4 kg (\( P < 0.05 \)), respectively. This was mainly due to an increase in total fat mass (TFM) of 1.0 ± 0.7 and 1.5 ± 0.5 kg (\( P < 0.05 \)), respectively. Further analyses of the

<table>
<thead>
<tr>
<th>Table 1. Example of a 1,500-kcal “snack package”</th>
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<tr>
<td><strong>g</strong></td>
</tr>
<tr>
<td><strong>Muffin</strong></td>
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<tr>
<td><strong>Hot chili peanuts</strong></td>
</tr>
<tr>
<td><strong>Snickers</strong></td>
</tr>
<tr>
<td><strong>Müsli bar</strong></td>
</tr>
<tr>
<td><strong>Chocolate milk</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
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</table>

15 min at 3,500 rpm, and plasma was stored at −20°C until further analysis. The plasma glucose enrichment was measured as previously described (31, 43). The glucose turnover rate of appearance (Ra) and rate of disappearance (Rd) were calculated assuming steady state as described by Flomgaard and coworkers (43).

Body composition was assessed using MRI and DXA scans. The MRI scan (Siemens Magnetom Total Imaging matrix magnetic resonance scanner, Erlangen, Germany) was performed in the evening to determine the amount of visceral fat by single-slice imaging (26, 46). The DXA scan (GE Medical Systems Lunar Prodigy Advance, Fairfield, CT) was performed immediately after the OGTT and was used to assess body composition regarding whole body fat and fat-free mass, as well as percentage of total android and gynoid fat (16).

An incremental exercise test to exhaustion was performed on a cycle ergometer (Monark 839E, Monark, Varberg, Sweden) to determine the respiratory exchange coefficient was above 0.95 and then every minute until the subjects were unable to maintain a cadence of 60 rpm.

Actiheart (Cambridge Neurotechnology Cambridge, Cambridge, UK) was used to determine TEE by measuring acceleration, heart rate (HR), HR variability, and electrocardiogram (EKG) magnitude. The Actiheart was attached to the chest with two standard ECG electrodes (Maxensor, MXC55, cloth tape/55 mm, Medimex). TEE was estimated using Actiheart Activity Analysis Software (V4.0.32, Cambridge, UK). For every participant, the Actiheart monitor was tested for adequate signals for 5–10 min before the Actiheart was set up to continuously record HR and movement for 24 h/day. Actiheart was used before the intervention and throughout the 14-day period of step reduction and overfeeding. To register the number of daily steps, the subjects were instructed to wear a pedometer (Digi-walker, CW-300, Yamax, Japan) placed in the waistline vertically above the knee. Diet records were used to assess the TEI and nutrient composition (DanKost Sport 2000, Danish Catering Centre, Herlev, Denmark) and further to set up an individual diet plan for the 14 days of intervention to ensure that the subjects maintained their habitual diet and TEI. In addition, during the 14 days of intervention, each subject ingested the content of a daily dietary supplementation snack package (1,500 kcal) containing different kinds of nuts, cakes, chocolates, chips, fruit juices, and soft drinks to increase their TEI by 50% (Table 1).

**Calculations.** Simultaneous measurements of HR and accelerations during a 60-s epoch period obtained with Actiheart were converted into energy using estimations calculated from “the Branched Model” (6). Using this model, TEE is calculated from resting energy expenditure (REE), diet-induced thermal effect (DIT), and the thermal effect of physical activity (AEE) by estimations from specific formulas (6).

Insulin sensitivity was calculated from a 180-min OGTT using the Matsuda index \( \left[ 10,000/\text{square root of (fasting glucose × fasting insulin)} \right] \times \left[ \text{mean (180 min)} / \text{mean (180 min)} \right] \) (35).

Insulin and glucose responses were calculated as area under the curve (AUC).

**Table 2. Activity level and energy**

<table>
<thead>
<tr>
<th>Activity Level</th>
<th>Pre-intervention</th>
<th>Inactivity</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steps, steps/day</td>
<td>10,278 ± 715</td>
<td>1,521 ± 131*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total energy expenditure, kcal/day</td>
<td>2,647 ± 132</td>
<td>2,273 ± 94*</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Total energy intake, kcal/day</td>
<td>2,762 ± 100</td>
<td>4,197 ± 97*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Energy balance, kcal/day</td>
<td>98 ± 158</td>
<td>1,978 ± 146*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± standard error of mean (SE). \( n = 9 \). *Significantly different from pre-intervention value.
Glucose and fat metabolism. Fasting plasma and serum levels describing glucose and fat metabolism are shown in Table 3. Further- more, the insulin response to the OGTT (Fig. 3B) as well as the insulin sensitivity (Fig. 3C) had returned to baseline levels, whereas the glucose response was lower compared with day 0 (P < 0.05) (Fig. 3A).

During the H-E clamps performed before and after the 14 days of step reduction and overfeeding, plasma glucose levels were ~5.0 mM (Fig. 4A), whereas the insulin levels were increased to a hyperinsulinemic level (day 0: 361.59 ± 22.88; day 14: 425.70 ± 26.09). Glucose infusion rate (GIR) during the 120-min clamps is presented in Fig. 4B. During the last 60 min of the clamps, GIR expressed by serum insulin concentration had reduced by 43.6 ± 11% (P < 0.05) (0.019 ± 0.003 vs. 0.011 ± 0.001 μg·kg⁻¹·min⁻¹·pM⁻¹, respectively) when days 0 and 14 are compared (Fig. 4C). Also, the intervention induced a reduction in insulin-stimulated glucose disappearance rate (RG/insulin) of 28 ± 4.3% (P < 0.05) during steady-state hyperinsulinemia (Fig. 4C). Endogenous hepatic glucose production rate (Rg insulin) was suppressed to the same extent before and after 14 days of intervention (99.5 ± 0.3 and 99.1 ± 0.7%, respectively).

Cytokines and inflammation. Plasma levels of leptin had increased at days 7 and 14, whereas plasma adiponectin had increased on day 14 only (P < 0.05). Plasma concentrations of TNF-α and IL-6 had not changed when days 7 and 14 are compared with day 0 (Table 3).

Day 30. After returning to a free living environment (day 30), aerobic fitness was no longer different from day 0 (Table 2). TBM, TFM, and percentage of fat in the different regions were still higher than the values on day 0 (P < 0.05) (Table 2). No differences were seen in fasting measures of metabolites or cytokines at day 30 compared with day 0 (Table 3). Furthermore, the insulin response to the OGTT (Fig. 3B) as well as the insulin sensitivity (Fig. 3C) had returned to baseline levels, whereas the glucose response was lower compared with day 0 (P < 0.05) (Fig. 3A).

DISCUSSION

The aim of the present study was to investigate changes in insulin sensitivity, body composition, and VO2 max during and after 14 days of reduced daily stepping combined with overfeeding. We demonstrated that 1) an impairment in the OGTT-

Table 3. Body composition and fitness level at Day 0, Day 3, Day 7, Day 14 and Day 30

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 30</th>
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<tbody>
<tr>
<td>Total body mass, kg</td>
<td>71.3 ± 3.5</td>
<td>71.8 ± 3.3</td>
<td>72.8 ± 3.4</td>
<td>72.9 ± 3.4</td>
<td>72.4 ± 4.3</td>
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<tr>
<td>BMI, kg/m²</td>
<td>21.6 ± 0.8</td>
<td>21.8 ± 0.8</td>
<td>22.3 ± 0.8</td>
<td>22.1 ± 0.8</td>
<td>21.9 ± 1.0</td>
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<tr>
<td>Total fat, %</td>
<td>14.3 ± 1.3</td>
<td>13.9 ± 1.6</td>
<td>15.3 ± 2.0</td>
<td>16.2 ± 1.2</td>
<td>16.4 ± 1.5</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>10.5 ± 1.3</td>
<td>10.6 ± 1.8</td>
<td>11.5 ± 1.9</td>
<td>12.0 ± 1.2</td>
<td>11.9 ± 1.6</td>
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<tr>
<td>Total fat free mass, kg</td>
<td>58.2 ± 6.9</td>
<td>60.9 ± 2.9</td>
<td>57.9 ± 7.5</td>
<td>57.9 ± 7.1</td>
<td>57.7 ± 8.3</td>
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<tr>
<td>Android fat, %</td>
<td>19.5 ± 1.8</td>
<td>18.0 ± 2.1</td>
<td>21.30 ± 2.3</td>
<td>22.8 ± 1.6</td>
<td>20.9 ± 1.9</td>
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<tr>
<td>Visceral fat, cm²</td>
<td>28.8 ± 13.5</td>
<td>28.1 ± 2.1</td>
<td>33.5 ± 6.3</td>
<td>43.1 ± 20.5*</td>
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<tr>
<td>V̇O₂max, ml·kg⁻¹·min⁻¹</td>
<td>3.39 ± 0.2</td>
<td>3.26 ± 0.2*</td>
<td>3.16 ± 0.2</td>
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<tr>
<td>VO2max, l/min</td>
<td>58.3 ± 2.4</td>
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<tr>
<td>Glucose, mM</td>
<td>4.3* 72.9</td>
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<tr>
<td>Insulin, pM</td>
<td>35.2* 4.81</td>
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<tr>
<td>C-peptide, pM</td>
<td>505.7* 4.3 (443.5–576.6)</td>
<td>696.1 (550.9–879.4)</td>
<td>625.4 (500.1–782.1)</td>
<td>428.8 (224.3–819.5)</td>
<td>526.3 (488.6–566.9)</td>
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<tr>
<td>TG, mM</td>
<td>0.92 (0.64–1.33)</td>
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<tr>
<td>FFA, µM</td>
<td>362.5 (267.5–491.2)</td>
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<tr>
<td>IL-6, pg/ml</td>
<td>1.89 (1.20–2.97)</td>
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<tr>
<td>TNF-α, pg/ml</td>
<td>2.75 (2.34–3.24)</td>
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<tr>
<td>Adiponectin, pg/ml</td>
<td>30.567 (25.538–36.585)</td>
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<tr>
<td>Leptin, pg/ml</td>
<td>1.401 (820–2.395)</td>
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Values are means ± SE [n = 9; dual X-ray absorptiometry scan (DXA) day 3, n = 5; day 7, n = 6]. BMI, body mass index; VO2max, maximal O2 uptake. *Significantly different from prevale.
Several studies have shown that a relatively short period of inactivity impairs glucose metabolism, including insulin sensitivity (21, 22, 36, 48, 50, 51, 57). However, to our knowledge, the time course of impairments has never been described during an intervention period to the same extent as in the present study. Furthermore, mimicking Western lifestyle by combining inactivity and overfeeding may give results that are more closely linked to reality than previous study designs aiming at inactivity. Our data suggest that, after only 3 days of reduced activity and overfeeding, undesirable metabolic changes were apparent, including impaired insulin sensitivity independent of changes in body composition. These implications had worsened after 7 days with an elevated fasting insulin level, a marked decrease in insulin sensitivity, and a compensatory increased insulin response to an oral glucose load to maintain glycemic control. Despite reduced insulin sensitivity, glucose levels were not altered by the intervention, probably due to the appropriate compensatory insulin response during the OGTT. Interestingly, these metabolic impairments had leveled off after 14 days of inactivity and overfeeding, indicated by a smaller increment in the insulin response during the OGTT and a fasting insulin level and insulin sensitivity index that were no longer different from baseline. Even though the Matsuda index has been shown to correlate with the rate of whole-body glucose disposal during an euglycemic insulin clamp (gold standard measure of insulin sensitivity) (35), the index is an indirect estimate of insulin sensitivity. This could explain why the results from the present clamp showed a reduction in insulin sensitivity after 14 days of intervention compared with baseline, whereas measures of fasting insulin levels and insulin response in a physiological postprandial setting still indicated a less impaired glucose metabolism after the entire 14-day intervention period compared with the study days during the intervention.

Aerobic fitness level (ml O$_2$·kg FFM$^{-1}·$min$^{-1}$) decreased after 14 days of intervention. Aerobic fitness previously has been described as a risk factor for and a precursor of T2DM (13, 24). However, there was no relationship between any of the measured metabolic markers and aerobic fitness level, indicating that reductions in fitness following reduced activity and overfeeding are not related to the impairments in insulin sensitivity. Additionally, it has previously been shown in healthy trained subjects that the decline in V$\text{O}_2$ max within the first weeks of reduced physical activity was due to a reduction in stroke volume rather than to metabolic impairments within the muscle tissue (12). Reduced arterio-venous differences of blood metabolites as a result of impaired tissue metabolism may, at least partly, explain a decreased muscle glucose uptake and thereby link V$\text{O}_2$ max and insulin sensitivity (12, 37). This further supports our assumption that the reduction in insulin sensitivity seen in our study cannot be explained by the decline in V$\text{O}_2$ max. Still, this conclusion should be made with caution.

Body fat, including the amount of visceral fat, increased gradually to reach statistical significance after 14 days of intervention. Interestingly, the accumulation of visceral fat did not seem to relate directly to increased total body mass (TBM), seeing that, in contrast to TBM, visceral fat continued to increase between days 7 and 14. An increase in TBM will also increase overall fat deposition; however, previous data indicate that visceral fat accumulation is not solely dependent on degree of obesity (19). Results from some studies indicate that inac-

![Fig. 3. Oral glucose tolerance test (OGTT) 180 min at days 0, 7, 14, and 30. A: blood insulin [area under the curve (AUC) pM]. B: blood glucose (AUC mM). C: Matsuda index. Data is presented ± SE (n = 9). *Significantly different from pre-value (P < 0.05).](http://jappl.org/11/11002/fig_3.png)
tivity per se may play an important role in the deposition of fat in the different depots (32, 42, 47). However, further studies are needed to clarify such a mechanism. Regardless of the reasons for the variation in fat distribution, both body fat (24) and especially amount of visceral fat (17, 55) have been linked to impaired insulin sensitivity and the development of insulin resistance and T2DM, which could be explained by the progression of chronic low-grade inflammation within and from these tissues (15, 18, 43). However, in the present study, disruptions in the regulation of glucose metabolism occurred before the increment in the amount of visceral adipose tissue and without changes in the systemic inflammatory markers TNF-α and IL-6. Taken together, these data show that reduced insulin sensitivity and changes in body composition did not occur in parallel as hypothesized. Although we only measured a limited number of inflammatory markers, the present study supports the notion that the initial steps in the development of disturbed glucose metabolism and impaired insulin sensitivity are not necessarily linked to systemic inflammation and the amount of visceral fat (52, 53).

When studied separately, both inactivity and overfeeding have been shown to impair insulin sensitivity (7, 10, 20–22, 36, 48, 50, 51, 57). Inactivity has been proposed as an important contributor to impaired glucose metabolism, including reduced insulin sensitivity, even though evidence of a causal link has not been found (49, 53). As previously reviewed, the effects of a sudden transition from a high to a low activity level, as in the present study, may be explained by ancient survivability mechanisms (5, 38, 53). Decreased insulin sensitivity and increased food storages may partly be regarded as an adaptation to a decreased activity level, hence a lower need for fuel in the muscle. This makes it possible to save fuel for periods with higher energy demands (5, 38, 53). The present data are in support of such a mechanism; however, the role of increased energy surplus should not be neglected. In our study, a shift from a high to a low activity level combined with overfeeding reduced insulin sensitivity and increased energy stored as fat, measured as an increase in TFM and percentage of fat. When shifting back to a free living environment, insulin sensitivity had normalized, independently of energy stored as

Fig. 4. Hyperinsulinemic euglycemic clamp 240 min pre- (day 0; □) and postintervention (day 14; ■). A: blood glucose (mM), B: GIR (mg·kg⁻¹·min⁻¹). C: GIR/insulin (µg·kg⁻¹·min⁻¹·pM⁻¹; left) and Rₘ/insulin (µg·kg⁻¹·min⁻¹·pM⁻¹; right) during steady-state hyperinsulinemia (60–120 min). Data is presented as means ± SE (n = 9). *Significantly different from pre-value (P < 0.05). **Significantly different from baseline (P < 0.05).
fat. However, without any direct measurements of energy balance after day 14, interpretation of data collected at day 30 after this period of returning to a free living environment must be made with caution. For the measurements to reflect the subjects’ self-selected free living lifestyle habits as much as possible, we chose not to intervene with their lifestyle after day 14; they received no instructions in regard to activity level and energy intake but simply returned to the laboratory at day 30 for the final study day. Thus we speculate that their condition in regard to lifestyle at day 30 resembles that observed before day 0. However, it is possible that the subjects differed with regard to the extent to which they returned to their prior relatively physically active lifestyle and eating behaviors, and some subjects might not have returned to their normal activity and eating behaviors at all. Therefore, for some subjects, the metabolic and body composition outcomes at day 30 may be a consequence of behavioral changes.

In the present model, the combination of inactivity and overfeeding provided a major increase in energy availability (+1,978 kcal/day) due to both decreased energy expenditure and increased energy intake. This high energy surplus potentially led to an accumulation of ectopic fat, e.g., in the liver and the muscle, inducing impaired insulin action within these tissues (11, 29, 30). When inactivity was previously studied alone, the intervention provided an energy surplus of ∼500 kcal/day. When this is compared with the energy surplus of ∼2,000 kcal/day from our present intervention, it is likely that combining inactivity and overfeeding would induce an even larger accumulation of fat in muscle and liver compared with an intervention where inactivity was performed alone. Furthermore, one might speculate that impairments in insulin sensitivity would be located in both liver and muscle, given a more pronounced decrease in whole-body insulin sensitivity. In contrast to our laboratory’s previous study in which step reduction alone was used as intervention (31), we found that TBM and TFM increased in the present study. In addition, the increase in the amount of visceral fat appeared to be much larger in response to the present intervention (7% vs. 49%). Also, fasting insulin levels increased when inactivity and overfeeding were combined, whereas no differences were observed in response to physical inactivity alone (31). Finally, the decrease in insulin sensitivity was more pronounced in the present study compared with step reduction alone, with reductions in glucose infusion rate of 44% and 17%, respectively (31). We do realize that direct comparisons cannot be made between these two independent studies, although the study outcomes were measured using the same methods. However, we speculate that a combination of inactivity and overfeeding results in a more unfavorable health profile than inactivity alone. Although inactivity has been linked with impaired insulin sensitivity in muscle tissue, impairment in insulin sensitivity after a few days of overfeeding have been located in the liver (7, 10). Although hepatic fat content was not measured, the changes in energy availability, TBM, TFM, amount of visceral fat, fasting insulin level, and whole-body insulin sensitivity from the present intervention could be seen as an indication of an increased amount of fat in this organ (29, 45), which further suggests that hepatic insulin sensitivity might be impaired. However, use of stable isotopes revealed that the insulin-stimulated glucose uptake in muscle was reduced by 28%, whereas the endogenous glucose production in the liver (Rg) was suppressed by insulin before and after 14 days of intervention (99.5 ± 0.3% vs. 99.1 ± 0.7%, respectively).

Hence, from these data, we cannot conclude whether our intervention with 14 days of inactivity and overfeeding affected the hepatic insulin sensitivity as proposed above. Since plasma levels of adiponectin have been found to correlate inversely with hepatic fat content and insulin sensitivity in obese individuals (4, 23, 28), the increased plasma levels of adiponectin in the present study (at day 14) could indicate that the liver was not affected by the intervention. Adiponectin is thought to play a role in the suppression of metabolic derangements related to development of T2DM, perhaps by decreasing hepatic lipogenesis and by increasing FFA oxidation and hepatic insulin sensitivity (23, 56). Thus reduced adiponectin levels in diabetic individuals compared with nondiabetic individuals may explain the accumulation of fat and development of insulin resistance in the liver in these patients (33, 34). The increased adiponectin plasma levels and the fat mass gained in the present study seem contradictory, seeing that the adiponectin level is often found to be reduced with obesity. When the increased levels of leptin after only 7 days of step reduction and overfeeding are taken into consideration, changes in adiponectin and leptin levels may represent a mechanism that induces metabolic compensatory changes in response to an increased positive energy balance. Leptin is known to be an appetite regulator, thereby restricting energy intake (27). Circulating leptin levels are directly proportional to the amount of body fat (9) and fluctuate with changes in caloric intake and the amount of energy stored in adipocytes to maintain energy homeostasis (8, 27). However, our subjects were instructed to increase their energy intake by following a fixed diet supplemented with snack packages; thus the physiological mechanism to reduce energy intake by increasing leptin levels and thereby also satiety may have been overruled. To further clarify an additional effect of overfeeding, an intervention study applying this parameter alone in the same population group would be needed.

The present intervention lowered insulin sensitivity, and it is possible that inactivity and overfeeding work in parallel to impair insulin sensitivity. The inactivity model (31) resembles the lifestyle adhered to by a growing part of our population who are engaged in a minimum of physical activity (54), and
the combination with overfeeding gives an even more realistic reflection of an unhealthy lifestyle as practiced in real life (14). This is a major strength of the present study. However, the impairment in the present study in insulin sensitivity observed after a short-term lifestyle change does not provide a direct link between inactivity and overfeeding and the development of Type 2 diabetes. In our study, the reduction in insulin sensitivity is transient, whereas it takes an extended period of time for a chronic disease to develop. Thus we can only hypothesize that after long-term inactivity and overfeeding insulin resistance will reach a pathological state. Also, the studied population was young and healthy, and it cannot be excluded that the intervention would have a different impact in a middle-aged, overweight population considering the age-related onset of Type 2 diabetes. Taken together, this underlines the complexity and difficulties in investigating the role of an unhealthy lifestyle in the development of lifestyle-related diseases.

In conclusion, our study shows that disruptions in the regulation of glucose metabolism, including deterioration in insulin sensitivity, occur after only 3 days of reduced daily stepping and overfeeding, with a more pronounced effect observed after 7 days of intervention. The impaired insulin sensitivity preceded changes in body composition, supporting the notion that the initial steps in impairment of insulin sensitivity may be linked directly to the effects of inactivity and a high calorie intake.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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