Normal physical activity obliterates the deleterious effects of a high-caloric intake

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doi:10.1152/japplphysiol.00155.2013.—A high-caloric intake combined with a sedentary lifestyle is an important player in the development of type 2 diabetes mellitus (T2DM). The present study was undertaken to examine if the level of physical activity has impact on the metabolic effects of a high-caloric (+2,000 kcal/day) intake. Therefore, healthy individuals on a high-caloric intake were randomized to either 10,000 or 1,500 steps/day for 14 days. Step number, total energy expenditure, dietary records, neuropsychological tests, maximal oxygen uptake (V˙O₂max), whole body dual-energy X-ray absorptiometry (DXA) and abdominal magnetic resonance imaging (MRI) scans, continuous glucose monitoring (CGM), and oral glucose tolerance tests (OGTT) with stable isotopes were performed before and after the intervention. Both study groups gained the same amount of body weight. However, the inactive group accumulated significantly more visceral fat compared with the active group. Following the 2-wk period, the inactive group also experienced a poorer glycemic control, increased endogenous glucose production, decreased hepatic insulin extraction, increased baseline plasma levels of total cholesterol and LDL, and a decreased cognitive function with regard to capacity of attention. In conclusion, we find evidence to support that habitual physical activity may prevent pathophysiological symptoms associated with diet-induced obesity.

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IN 2009, THE WORLD HEALTH ORGANIZATION (WHO) estimated that more than 200 million people worldwide have type 2 diabetes mellitus (T2DM) (2). In addition, T2DM now seems to emerge in young adults, most likely driven by increasing rates of obesity, decreased physical activity, genetic predisposition, and ethnicity (39, 41, 50).

Epidemiological studies (16) as well as both longitudinal animal (28, 30) and human inactivity studies (27, 34) indicate that low physical activity is associated with the pathophysiology of T2DM and obesity, and recently it has been estimated that physical inactivity (worldwide) causes 7% of the burden of disease related to, e.g., T2DM (31). In addition, elimination of physical inactivity would increase life expectancy by 0.68 years worldwide, making physical inactivity comparable to the established risk factors of smoking and obesity (31). Physical inactivity (37, 40), a high-energy dietary intake (17), and T2DM (7, 15) are also associated with dementia, depression, and impaired cognitive function. It is critical that we understand how inactivity alters body composition, glucose and lipid metabolism, and cognitive function, and if normal physical activity can prevent these changes.

Previous studies estimate that ∼17% of US adults take below 2,500 steps/day (48). A decrease in ambulatory physical activity (compared with, e.g., bed-rest studies) is one of the most valid models for studying the role of inactivity on the development of metabolic disease (46), and it has been shown that a reduction in steps per day from approximately 10,000 to 1,500 for 2 wk decreases insulin sensitivity and increases the amount of visceral adipose tissue (27, 34).

Epidemiological studies have shown that a high level of both fitness (21, 44) and self-reported physical activity (6, 22) reduce overall mortality, and that this reduction is independent of body mass index (BMI). This could, in part, be explained by the fact that it is not the total amount of adipose tissue but the amount of visceral adipose tissue that plays a key role in the development of T2DM (8).

Not only physical inactivity but also overeating induces insulin resistance (5, 9), and studies examining the combination of a high-caloric intake with a sedentary lifestyle also conclude that there is an important causal link to the development of T2DM (25, 55). An animal study has shown that voluntary running rescues high-fat-fed mice from obesity (10); however, it is still unknown whether normal physical activity can prevent diet-induced changes in metabolic parameters and cognitive function and to what extent a sedentary lifestyle influences these parameters in humans. The present study was undertaken to examine if the level of physical activity has impact on the metabolic effects of a high-caloric intake. Therefore, healthy individuals on a high-caloric intake were randomized to either 10,000 or 1,500 steps/day for 14 days.

MATERIALS AND METHODS

Subjects

Twenty healthy males [median age: 22 yr (range 18–29 yr)] participated in the study. Before inclusion all 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 walked \( \sim 10,000 \) steps/day, and were normally trained (i.e., not athletes, not inactive) (Table 1).

All subjects gave written informed consent to participate, and the study was approved by the Scientific Ethics Committee of the Capital Region of Denmark (file number H-4-2009-082) in accordance with the Helsinki Declaration.

### Study Design

The primary outcome of the study was to assess the metabolic consequences of the intervention (cardiovascular fitness, body composition, and glucose/lipid metabolism). Secondary outcomes were liver function, cognitive function, and inflammation. The current study sample was based on previous studies (25, 27, 34) that have shown significant changes in metabolic parameters using different kinds of nuts, cakes, chocolates, chips, fruit juices, and sodas/soft drinks to increase the total energy balance by approximately \( 2,000 \) kcal in both groups.

Dietary records were used to assess the TEI and nutrient composition (DanKost Sport 2000, Danish Catering Centre, Herlev, Denmark). Using these records, an individual diet plan was made for the entire intervention period, to make sure that the subjects as a baseline-intake maintained habitual diet. In addition, daily dietary suppletions as described above were given in snack packages containing different kinds of nuts, cakes, chocolates, chips, fruit juices, and sodas/soft drinks to increase the total energy balance by approximately \( 2,000 \) kcal in both groups.

To determine aerobic fitness, maximal oxygen uptake (\( V_{O_2,max} \)) was measured during an incremental exercise test performed on a cycle ergometer (Monark 839E, Monark, Varberg, Sweden) using an Incremental Exercise Test Start with \( 80 \% \) of the predicted \( V_{O_2,max} \). The test continued until voluntary exhaustion or \( 70 \% \) VO\(_2\max\) was reached. The test was terminated if the subject experienced symptoms of exhaustion or if the subject requested to stop.

### Measurements

Number of steps per day was measured using a simple pedometer (Yamax Digi-Walker SW-200, London, UK). Steps were recorded daily for 3 days prior to the intervention as well as during the intervention.

TEE was measured using the Actiheart monitor (Cambridge, UK) as previously described (25). The Actiheart was set up to record heart rate (HR) and movement continuously for 3 days before initiation of the study and two times during the inactivity period (the first and the last 72 h).

Dietary records were used to assess the TEI and nutrient composition (DanKost Sport 2000, Danish Catering Centre, Herlev, Denmark). Using these records, an individual diet plan was made for the entire intervention period, to make sure that the subjects as a baseline-intake maintained habitual diet. In addition, daily dietary suppletions as described above were given in snack packages containing different kinds of nuts, cakes, chocolates, chips, fruit juices, and sodas/soft drinks to increase the total energy balance by approximately \( 2,000 \) kcal in both groups.

### Table 2. Content of snack packages

<table>
<thead>
<tr>
<th></th>
<th>AHCD</th>
<th>IHCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snack package (example), kcal</td>
<td>563</td>
<td>563</td>
</tr>
<tr>
<td>Cookies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cashew nuts</td>
<td>577</td>
<td>577</td>
</tr>
<tr>
<td>Chocolate</td>
<td>235</td>
<td>235</td>
</tr>
<tr>
<td>Soft drinks</td>
<td>139</td>
<td>139</td>
</tr>
<tr>
<td>Twix</td>
<td>418</td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2,002</td>
<td>1,514</td>
</tr>
</tbody>
</table>

### Data are presented as means ± SE. Shown are an example of the content and the corresponding amount of kcal (top) and amount of nutrients (middle) in one of the snack packages. The mean of the total amount of nutrients in all snack packages is presented at bottom.
indirect calorimetry (COSMED Quark b2, Pavona di Albano, Rome, Italy), as previously described (25). Before the study, subjects performed a familiarization test, and the actual tests were performed before and after intervention.

Fat and fat-free tissue masses were measured using a dual-energy X-ray absorptiometry (DXA) scanner, Lunar Prodigy Advance (GE Healthcare, Madison, WI). Android and gynoid fat mass was estimated from these DXA scans by the same (blinded) person.

Intra-abdominal and subcutaneous fat content was determined by 3-T MRI-scans (Siemens Magnetom Total imaging matrix magnetic resonance scanner, Erlangen, Germany) as previously described (34). Images displaying fat were analyzed using MANGO (Multi-Image Analysis GUI) version 2.5 developed at Research Imaging Center, The University of Texas Health Science Center, San, Antonio, TX. Any adipose tissue located from the diaphragm to the first sacral vertebra except subcutaneous tissue were characterized as visceral fat. Subcutaneous fat was determined using the single slice method at the level just below the umbilicus. Analyses were performed by the same (blinded) person.

Due to the relatively short intervention period a possible measurable change in cognitive function was suspected to be in the area of attention. In addition, the present study was designed to perform repetitive testing and it was of great importance that the applied test could be performed several times with no opportunity of learning. Based on this, the computer controlled Conner’s Continuous Performance Test II 2000 (CPTII) (Conners, Toronto, Canada: Multi-Health Systems) was used to identify changes in attention. Before the study, subjects performed a familiarization test, and the test was performed before and after intervention. The CPTII lasts for 14 min and is divided into six blocks, each containing 20 trials (letters). The subjects were asked to respond by pressing the space button whenever any letter appeared on the computer screen but were told to inhibit the response when an X appeared on the screen. The results are presented as T scores (age- and sex-corrected raw data) of commission (the number of times the subject pressed space when an X appeared) and omission (the number of times a letter was presented, but the subject did not press space).

To measure the average 24-h glucose levels, glucose concentrations were monitored continuously using the Guardian REAL-Time Continuous Glucose Monitor (CGM) System (Medtronic, Northridge, CA). The CGM System consists of a glucose sensor, a transmitter, and a small external monitor. The glucose sensor was inserted under the skin to measure glucose levels in the tissue fluid. The glucose sensor produces an electronic signal that is related to the amount of glucose present in the blood. The CGM transmitter, which is a small device that attaches to the glucose sensor, gathered the glucose data, and sent it wirelessly to the glucose monitor unit which was attached to a waistband. The CGM system was calibrated by the subject with finger-stick blood glucose three times per day. The CGM system recorded during the same time periods as the Actiheart. Using this method glucose was measured every 5 min during these 3 days. For each 3-day monitoring, mean, maximum, minimum, and the difference between maximum and minimum (delta max-min) glucose levels were calculated for 24 h.

Oral glucose tolerance tests (OGTTs) were performed after an overnight fast. Peripheral catheters were placed in an antecubital vein for blood sampling and in the contralateral antecubital vein for infusion of stable isotopes. At 8:00 am (t = 0 min) a primed (20 μmol/kg), continuous (0.20 μmol·kg⁻¹·min⁻¹) infusion of [6,6-²H₂]glucose (Cambridge Isotopes, Laboratories, Cambridge, MA) was initiated. Two hours later (t = 120 min), we gave the subject 75 g of glucose as a bolus (73 g dextrose anhydride + 2 g [U-¹³C]glucose; Cambridge Isotopes, Laboratories) dissolved in 300 ml water was administered. Following oral glucose ingestion the infusion rate of [6,6-²H₂]glucose was sequentially lowered to 0.06 μmol·kg⁻¹·min⁻¹ to mimic expected suppression in endogenous glucose production (47). For determination of plasma glucose tracer enrichment, venous blood plasma samples were collected at t = 120, 20, 10, and 0 min, and then every 10 min until t = 180 min, at which point the tracer infusion was ceased. Plasma and aliquots from the tracer infusates were stored at −80°C until analysis.

Plasma concentrations of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), alanine transaminase (ALT), aspartate transaminase (AST), and gamma glutamyl transpeptidase (GGT) were measured at baseline (enzymatic assays; Roche p-Modular system, Switzerland). Plasma cytokine levels (TNF-alpha and IL-6) were measured using ELISA (R&D Systems). Measured intra-assay CV values were 5% with regard to IL-6 and 4% with regard to TNF-alpha. All samples were run in duplicates and the mean value was calculated.

During the OGTT, plasma glucose concentrations were measured at baseline and every 10 min from t = 0 min [using an automatic analyzer (Cobas Faro, Roche, Basel, Switzerland)]. Concentrations of free fatty acids (FFA) (Cobas Faro, Roche, Basel, Switzerland), insulin, and C-peptide (electro-chemiluminescence immunoassay; Roche e-Modular System), and intact glucose-dependent insulinotropic polypeptide (GIP) and total glucagon-like peptide 1 (GLP1) [radioimmunoassay as previously described (26, 35)] were measured at baseline and every 20 min from t = 0 min.

Plasma glucose tracer enrichments (for M + 2 and M + 6 ions) were determined using liquid-chromatography tandem mass spectrometry (API 3000 LC-MS/MS; Applied Biosystems, Foster City, CA), performed on a hexobenzoyl derivative of glucose (33). Glucose kinetics were calculated using a non-steady-state single-pool model, as previously described (43).

Statistical Analyses

All baseline data as well as area under the curve (AUC) (calculated with regard to data obtained during the OGTT) were analyzed using parametric methods on the absolute data. Paired t-tests were applied to test before vs. after intervention in each group, and unpaired t-test was performed on the delta values (before minus after) to compare differences between groups (called the delta P value). Reported values are mean with SE. P < 0.05 was considered statistically significant. Analyses were performed using a statistical software package (SAS version 9.1.3 SAS Institute).

RESULTS

Pedometer, Actiheart, and Dietary Records

For the 2-wk period the subjects in the IHCD group significantly reduced their number of daily steps (P < 0.01), resulting in a reduced energy expenditure (P < 0.0001). In the AHCD group, subjects continued their daily number of steps, causing no significant difference in energy expenditure (Table 1). Both number of steps and TEE were significantly different between groups (delta P < 0.0001 and < 0.01, respectively). TEI in both groups increased significantly (Table 1), resulting in a positive energy balance in the IHCD and the AHCD group of 1,577 ± 227 and 2,147 ± 78 kcal/day, respectively (delta P < 0.01).

Cardiovascular Fitness

A significant reduction in absolute VO₂max (ml/min) as well as in relative VO₂max [ml·min⁻¹·kg⁻¹] of total fat free mass (FFM) was found in the IHCD group (P < 0.05 for both absolute and relative VO₂max), with no difference in the AHCD group. In addition, there was a significant difference between groups (delta P < 0.05 for both absolute and relative VO₂max) (Table 1).
Body Composition, DXA, and MRI Scans

Both groups increased significantly in body weight (delta P: nonsignificant (NS)), BMI (delta P: NS), total fat mass (TFM) (delta P < 0.05), and glycoind fat mass (delta P: NS), whereas only the IHCD group increased in android fat mass (delta P = 0.01). There was no difference in FFM in either group before and after intervention (Table 1) (delta P: NS). The increment in android fat mass is likely due to an increase in visceral adiposity since results from the MRI scans showed a significantly 30% increase in the amount of visceral adipose tissue in the IHCD group (delta P < 0.01), with no change in the AHCD group combined with a significant increase in abdominal subcutaneous adipose tissue in both groups (~6% in the AHCD group and ~9% in the IHCD group), with no difference between groups (Table 1).

Cognitive Function

Although there was no significant difference between groups (delta P: NS, with regard to both omission and commission), there was an overall loss of attention and concentration in the IHCD group since both omission (misses) (P < 0.01) and commission (non-target response, borderline significant, P = 0.06) increased in the IHCD group, whereas there was no significant change before and after intervention in the AHCD group (Table 1).

Glucose Metabolism

CGM system. The mean 24-h glucose level, measured by the CGM system, was significantly increased in the IHCD group (P < 0.01), whereas no difference was found in the AHCD group (Table 3). These data was supported by an increase in maximum 24-h blood glucose (delta P: NS), whereas only the IHCD group increased in android fat mass (delta P = 0.01). There was no difference in FFM in either group before and after intervention (Table 1) (delta P: NS). The increment in android fat mass is likely due to an increase in visceral adiposity since results from the MRI scans showed a significantly 30% increase in the amount of visceral adipose tissue in the IHCD group (delta P < 0.01), with no change in the AHCD group combined with a significant increase in abdominal subcutaneous adipose tissue in both groups (~6% in the AHCD group and ~9% in the IHCD group), with no difference between groups (Table 1).

Lipid Metabolism

There was a reduction in baseline plasma levels of FFA after intervention in both groups (P < 0.05, delta P: NS). The total AUC of FFA during the OGTT decreased significantly in the AHCD group (P = 0.01, delta P: NS), whereas there was no difference in either group in the insulin-suppressed concentrations of FFA [AUC during the last 2 h of the OGTT (AUC(60–120min)), delta P: NS]. Baseline plasma levels of total cholesterol (P = 0.005) and LDL (P = 0.01) were increased in the IHCD group when comparing before vs. after intervention, whereas there was no difference in the AHCD group and no difference between groups (delta P: NS). There was no difference in

Table 3. Glucose metabolism

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AHCD Before</th>
<th>AHCD After</th>
<th>P value</th>
<th>IHCD Before</th>
<th>IHCD After</th>
<th>P value</th>
<th>Delta P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 24-h glucose, mmol/l</td>
<td>5.1 ± 0.5</td>
<td>5.2 ± 0.3</td>
<td>0.8</td>
<td>4.9 ± 0.2</td>
<td>5.4 ± 0.3</td>
<td>0.01</td>
<td>0.5</td>
</tr>
<tr>
<td>Minimum glucose, mmol/l</td>
<td>3.4 ± 0.3</td>
<td>3.9 ± 0.2</td>
<td>0.1</td>
<td>4.0 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Maximum glucose, mmol/l</td>
<td>7.8 ± 1.0</td>
<td>7.0 ± 0.3</td>
<td>0.5</td>
<td>6.5 ± 0.2</td>
<td>7.1 ± 0.3</td>
<td>0.01</td>
<td>0.3</td>
</tr>
<tr>
<td>Delta (max minus min), mmol/l</td>
<td>4.5 ± 0.8</td>
<td>3.1 ± 0.3</td>
<td>0.2</td>
<td>2.5 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>AUC C-peptide/AUC insulin</td>
<td>9.1 ± 0.5</td>
<td>8.8 ± 0.3</td>
<td>0.3</td>
<td>8.2 ± 0.4</td>
<td>7.0 ± 0.3</td>
<td>0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>AUC GIP, min×pmol/l</td>
<td>2,288 ± 177</td>
<td>2,354 ± 125</td>
<td>0.6</td>
<td>2,149 ± 120</td>
<td>2,206 ± 72</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>AUC GLP-1, min×pmol/l</td>
<td>4,743 ± 311</td>
<td>4,600 ± 139</td>
<td>0.6</td>
<td>3,595 ± 259</td>
<td>3,535 ± 385</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>AUC Rd Glucose, mg/kg</td>
<td>983.4 ± 52.5</td>
<td>904.9 ± 33.9</td>
<td>0.2</td>
<td>1,009.9 ± 36.3</td>
<td>1,046.4 ± 55.9</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Baseline insulin, pmol/l</td>
<td>26.2 ± 3.1</td>
<td>30.5 ± 2.7</td>
<td>0.2</td>
<td>34.0 ± 4.0</td>
<td>45.3 ± 5.6</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Baseline C-peptide, pmol/l</td>
<td>423.1 ± 34.8</td>
<td>494.8 ± 35.7</td>
<td>0.02</td>
<td>474.7 ± 32.0</td>
<td>513.1 ± 29.4</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Baseline glucose, mmol/l</td>
<td>5.0 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>0.9</td>
<td>5.0 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td>0.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Data obtained from the continuous glucose monitoring (CGM) and from the oral glucose tolerance tests (OGTTs) before and after intervention in both groups. Baseline values equals fasting levels on the respective test days. AUC, area under the curves; Active GIP, glucose-dependent insulinotropic polypeptide; total GLP-1, glucagon-like peptide 1; Rd glucose, rate of disappearance of glucose. P value, paired t-test within trial; delta P value, unpaired t-test on delta values (before minus after) from each group, to test differences between trials.
baseline plasma levels of TG and HDL in either group and no difference between groups with regard to these parameters (delta P: NS) (Table 4).

Liver Enzymes

ALT, AST, and GGT were unchanged in both groups (Table 4), and there were no differences between groups (delta P: NS).

Inflammatory Markers

Baseline plasma levels of TNF-alpha and IL-6 were unchanged in both groups, and there were no differences between groups (delta P: NS, data not shown).

DISCUSSION

The major novel finding of the present study is that the level of physical activity determines to which degree a high-caloric diet influences fat distribution. Thus the group undertaking only 1,500 steps/day had a more pronounced increase in the amount of visceral fat compared with the group, who continues normal physical activity equivalent to 10,000 steps/day. In addition, the present study suggests that physical inactivity has a negative effect on glucose and lipid metabolism, as well as on the ability to concentrate, compared with normal physical activity.

A subgroup (~30%) of obese people has been characterized as “healthy obese” or “metabolically healthy obese,” meaning obese persons with, e.g., preserved insulin sensitivity and relatively low visceral fat mass (8, 38). In the present study, where all individuals were exposed to a high-caloric diet, a sedentary lifestyle induced an increase in body weight, BMI, TFM, gynoid and android fat mass, subcutaneous abdominal fat as well as an increase in the amount of visceral adipose tissue, whereas a normal physically active lifestyle only increased body weight, BMI, TFM, gynoid fat mass, and subcutaneous abdominal fat. The findings are supported by a significant difference between groups with regard to TFM, android fat mass, and visceral adipose tissue, indicating that central adipose storage that can be prevented by normal physical activity. This main finding is of great importance; it is well described that the amount of visceral adipose tissue is a key prognostic factor for premature death as well as severe comorbidity such as hypertension, insulin resistance, dyslipidemia, and T2DM (24). In addition, our findings strongly support the common recommendations on daily physical activity of ~10,000 steps/day (3). As previously shown (25, 27), there was no concomitant increase in baseline plasma cytokine levels in either group afterward. Supported by previous work (25), there was no change in FFM in either group after intervention. However, it has been shown that inactivity alone (energy surplus of ~500 kcal/day) induced a decrease in FFM of ~1 kg (34), suggesting that a high-caloric intake is able to maintain muscle mass during inactivity. It could be speculated that fitness could play an important role in keeping “healthy obese” metabolically healthy. Recently Ortega et al. (36) have shown that the metabolically healthy obese have a higher fitness level than the metabolically unhealthy obese. In the present study, \(V_{O2\max}\) decreased in the IHCD group (with a significant difference between groups). The present study was not powered
for correlation analyses but according to the above-mentioned results, the reduction in fitness is considered as an important clinical marker in these individuals. Although with only borderline significant differences between groups, 2 wk of intervention in the IHCD group worsened glycemic control (increased plasma glucose during OGTT), increased endogenous glucose production, induced a compensatory increased insulin response during the OGTT, as well as increased baseline insulin concentrations. The latter was probably explained by reduced hepatic insulin extraction (decreased AUC C-peptide/AUC insulin) and not by increased insulin secretion. Previous studies on both physical inactivity (27, 34) and inactivity combined with excessive caloric intake (25) have shown the same tendency. Interestingly, the present study reveals that normal physical activity (AHCD group) can prevent these changes; the pancreas had an adequate beta-cell response in the AHCD group (increased AUC C-peptide) as well as a normal glucose and insulin response during the OGTT, which partially can be explained by a reduced glucose Ra from the gut. A clear explanation for this novel finding is pending, but one explanation could be a compensatory slowing of gastric emptying (52, 54). Even though there were no

Table 4. Lipid metabolism and liver enzymes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AHCD</th>
<th>IBHD</th>
<th>Delta P Value</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
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<tr>
<td>FFA, μmol/l</td>
<td></td>
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<tr>
<td>TG, mmol/l</td>
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<tr>
<td>Cholesterol, mmol/l</td>
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<td></td>
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<tr>
<td>LDL, mmol/l</td>
<td></td>
<td></td>
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<tr>
<td>HDL, mmol/l</td>
<td></td>
<td></td>
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<tr>
<td>ALT, U/l</td>
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<tr>
<td>AST, U/l</td>
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<tr>
<td>GGT, U/l</td>
<td></td>
<td></td>
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<tr>
<td>OGTT</td>
<td></td>
<td></td>
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<tr>
<td>AUC FFA 0–180 min, min×μmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC FFA 60–180 min, min×μmol/l</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Baseline plasma values before and after intervention (top of the table) as well as free fatty acid (FFA) plasma levels during the oral glucose tolerance test (OGTT), presented as area under the curve (AUC) at the bottom of the table. Data are presented for both groups. TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma glutamyl transpeptidase. P value: paired t-test within trial. Delta P value: unpaired t-test on delta values (before minus after) from each group, to test differences between trials.
significant differences between groups, a possible decreased glycemic control in the IHCD group is supported by data obtained from the CGM system, showing significant increases in mean and maximum CGM glucose (Table 3). It is known that hyperglycemia in T2DM is associated with cardiovascular disease (29), and that glucose levels (fasting, 1-h, and 2-h OGTT glucose levels) that are below the diabetic threshold (but still above normal) are risk factors for cardiovascular disease (13). In addition, elevated glycemic variability is associated with atherosclerosis (45), and the decreased glycemic control (OGTT data) and increased glycemic variability (CGM system data) in the IHCD group are therefore interpreted as serious clinical risk markers which, however, may be counteracted by physical activity.

Surprisingly, the differential group responses in insulin secretion were not explained by changes in secretion of incretin hormones. It has previously been shown in healthy humans, that a 12-day intervention with prednisolone treatment, relative physical inactivity, and a high-caloric diet induced an increased GIP, but not GLP-1, response during an OGTT (19). In addition, short-term dexamethasone treatment of healthy first-degree relatives of patients with T2DM induced an increased GLP-1 and GIP plasma response during an OGTT (23). In this perspective, glucocorticosteroids could be speculated to have a more severe impact on the incretin response during an OGTT than physical inactivity and overeating per se.

In the present study Rd glucose does not explain the group differences in glycemic control. Previous studies, using almost the same intervention [1,500 steps/day for 2 wk and isocaloric diet (27) and 1,500 steps/day for 2 wk and high-caloric diet (25)] have shown a reduction in the rate of disappearance of glucose. In these studies hyperinsulinemic euglycemic clamps combined with stable isotopes were used to assess Rd glucose. It could be speculated that the difference in use of method (OGTT vs. clamp) could explain the disparate results.

The National Cholesterol Education Program (NCEP) treatment goals focus on LDL and vary depending on the risk for developing coronary heart disease (CHD). LDL levels below 4.1 mmol/l are considered low risk (1). Although below this threshold, there was an increase in baseline LDL levels in the IHCD group, with no change in the AHCD group in the present study. Population studies (42, 51) have found a direct relationship between levels of LDL cholesterol and the rate of new-onset CHD in men and women, and in this perspective (although there was no difference between the groups) it is concerning that only 2 wk of sedentary lifestyle combined with a high-caloric intake can increase LDL levels significantly, whereas it is reassuring that even normal physical activity seems to obliterate this effect despite a high-caloric intake, at least on the short term.

In accordance with previous studies using high-caloric diets (9, 12), there were decreased baseline FFA plasma levels in both groups after intervention. Since baseline insulin plasma levels only increased significantly in the IHCD group, and since there was no change in FFA AUC60–180min during the OGTT in either group (indicating normal insulin sensitivity of the adipose tissue) the decreased baseline levels of FFA are most likely not due to an insulin-mediated suppression of lipolysis per se, but we speculate may reflect an increased triglyceride synthesis within the adipose tissue, e.g., through effect of the hormone acylation-stimulating protein (ASP) (20, 49).

The decreased neuropsychological attention in the IHCD group is in agreement with animal studies as well as human epidemiological studies showing that physical inactivity and/or excessive dietary energy intake can decrease cognitive function (4, 32). Acute and long-term transient hyperglycemia have similar effect (14), possibly due to reduced neurogenesis (53). The results indicate that normal physical activity of 10,000 steps/day can prevent a decrease in attention, and this conclusion is supported by data from human intervention studies showing increased cognitive function, larger hippocampal volumes, and increased cortical volumes after exercise (11, 18).

The present study has been performed during 2 wk of intervention, and although the results point toward a beneficial effect of normal physical activity, it is not possible to extrapolate to a life-long exposure of physical inactivity and high-caloric diet. Although the present study shows marked changes in the distribution of adipose tissue, in cognitive function, and in lipid and glucose metabolism in the IHCD group, only a significant effect of normal physical activity was proven with regard to the distribution of adipose tissue and fitness ($V_{\text{O}}_{\text{2max}}$) (significant differences between trials). This could be due to the relatively short duration of the intervention, the small number of subjects or, most importantly, to the fact that the increase in total energy balance was lower in the IHCD group than expected (IHCD group: 1,577 ± 227 kcal/day; AHCD group: 2,147 ± 78 kcal/day). In addition the participants were probably more physically active than many individuals. It is likely that more sedentary individuals would experience even worse metabolic outcomes going through the same interventions.

In conclusion, we find evidence to support that normal daily physical activity can prevent increases in the amount of visceral adipose tissue, even in the presence of overeating. In addition, we suggest that a normal active lifestyle can inhibit the deterioration of glucose and lipid metabolism, and the decline in attention that is induced by a hypercaloric diet. This indicates that sufficient habitual activity may prevent pathophysiological symptoms associated with diet-induced obesity.

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**DISCLOSURES**

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


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