A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity

Rikke Krogh-Madsen,1 John P. Thyfault,2 Christa Broholm,1 Ole Hartvig Mortensen,1 Rasmus H. Olsen,1 Remi Mounier,1 Peter Plomgaard,1 Gerrit van Hall,1 Frank W. Booth,3 and Bente K. Pedersen1

1Centre of Inflammation and Metabolism at Department of Infectious Diseases and Copenhagen Muscle Research Centre, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; 2Harry S. Truman Memorial Veterans Hospital, Health Activity Center, Departments of Nutrition and Exercise Physiology and Internal Medicine, University of Missouri, Columbia, Missouri; and 3Health Activity Center, Departments of Biomedical Sciences and of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri

Submitted 30 August 2009; accepted in final form 29 December 2009


A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity. J Appl Physiol 108: 1034–1040, 2010. First published December 31, 2009; doi:10.1152/japplphysiol.00977.2009.—US adults take between ~2,000 and ~12,000 steps per day, a wide range of ambulatory activity that at the low range could increase risk for developing chronic metabolic diseases. Dramatic reductions in physical activity induce insulin resistance; however, it is uncertain if and how low ambulatory activity would influence peripheral insulin sensitivity. We aimed to explore if healthy, nonexercising subjects who went from a normal to a low level of ambulatory activity for 2 wk would display metabolic alterations including reduced peripheral insulin sensitivity. To do this, ten healthy young men decreased their daily activity level from a mean of 10,501 ± 808 to 1,344 ± 33 steps/day for 2 wk. Hyperinsulinemic-euglycemic clamps with stable isotopes and muscle biopsies, maximal oxygen consumption (V\textsubscript{O\textsubscript{2 max}}) tests, and blood samples were performed pre- and postintervention. A reduced number of daily steps induced a significant reduction of 17% in the glucose infusion rate (GIR) during the clamp. This reduction was due to a decline in peripheral insulin sensitivity with no effect on hepatic endogenous glucose production. The insulin-stimulated ratio of pAkt\textsuperscript{ Ser308}/total Akt decreased after step reduction, with a post hoc analysis revealing the most pronounced effect after 4 h of insulin infusion. In addition, the 2-wk period induced a 7% decline in V\textsubscript{O\textsubscript{2 max}} (ml/min; cardiovascular fitness). Lean mass of legs, but not arms and trunk, decreased concurrently. Taken together, one possible biological cause for the public health problem of Type 2 diabetes has been identified. Reduced ambulatory activity for 2 wk in healthy, nonexercising young men significantly reduced peripheral insulin sensitivity, cardiovascular fitness, and lean leg mass.

Address for reprint requests and other correspondence: R. Krogh-Madsen, Centre of Inflammation and Metabolism, Rigshospitalet-Section 7641, Blegdamsvej 9, DK-2100 Copenhagen, Denmark (e-mail: krogh-madsen@inflammation-metabolism.dk).

and at the low range may increase risk for developing chronic metabolic disease(s) (4). We postulated that healthy, nonexercising subjects who transitioned from a high to low level of ambulatory activity (from >10,000 to <2,000) would quickly display metabolic alterations. Our initial findings showed that healthy young men who reduced their daily steps from an average of 10,501 ± 808 to 1,344 ± 33 for a 2-wk period displayed a clustering of metabolic alterations including increased insulin response to an oral glucose tolerance test (OGTT), increased plasma triglyceride response to an oral fat tolerance test, and a 7% increase in visceral fat (21). These findings led us to question if the loss of insulin sensitivity induced by reduced ambulatory activity was due to peripheral or hepatic decreases in insulin sensitivity. Moreover, if there was a decline in peripheral insulin sensitivity we questioned if it was associated with reduced activation of the insulin signaling pathway in skeletal muscle as has been shown in rodent models in which daily physical activity was ceased for acute periods of time (16). We additionally wished to determine if reduced ambulatory activity would negatively impact lean body mass or cardiorespiratory fitness [maximal oxygen consumption (V\textsubscript{O\textsubscript{2 max}})], two factors strongly linked to metabolic health and mortality risk. Herein we provide evidence that 2 wk of reduced daily ambulatory activity in healthy young, nonexercising subjects decreases peripheral insulin sensitivity and insulin stimulation of Akt in skeletal muscle without change in rate of glucose appearance during a hyperinsulinemic-euglycemic clamp. In addition, we show evidence that reduced ambulatory activity significantly lowers both lean body mass and cardiorespiratory fitness.

METHODS

Ethical approval. All subjects gave oral and written consent, and the study was approved by the Scientific-Ethics Committee of Copenhagen and Frederiksberg Municipalities (file no. KF01268925) in accordance with the Helsinki Declaration.

Subjects. Ten healthy human males (mean age 23.8 ± 1.5; body mass index of 22.1 ± 0.7 kg/m\textsuperscript{2}) participated in the study [reported in a preliminary report (21)]. Before the study all 10 subjects underwent a thorough clinical examination. All subjects were nonsmokers, asymptomatic, with no family history of diabetes, did not take medications, and revealed no physical abnormalities during examination. All subjects had normal resting values of blood pressure, normal plasma levels of glucose, insulin, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG), normal hematological parameters including leukocyte counts, and normal hepatic, thyroid, and renal
parameters. None of the participants performed planned exercise sessions of >2 h/wk or walked <3,500 steps/day.

**Study design.** In a free-living environment, participants were instructed to nightly record their daily steps (cycling not included) for 1 inclusion-week and then reduce steps to 1,500 per day for 14 days (cycling not allowed). Step number was measured using a simple pedometer (Yamax Digi-Walker SW-200, London, UK); daily physical activity was also monitored by an Actiheart monitor (Cambridge, UK). Subjects did not exercise more than 2 h/wk on a recreational level. Subjects who walked less than 3,500 steps per day or performed regular exercise were excluded. All subjects agreed to refrain from vigorous physical activity and only performed physical activity corresponding to their normal routines, for 2 days preceding the first test day. The number of steps was registered every night at bedtime. Dietary records were taken during the inclusion-week. Subjects carefully noticed what they consumed day by day, and they maintained their usual dietary habits during the whole study. Subjects were not allowed to consume alcohol 2 days preceding the first test and during the study period. Subjects who performed exercise were excluded. The subjects were asked to weigh and register consumed food the day before pre- and post-insulin clamp and the VO₂ max tests. Figure 1 illustrates testing times.

**Determinations.** An incremental exercise test was performed on a cycle ergometer (Monark 839E, Monark Ltd, Varberg, Sweden) to obtain VO₂ max for each participant with indirect calorimetry system (Moxus modular VO₂ system, AEI Technologies, Pittsburgh, PA) as previously described (1). Preceding the study, subjects performed a familiarization test.

The Actiheart monitor [used to measure daily energy expenditure (7)] is attached to the chest with two standard electrocardiogram (ECG) electrodes and is able to measure acceleration, heart rate (HR), HR variability, and ECG magnitude for epoch settings of 15, 30, and 60 s and can be recorded for a set time. Data on interbeat intervals (IBI logging) and ECG waveforms can also be recorded. Acceleration is measured by a piezoelectric element within the Actiheart with a frequency range of 1–7 Hz (3 dB). For every participant, the Actiheart monitor was tested for adequate HR pickup by recording ECG waveforms for ~30 s before the rest test. If pickup was adequate, the Actiheart was set up to record HR and movement continuously for 1 wk, after which it was reloaded for the rest of the study period.

Fat and fat-free tissue masses for the whole body, trunk, and extremities were measured using a dual-energy X-ray absorptiometry (DXA) scanner, Lunar Prodigy Advance (GE Healthcare, Madison, WI) (21).

Hyperinsulinemic-euglycemic clamps were performed after an overnight fast (Fig. 1). Peripheral catheters were placed in an antecubital vein for blood sampling, in the contralateral antecubital vein for infusion of glucose, insulin, and stable isotopes, and in a dorsal hand vein for blood sampling; the catheterized hand was wrapped in a heating blanket to obtain arterialized venous blood for measurement of glucose and potassium during the clamp. The experiment was commenced by priming the glucose pool by administrating a bolus of 17.5 μmol/kg of [6,6-²H₂]glucose (Cambridge Isotopes Laboratories). This was followed by a continuous infusion (rate 0.4 μmol·kg⁻¹·min⁻¹) for 2 h before the start of the clamp.

Insulin (Actrapid, Novo Nordisk Insulin, 100 IE/ml) was infused continuously at a rate of 40.0 mU·min⁻¹·m⁻², and the plasma glucose concentration was kept at 5.0 mM by a coinfusion of glucose (200 g/1,000 ml, enriched with [6,6-²H₂]glucose to 2.5%) at a variable rate. During the 4-h clamps the infusion rate of [6,6-²H₂]glucose was reduced to 0.08 μmol·kg⁻¹·min⁻¹. Arterialized blood was analyzed.
for glucose and potassium concentrations at intervals of 5 min during the first hour, and every 10 min during the last 3 h of the clamp. Blood samples were collected in heparin-containing tubes and immediately centrifuged for 15 min at 3,500 rpm, and the plasma was stored at −20°C until further analysis.

The plasma glucose enrichment was measured as previously described (23). The glucose turnover rate of appearance (Ra) and rate of disappearance (Rd) were calculated assuming steady state:

\[
Ra = \frac{F_{\text{infusion}}}{E_{\text{glucose}}} \quad \text{and} \quad Rd = \frac{F_{\text{total}} - GIR}{E_{\text{glucose}}}
\]

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 lean body mass; \(E_{\text{glucose}}\) is the enrichment of glucose in plasma; \(F_{\text{total}}\) is the sum of \(F_{\text{infusion}}\) and the [6,6-\(^2\text{H}_2\)]glucose infused by the clamp; GIR is the glucose infusion rate; and kilograms refers to lean body mass.

Baseline blood samples were obtained before initiation of the stable isotope infusion (time point −2 h, Fig. 1). Glucose, triglyceride (TG), and free fatty acids (FFA) were measured with an automatic analyzer (Cobas Faro, Roche, Basel, Switzerland); insulin and C-peptide by ELISA (DAKO, Glostrup, Denmark); tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\), interleukin (IL)-6, IL-15, and leptin by ELISA (R&D Systems); and adiponectin by a RIA kit (LINCO Research).

Vastus lateralis muscle biopsies were taken before and 1 and 4 h after the starting of the clamp. Muscle was quickly frozen in liquid nitrogen and stored at −80°C until later analysis. Muscle tissue was homogenized in ice-cold buffer containing protease inhibitors and phosphatase inhibitors as previously referenced (13, 29). Insulin receptor \(\beta\) (IR\(\beta\)), phosphorylation of Akt at threonine 308 (pAkt\(^{308}\)), total Akt, phosphorylation of AS160 (pAS160), interleukin (IL)-6, IL-15, and leptin by ELISA (R&D Systems); and adiponectin by a RIA kit (LINCO Research).

RESULTS

Pedometer and Actiheart data. For a 2-wk period, participants reduced the number of daily steps by more than 85%

Table 1. Correlation coefficients

<table>
<thead>
<tr>
<th>ΔPhysiological Variables</th>
<th>ΔDaily Steps</th>
<th>ρ</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔDaily energy expenditure, kJ/day</td>
<td>0.922</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>ΔVo_{2\text{max}}, ml·kg(^{-1})·min(^{-1})</td>
<td>0.921</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>ΔVo_{2\text{max}}, ml·min(^{-1})</td>
<td>0.960</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>ΔLeg lean mass, kg</td>
<td>0.262</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ΔGlucose infusion rate, mg·kg(^{-1})·min(^{-1})</td>
<td>−0.225</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Pearson correlation coefficients (\(\rho\)) are given between Δ in daily steps and Δ in physiological variables, where Δ represents a decrease from pre- to postintervention. No other but the mentioned factors correlated. Vo_{2\text{max}}, maximal oxygen consumption; NS, nonsignificant; \(n = 10\).
Table 2. Pre- and post-2-wk intervention results

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Preintervention</th>
<th>Postintervention</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2max test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V̇O2max, ml/min</td>
<td>3,435</td>
<td>149</td>
<td>3,194</td>
</tr>
<tr>
<td>V̇O2max, ml·min⁻¹·kg⁻¹</td>
<td>47.7</td>
<td>1.3</td>
<td>45.1</td>
</tr>
<tr>
<td>Maximal power, W</td>
<td>259</td>
<td>11</td>
<td>243</td>
</tr>
<tr>
<td>Maximal HR, beats/min</td>
<td>194</td>
<td>3</td>
<td>193</td>
</tr>
<tr>
<td>DXA scan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body mass, kg</td>
<td>70.9</td>
<td>1.9</td>
<td>69.7</td>
</tr>
<tr>
<td>Trunk lean mass, kg</td>
<td>26.3</td>
<td>0.8</td>
<td>25.6</td>
</tr>
<tr>
<td>Arm lean mass, kg</td>
<td>6.7</td>
<td>0.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Leg lean mass, kg</td>
<td>18.6</td>
<td>0.5</td>
<td>18.1</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.39</td>
<td>0.11</td>
<td>5.26</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>31.9</td>
<td>3.7</td>
<td>31.8</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>337.6</td>
<td>27.5</td>
<td>335.4</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>1,001</td>
<td>199</td>
<td>1,266</td>
</tr>
<tr>
<td>TG, µmol/l</td>
<td>617</td>
<td>56</td>
<td>647</td>
</tr>
<tr>
<td>TNF, pg/ml</td>
<td>1.31</td>
<td>0.13</td>
<td>1.38</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>0.79</td>
<td>0.22</td>
<td>1.02</td>
</tr>
<tr>
<td>IL-15, pg/ml</td>
<td>1.29</td>
<td>0.1</td>
<td>1.27</td>
</tr>
<tr>
<td>Adiponectin, ng/ml</td>
<td>5,056</td>
<td>803</td>
<td>4,841</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>2,334</td>
<td>389</td>
<td>2,676</td>
</tr>
</tbody>
</table>

VO2max test results, dual-energy X-ray absorptiometry (DXA) scans, and plasma concentrations on lipid and glucose metabolism as well as inflammation are shown. Total lean mass decreased significantly post-2 wk intervention vs. preintervention (see Ref. 21). HR, heart rate; FFA, free fatty acids; TG, triglycerides. n = 10.

Although the evidence that exercise cessation or reduced daily ambulatory activity negatively impacts insulin sensitivity, previous examinations have found that exercise cessation in endurance athletes has a similar impact. Heath et al. (9) showed that 10 days of exercise cessation in endurance athletes dramatically increased insulin responses to an OGTT. A similar result was witnessed in master endurance athletes (~61 yr old) who ceased exercise training for 10 days (25). King et al. (14) showed that endurance athletes who cease training for 10 days have a reduction in peripheral insulin sensitivity measured by hyperinsulinemic-euglycemic clamp. They further determined that this was due to a loss of insulin sensitivity and not a total loss of insulin responsiveness as a much larger infusion of insulin actually sustained insulin sensitivity after 10 days of exercise cessation. In a study from another group, the cessation of exercise in endurance athletes for only 60 h lowered insulin sensitivity to what was measured in sedentary age-matched controls (6). Similar results have been found in rodents. Kump and Booth (16) have shown that rats allowed daily access to voluntary running wheels for 3 wk (average daily run distance equaled 5.7 km) display rapid losses of insulin-stimulated glucose uptake and insulin signaling in skeletal muscle after their daily running was inhibited by wheel lock for only ~2 days (53 h).

Although the evidence that exercise cessation or reduced ambulatory activity leads to a rapid decline in skeletal muscle

DISCUSSION

Major novel findings of this study were that 2 wk of reduced daily ambulatory activity in a free-living condition resulted in decreases in insulin-stimulated Akt phosphorylation, peripheral insulin sensitivity, leg lean mass, and V̇O2max in young healthy men who had not been previously exercise trained. As shown by others in previous studies, the latter three aforementioned decreases are primary decrements that can lead to chronic metabolic disorders and premature mortality.

Importantly, the experimental design, used here, models the extremes (~2,000 to ~12,000 (4)) in numbers of daily steps in the majority of free-living adults in developed countries, most of whom do not partake in structured exercise programs (4). Therefore, the present study is distinctly different from previous studies in which nontypical and extreme changes in activity levels were deployed including continuous bed rest in which subjects are not allowed to stand or walk (8) and detraining studies in which intense structured-exercise programs ceased (2, 18). Furthermore, subjects served as their own control, eliminating potential gene differences that might occur by comparing separate groups of active and less active subjects.

The attenuation of peripheral insulin sensitivity after 2 wk of the decreased ambulatory activity occurred without any detectable alteration in endogenous hepatic glucose production, implying that a peripheral decrement in insulin sensitivity is primary to hepatic insulin resistance on reductions in daily stepping. These findings further reinforce the dogma that glucose entry is exquisitely matched with substrate usage in skeletal muscle, in part by modulating muscle insulin sensitiv-
Insulin sensitivity in human and animal models is very strong, it remains underappreciated as an initiator of metabolic disease and an underused model to study initial declines in insulin sensitivity that likely precede overt insulin resistance. The most common experimental model for studying insulin resistance is acute and long-term high-fat diets, which, as we have already stated, appear to be effective in sedentary conditions only. A recent study by Brons et al. (5) showed that a 5-day

**Fig. 2. Glucose values during hyperinsulimemic-euglycemic clamp.**  
**A:** glucose infusion rate (GIR) during the euglycemic clamp. **B:** blood glucose concentration just before (time point 0) and during the euglycemic clamp. **C:** area under the curve (AUC) for glucose rate of appearance (Ra) and rate of disappearance (Rd) during the euglycemic clamp (120–240 min). *Significant difference between preintervention (Pre) and postintervention (Post) (paired t-test, \( P < 0.01 \)). Preintervention is before reduced daily steps; postintervention is final 3 days of 14-day period of reduced daily steps. \( \bullet \) Preintervention determination; \( \square \) postintervention determination; filled bars, preintervention determination; open bars, postintervention determination. Data are presented as mean ± SEM. \( n = 10 \).

**Fig. 3. Insulin signaling in skeletal muscle during hyperinsulimemic-euglycemic clamp.** Akt, AS160, and insulin receptor (IR)-β protein content and phosphorylation (p) were examined in biopsies taken from vastus lateralis muscle pre- and post-euglycemic clamp. Muscle biopsies were obtained at time points 0 h (before insulin clamp) and 1 h and 4 h (after initiation of insulin clamp); labels on x-axis indicate these times. Labels on y-axis indicate: phosphorylation of IR-β expressed relative to IR-β protein (A); phosphorylation of Akt at threonine308 expressed relative to Akt protein (B); phosphorylation of AS160 expressed relative to AS160 protein (C); and representative immunoblots of signaling proteins. pTyIRβ, tyrosine phosphorylated IRβ. Data are expressed as geometric means (95% confidence interval). *Post hoc analysis, \( P = 0.03 \) (difference between pre- and postintervention). **Post hoc analysis, \( P < 0.05 \) (different from 0 h). \( n = 10 \).
high-fat/high-calorie diet in young healthy men (daily activity levels were not reported) induced hepatic but not peripheral insulin resistance measured by a hyperinsulinemic-euglycemic clamp. This suggests that diet-induced insulin resistance may initially be activated through hepatic disturbances while reductions in activity lead to declines in skeletal muscle insulin sensitivity. Type 2 diabetes has been linked to both increased consumption of dietary fats and a sedentary lifestyle, making it plausible that both of these lifestyle factors work in parallel to impair both hepatic and skeletal muscle insulin sensitivity and are an underlying feature of the dramatic increase in rates of Type 2 diabetes.

The loss of 2.8% in leg lean mass with 2 wk of reduced daily ambulatory activity was unanticipated as most literature suggests that reduced muscle loading must be drastic, such as limb immobilization or bed rest, to observe muscle atrophy. Thus it is likely that decreases in leg lean mass and in the rate of glucose disappearance per kilogram of whole body lean mass (Fig. 2C) both contribute to decreased glucose infusion rate in a hyperinsulinemiec-euglycemic clamp (Fig. 2A) after 2 wk of reduced ambulatory activity.

A 7% suppression of cardiovascular fitness (V̇O₂ max) after lowered ambulatory activity was also unanticipated based on our notion that the loss in ambulatory activity would be of insufficient magnitude and duration to produce a loss. Because of the previously established strong connection between reduced cardiorespiratory fitness and premature mortality, the rapid decline in V̇O₂ max is clinically significant in these young, healthy subjects after only 2 wk of reduced stepping.

The parallel losses of peripheral insulin sensitivity, leg lean mass, and V̇O₂ max are clinically relevant as all three independently increase mortality. While low cardiovascular fitness is the best predictor of mortality (28), low skeletal muscle strength also increases mortality (20), and insulin resistance is believed to be a primary initiator of many chronic diseases (24), which also increase mortality. Taken together, we speculate that reduced ambulatory activity may be primary and predispose to other environmental–gene interactions, especially in at-risk populations such as overweight, elderly, or genetically predisposed humans, that lead to the early development of chronic diseases and/or premature mortality. Chronic diseases take years to become overtly clinical. Our observations suggest that reductions in daily steps or physical activity may initiate or contribute to progressive declines in metabolic function.

ACKNOWLEDGMENTS

All authors have contributed to the conception and design, or the analysis and interpretation of data; the drafting of the article or revising of the article; and the final approval of the version of the article to be published. Ruth Rousing and Hanne Villumsen are acknowledged for technical assistance.

GRANTS

The study was supported by the Commission of the European Communities (contract no. LSHM-CT-2004-005272 EXGENESIS) and by grants from the Augustinus Foundation, The Novo Nordisk Foundation, and from Unlever. R. H. Olsen received a scholarship from the Danish Research Council. The Centre of Inflammation and Metabolism is supported by a grant from the Danish National Research Foundation (DG 02-512-555).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


