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Effects of 1 day of inactivity on insulin action in healthy men and women: interaction with energy intake

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Abstract

Prolonged periods of limited muscle activity can reduce insulin action. Acute changes in low muscle activity (ie, sitting) have not been assessed. In addition, unless energy intake is reduced during sitting to match low expenditure, the concurrent energy surplus may explain lower insulin action. The objective of the study was to evaluate the acute effect of sitting, with and without energy surplus, on insulin action. Fourteen young (26.1 ± 4.5 years, mean ± SD), nonobese (23.7% ± 7.1% fat), fit (peak oxygen consumption = 49.1 ± 3.3 $mL \cdot kg^{-1} \cdot min^{-1}$) men (n = 7) and women (n = 7) completed three 24-hour conditions: (1) an active, no-sitting condition (high energy expenditure of 2944 ± 124 kcal with energy intake matched to expenditure) = NO-SIT; (2) low energy expenditure (sitting) of 2195 ± 121 kcal with no reduction in energy intake (energy surplus) = SIT; and (3) sitting with energy intake reduced to 2139 ± 118 kcal to match low expenditure (energy balance) = SIT-BAL. Insulin action was measured the following morning during a continuous infusion of [6,6-^{2}H]-glucose. Data were analyzed using linear mixed-effects models with planned contrasts. Compared with NO-SIT, insulin action, defined as whole-body rate of glucose disappearance normalized to mean plasma insulin, was reduced by 39% in SIT ($P < .001$) and by 18% in SIT-BAL ($P = .07$). Insulin action was higher in SIT-BAL compared with SIT ($P = .04$). One day of sitting considerably reduced insulin action; this effect was minimized, but not prevented, when energy intake was reduced to match expenditure. Strategies to limit daily sitting may reduce metabolic disease risk.

1. Introduction

Recent epidemiologic data suggest a positive relationship between time spent in sedentary behaviors and insulin resistance and risk for type 2 diabetes mellitus [1-4]. Sustained absence of physical activity imposed by hind limb suspension in rodents or bed rest in humans can reduce insulin-mediated glucose uptake (ie, insulin action) [5-11]. The epidemiologic studies have consistently reported that time spent in tasks that require little muscle activity (low accelerometry counts, self-reported television time or computer use) is inversely related to insulin action [1-3]. A recent study also reported lower insulin action following 2 weeks of reduced daily walking in conjunction with increased body fat, decreased fat-free mass, and unsupervised food intake in free-living men [12]. In contrast, there are no published interventional studies that have examined the acute impact of prolonged sitting in a controlled setting with strict dietary control. This novel paradigm is distinct from sedentary lifestyle, which usually refers to the avoidance of moderate-vigorous exercise. Prolonged sitting, on the other hand, minimizes time spent standing, ambulating, and other low-intensity activities. Many people spend

Institutional approval: The study protocol was approved by the Institutional Review Board at the University of Massachusetts Amherst and the University of Missouri prior to initiation of the study. All subjects gave their verbal and written informed consent before participating.

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considerable amounts of the waking day sitting, which, over time, likely contributes to increased risk for obesity and metabolic disorders. Therefore, it is important to examine the acute metabolic impact of prolonged sitting to identify early processes involved in the development of metabolic disease.

Factors that may account for impaired insulin action after periods of low muscle activity are not well characterized. Less standing and ambulation reduce daily energy expenditure and lead to energy surplus unless dietary energy intake is reduced to match the low expenditure. Energy surplus reduces insulin action [13,14]. The effects attributed to low muscle activity may be partly mediated by the concurrent energy surplus, but this hypothesis has not been tested. Therefore, this study was designed to evaluate (1) the metabolic response to 1 day of prolonged sitting with no change in diet and (2) the contribution of energy surplus to the metabolic response to sitting. We hypothesized that, relative to a condition in which sitting was minimized, prolonged sitting and energy surplus would reduce insulin action. Based on the independent effects of energy surplus on metabolism, we hypothesized that reducing energy intake to match low expenditure would attenuate the sitting-induced decline in insulin action.

2. Subjects and methods

2.1. Subjects

Fourteen recreationally active men (n = 7) and women (n = 7) between the ages of 19 and 32 years from the University of Massachusetts surrounding area participated in this study. Subject characteristics are presented in Table 1. All volunteers were nonobese, in good health, nonsmoking, free of known disease, not following a very low or very high carbohydrate diet (<30% or >70% carbohydrate, respectively), and not taking any medications or supplements known or suspected to alter carbohydrate or lipid metabolism. Four women were taking monophasic birth control. For the remaining women, based on self-reported onset and cessation of menses, one completed all trials in the luteal phase; one completed 2 conditions in the luteal phase and the third condition (NO-SIT) in the follicular phase. For one woman with irregular menses, we could not determine menstrual cycle phase during any experimental condition. The study protocol was approved by the Institutional Review Board at the University of Massachusetts Amherst and the University of Missouri before initiation of the study, and all subjects gave their verbal and written informed consent before participating.

Table 1
Subject characteristics[a]

	Mean ± SD	Range
Age (y)	26.1 ± 4.5	19.8-32.2
Weight (kg)	69.5 ± 13.2	49.6-89.7
Height (cm)	170.9 ± 10.1	152.0-188.0
BMI (kg/m^2)	23.6 ± 3.0	18.8-29.2
% Fat	23.7 ± 7.1	13.0-36.6
Lean mass (kg)	53.4 ± 13.4	35.6-78.0
Vo_{2peak} (mL·kg^{-1}·min^{-1})	49.1 ± 3.3	40.5-52.4
Physical activity (h/wk)[b]	2.8 ± 1.2	1.5-8.0

BMI indicates body mass index; Vo_{2peak}, peak oxygen consumption.

[a] N = 14; 7 men, 7 women.

[b] Defined as number of hours per week of planned moderate to vigorous exercise (ie, ≥4.0 METS). Activity was self-reported.

2.2. Overall design

To test the aims of this study, a counterbalanced, crossover design in which participants served as their own controls was used (Fig. 1). The intent of the study design was to compare subjects when physically active and inactive while consuming the same caloric intake and to also compare these trials to inactivity with energy intake reduced to approximate low energy expenditure. Insulin action was assessed in the morning following three 24-hour experimental conditions: (1) an active condition with minimal sitting (ie, high energy expenditure with high energy intake) = NO-SIT; (2) reduced energy expenditure (prolonged sitting) without a concomitant reduction in energy intake (energy intake > expenditure; ie, energy surplus) = SIT; and (3) inactivity with energy intake reduced by approximately 1000 kcal (14.1 ± 1.0 kcal/kg/d, mean ± SD) = sitting, energy balance (SIT-BAL). Energy intake and expenditure across the 3 conditions are presented in Table 2.

2.3. Preliminary testing

Before participating in the experimental protocol, body composition (fat mass, fat-free mass, and percentage body fat) was assessed by dual energy x-ray absorptiometry (Lunar, Madison, WI). Resting energy expenditure (REE) was also measured in the morning after an overnight fast before the start of the study. Upon arrival, subjects lay supine in a quiet room for 30 minutes. A ventilated hood was placed over the subject's head, and respiratory gases were collected using indirect calorimetry (TrueMax2400 Metabolic Measurement System; Parvomedics, Salt Lake City, UT) for 30 minutes. Subjects also completed a graded, continuous exercise test on a treadmill (9100 HR; LifeFitness, Schiller Park, IL) to assess peak oxygen consumption. The test commenced at a low work rate (eg, 8 km/h), with incremental increases in treadmill grade (eg, +2% every 2 minutes) and/or speed (eg, +1 km/h) until a peak voluntary effort was achieved. Gas exchange measurements were obtained continuously throughout the test by open-circuit spirometry (TrueMax2400 Metabolic Measurement System, Parvomedics). Heart rate was measured and recorded throughout the test by telemetry using a Polar monitor (Polar Electro, Kempele, Finland).

2.4. Experimental protocol

Subjects completed three 24-hour visits to the laboratory in a counterbalanced order with at least a week between

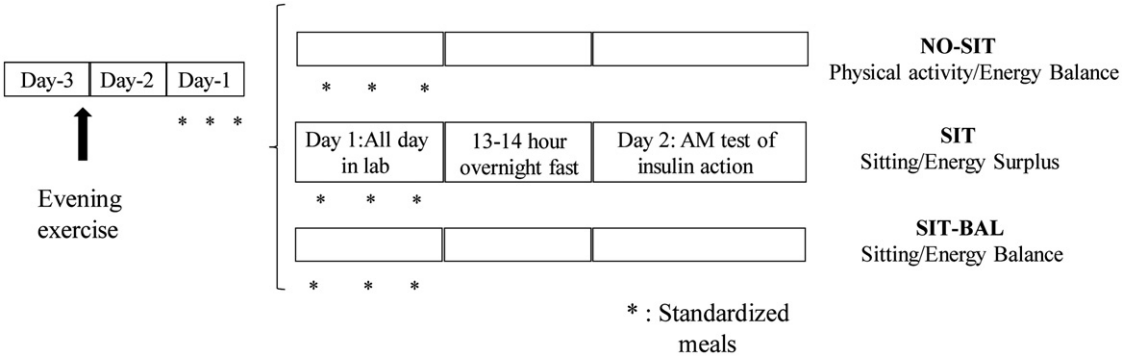

Fig. 1. Overview of study design.

visits. Three days before arrival in the laboratory (day −3), subjects were asked to perform 30 minutes of moderate exercise (eg, jogging, cycling) at approximately 8:00 PM. Subjects were instructed to perform the same exercise bout (ie, same mode, duration, and intensity) before each visit. For the 2 days before each 24-hour intervention, subjects were instructed to refrain from any structured exercise (ie, no physical activity beyond activities of daily living). Subjects were also instructed to refrain from alcohol and caffeine consumption for 24 hours before each experimental trial. All meals were provided the day before each 1-day visit (day −1) and were standardized across conditions. To estimate total daily energy expenditure and energy requirements for day −1, the REE was multiplied by an activity factor based on reported habitual activity (activity factor range, 1.5-1.7). Energy intake (mean ± SEM) on day −1 was 2314 ± 79 kcal; and macronutrient composition was 55% carbohydrate, 29% fat, and 16% protein. At 8:00 AM on day 1, subjects reported to the Energy Metabolism Laboratory for the 1-day visit. Subjects were given a standardized breakfast to eat and were shown to their designated room equipped with a chair, desk, and futon. Subjects were provided access to a computer with Internet service, books and magazines, or movies throughout the day and evening. A standardized lunch and dinner were given at approximately 12:00 PM and 5:00 PM, respectively. Subjects were allotted 25 minutes to eat each meal. After an overnight stay in the laboratory, insulin action was assessed in the morning (day 2), approximately 13 to 14 hours following the evening meal (details provided below).

Table 2
Average energy intake and expenditure across conditions[a]

	NO-SIT	SIT	SIT-BAL
Energy intake (kcal/d)	3106 ± 158*	3133 ± 168*	2109 ± 119†
Energy expenditure (kcal/d)	2944 ± 124*	2195 ± 121†	2139 ± 118†
Energy balance (kcal/d)[b]	162 ± 66*	938 ± 57†	-30 ± 23‡

Differences in outcome variables between groups were assessed by linear-mixed effects models using condition as a fixed-effect covariate and subject as a random effect. Values in a row with different superscript symbols are significantly different; $P < .05$.

[a] All values are means ± SEMs.

[b] Defined as energy intake − energy expenditure.

2.5. Posture and physical inactivity

Postural allocation (sitting or standing), walking, and other types of physical activity were quantified using an *activ*PAL professional physical activity monitor (PAL Technologies, Glasgow, Scotland). The *activ*PAL is a single-unit monitor based on a uniaxial accelerometer that is worn midline on the anterior aspect of the thigh. The monitor produces a signal related to thigh inclination and has been shown to be a valid and reliable measurement tool for determining posture and motion during activities of daily living in a healthy population [15]. The monitor also records step count and cadence with greater sensitivity than pedometers. Subjects were instructed to wear the monitor for 2 to 4 days before and during each 1-day trial. These data were used to estimate energy expenditure from 8:00 AM on day 1 to 8:00 AM on day 2 during the 3 conditions. These data were also used to determine any potential impact of prior physical activity and energy expenditure on outcome variables measured during the study.

2.6. Control of energy intake

Standardized meals consisted of commercially prepared frozen entrees and foods prepared and weighed in the Energy Metabolism Laboratory (eg, cereal, bagel, fruit, peanut butter). The energy content and composition of the evening meal (3 personal cheese pizzas and a Swiss cheese wedge) were identical both between subjects and across all conditions (1030 kcal; 43% carbohydrate, 39% fat, and 18% protein) to standardize the effect of the previous meal on insulin action. This meal was well tolerated by all subjects. The carbohydrate composition of breakfast and lunch was increased so that average daily macronutrient composition on day 1 was 54% carbohydrate, 29% fat, and 16% protein. Breakfast and lunch consisted of solid foods, juice, or noncaffeinated soft drink beverages. The energy content of breakfast and lunch on day 1 was lower in SIT-BAL compared with SIT and NO-SIT. Energy intake during NO-SIT and SIT was set exactly the same for both trials (44.8 ± 2.3 kcal/kg/d, mean ± SD), which was approximately 1000 kcal greater (14.1 ± 1.0 kcal/kg/d, mean ± SD) than the SIT-BAL trial (Table 2). For all conditions, the dietary

composition, the timing of meals, and the time interval between the evening meal and the measurement of insulin action were held constant.

2.7. Control of energy expenditure

During NO-SIT, total sitting time throughout the day was restricted; subjects stood while reading, talking on the phone, playing board games or cards, and working on the computer. Subjects walked intermittently at a low to moderate intensity (≤5 km/h, ~10,000 steps per day, Table 3) and performed standardized tasks and activities for a set amount of time at specific times throughout the day. To simulate an active day experienced by healthy young adults, the energy expenditure goal for 24 hours was 2.05 ∗ REE. However, we achieved a slightly lower energy expenditure in these subjects (1.85 ± 0.06 ∗ REE). Therefore, subjects were in a slight energy surplus of 162 ± 66 kcal (mean ± SEM, Table 2) in the NO-SIT condition. Tasks and activities were selected from a list of low-intensity (≤3.8 metabolic equivalents [METS]) household activities (eg, sweeping, dusting, folding laundry, vacuuming, dish washing) with directly measured MET values [16] and from the Physical Activity Compendium (playing darts, take out trash, put away groceries) [17]. Based on accelerometer data, the average estimated energy expenditure ranged from 1.1 to 2.7 METS on a minute by minute basis (low-intensity range). During SIT and SIT-BAL, walking and standing were restricted and subjects spent much of the waking day sitting (Table 3). Daily energy expenditure during these 2 sitting conditions was approximately the same and was significantly lower (by 24%-29%) than energy expenditure during NO-SIT (Table 2).

Energy expenditure during each 1-day trial was calculated using the *activ*PAL professional 15-second epoch data. In a similar group of subjects (n = 8; 30.2 ± 12.6 years, 72.8 ± 13.7 kg body weight, 171 ± 6.4 cm height, 24.9 ± 4.0 kg/m^2 BMI; mean ± SD), we determined the relationship between uniaxial accelerometry counts and METS during sitting and during 7 low- to moderate-intensity standing activities, including standing quietly, swaying, and changing clothes. In addition, the relationship between stepping rate and METS was determined during slow- (1.6 km/h) and moderate- (4.8 km/h) intensity walking. Separate equations derived from accelerometry counts and step rate for standing activities and walking, respectively, were applied to each epoch to calculate energy expenditure and then summed over the 24-hour trials. We estimated that average sitting energy expenditure during the trials was 1.1 METS. Sleep time was noted by the investigators and assumed to be equal to REE.

Table 3
Total sitting, standing, and stepping time, and number of steps during the three 24-hour conditions[a]

	NO-SIT	SIT	SIT-BAL
Sit time (h/d)[b]	5.8 ± 0.5*	16.9 ± 0.3†	16.8 ± 0.1†
Stand time (h/d)[b]	9.8 ± 0.1*	0.2 ± 0.0†	0.3 ± 0.0†
Step time (h/d)[b]	2.2 ± 0.1*	0.1 ± 0.0†	0.1 ± 0.0†
Total steps (steps/d)[b]	9914 ± 482*	264 ± 72†	251 ± 54†

Differences in outcome variables between groups were assessed by linear-mixed effects models using condition as a fixed-effect covariate and subject as a random effect. Values in a row with different superscript symbols are significantly different; $P < .05$.

[a] All values are means ± SEMs.

[b] Calculated from *activ*PAL output from 8:00 AM on day 1 to 8:00 AM on day 2.

2.8. Assessment of insulin action and other metabolic variables

Thirteen to 14 hours after the evening meal, insulin action was assessed using a 1-hour continuous infusion of 20% glucose that contained 2% [6,6-^{2}H]-glucose (Cambridge Laboratories, Andover, MA), as previously described [18,19]. Indwelling catheters were placed in a superficial vein of each forearm for venous blood sampling and continuous infusion of [6,6-^{2}H]-glucose. Venous blood samples were collected to determine naturally occurring levels of isotopic enrichment before the infusion. A priming bolus of 200 mg [6,6-^{2}H]-glucose was given followed by a 90-minute infusion of 2.0% [6,6-^{2}H]-glucose at a rate of 3.0 mg/min delivered by a peristaltic infusion pump (Pump 22; Harvard Apparatus, Holliston, MA). Venous blood samples were collected at 0, 75, and 90 minutes. At 90 minutes, the infusate was changed to 20% dextrose containing 2.0% [6,6-^{2}H]-glucose delivered at a rate of 8.45 mg/kg fat-free mass (FFM) per minute for an additional 60 minutes (ie, from 90-150 minutes). Blood samples were collected at 140, 145, and 150 minutes to determine glucose rate of appearance (R_a) and disappearance (R_d), as well as the mean plasma concentrations of glucose (MPG) and insulin (MPI). Insulin action was determined using the isotopically determined glucose uptake normalized to MPI concentrations during the continuous [6,6-^{2}H]-glucose infusion. This procedure for the assessment of insulin action was identical among the 3 different treatment conditions.

2.9. Blood collection and biochemical analysis

Venous blood was collected in vacutainers (Becton, Dickinson, Franklin Lakes, NJ) containing sodium fluoride to inhibit glycolysis and potassium oxalate for analysis of glucose and glucose isotopic enrichment. Vacutainers containing EDTA were used for the analysis of insulin. After collection, samples were immediately centrifuged at 3300 rpm with a maximum force of 1380*g* for 10 minutes. Plasma aliquots were stored in 2-mL cryotubes at −80° C until analysis.

Plasma glucose concentrations were determined by the glucose oxidase method using a GL5 Analox Analyzer (Analox Instruments, Lunenberg, MA). Insulin concentrations were determined using a radioimmunoassay kit specific for human insulin (Linco Research, St Charles, MO). Glucose isotopic enrichment was determined using liquid chromatography–mass spectrometry as previously described [20].

2.10. Calculations

2.10.1. Isotope-derived glucose turnover

Glucose R_a =

$$\frac{F - V[(C_1 + C_2)/2][(IE_2 - IE_1)/(t_2 - t_1)]}{[(IE_2 + IE_1)/2]}.$$

Glucose R_d =

$$R_a - V[(C_2 - C_1)/(t_2 - t_1)].$$

F represents the isotopic infusion rate. IE_1 and IE_2 are the isotopic enrichments (ratio of [6,6-^{2}H]-glucose to total plasma glucose) at time points t_1 and t_2, respectively. C_1 and C_2 are the concentrations of plasma glucose at t_1 and t_2, and V is the estimated volume of distribution for glucose of 180 mL/kg.

Whole-body insulin action was defined as glucose R_d/MPI, where MPI is the mean plasma insulin concentration during the final stages of the infusion [18,19,21].

Hepatic insulin action was defined as the percentage suppression of basal hepatic glucose production (HGP) during the glucose infusion, where greater suppression indicates greater hepatic insulin action = [1 − ($HGP_{infusion}$/$HGP_{fasting}$)·100]. $HGP_{fasting}$ is equal to the basal R_a, whereas residual HGP during the infusion ($HGP_{infusion}$) is calculated as mean glucose R_a − [6,6-^{2}H]-glucose infusion rate.

2.10.2. Fat and carbohydrate oxidation

Whole-body substrate oxidation was estimated in the fasted condition and during the final 10 minutes of the [6,6-^{2}H]-glucose infusion via indirect calorimetry. Fat and carbohydrate oxidation rates were calculated using the formula of Péronnet and Massicotte [22]:

Fat oxidation rate (in grams per minute)
= 1.6946 VO_2 − 1.7012 VCO_2

Carbohydrate oxidation rate (in grams per minute)
= 4.5850 VCO_2 − 3.2255VO_2.

2.10.3. Oxidative and nonoxidative glucose disposal

Glucose oxidative disposal was assumed to equal the carbohydrate oxidation rate during the [6,6-^{2}H]-glucose infusion. Nonoxidative glucose disposal, usually attributed to glucose storage, was calculated as glucose R_d − carbohydrate oxidation.

2.11. Statistical analysis

Differences in insulin action, glucose kinetics, substrate oxidation, and substrate and hormone variables among the 3 conditions were analyzed by means of linear mixed-effects models with planned contrast analyses using the R statistical software package, version 2.2.1 (R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2005, http://www.R-project.org). All nonnormally distributed data were log-transformed before analysis. All figures and tables include the original, nontransformed data. Statistical significance was accepted at $P < .05$. Subject characteristics are presented as mean ± SD; all other data are expressed as mean ± SEM.

3. Results

Fourteen participants commenced the study, with 12 completing all 3 trials. One man completed only 2 of 3 trials (NO-SIT and SIT) because of a minor adverse event, and one woman only completed the NO-SIT and SIT-BAL conditions because of time constraints. Therefore, the total number of observations for each of the 3 conditions for all outcome measures is as follows: NO-SIT, n = 14; SIT, n = 12; and SIT-BAL, n = 12.

3.1. Plasma glucose and insulin

Fasting plasma glucose concentrations were not significantly different among any of the 3 conditions (NO-SIT = 4.91 ± 0.08 mmol/L, SIT = 5.04 ± 0.09 mmol/L, SIT-BAL = 4.91 ± 0.08 mmol/L). Similarly, mean plasma glucose concentrations during the glucose infusion (NO-SIT = 9.3 ± 0.3 mmol/L, SIT = 9.4 ± 0.3 mmol/L, SIT-BAL = 9.2 ± 0.3 mmol/L) were similar among conditions ($P > .05$). Fasting insulin concentrations were 19% higher in SIT and approximately 10% higher in SIT-BAL compared with NO-SIT, but these differences were not statistically significant ($P > .05$) (Table 4). Compared with NO-SIT, MPI concentrations measured during the [6,6-^{2}H]-glucose infusion were 41% higher in SIT ($P < .001$) and 20% higher in SIT-BAL ($P = .08$) (Table 4). The MPI was 18% greater in SIT compared with SIT-BAL ($P = .02$) (Table 4).

3.2. Glucose turnover and hepatic insulin action

Whole-body glucose R_d is shown in Table 4. During the infusion, total glucose R_d was significantly lower in SIT compared with both NO-SIT ($P < .001$) and SIT-BAL ($P = .05$). Total glucose R_d was also lower in SIT-BAL compared with NO-SIT, although the P value of the difference was not less than 0.05 ($P = .08$). Hepatic glucose production in the fasted state ($HGP_{fasting}$) was similar among the 3 conditions (Fig. 2). In all 3 conditions, the glucose infusion suppressed HGP considerably, with residual HGP lowered to 27% to 38% of fasting values (Fig. 2). However, there were no significant differences in percent suppression of HGP among the 3 conditions ($P > .05$) (Table 4).

3.3. Whole-body insulin action

Whole-body insulin action, defined as R_d/MPI, was 39% lower in SIT ($P < .001$) and 18% lower in SIT-BAL (though not statistically significant, $P = .07$) relative to NO-SIT (Fig. 3). R_d/MPI was also lower in SIT compared with SIT-BAL ($P = .04$).

Table 4
Comparison of insulin concentrations, glucose turnover, hepatic insulin action, and substrate metabolism across the 3 conditions[a]

	NO-SIT	SIT	SIT-BAL
Insulin (pmol/L)			
Fasting	39.9 ± 3.1	47.6 ± 4.7	43.7 ± 6.4
Glucose infusion[b]	171.4 ± 24.3*	242.2 ± 32.3†	204.8 ± 37.4*
Glucose R_d (μmol·min^{-1}·kg FFM^{-1})			
Fasting	21.5 ± 1.1	21.7 ± 2.7	20.6 ± 1.5
Glucose infusion[c]	51.1 ± 2.1*	47.4 ± 2.6†	48.7 ± 3.4*†
NonOx glucose R_d (μmol·kg FFM^{-1}·min^{-1})	28.5 ± 3.0	25.6 ± 3.7	31.1 ± 4.7
Ox glucose R_d (μmol·kg FFM^{-1}·min^{-1})	22.6 ± 2.1*	21.8 ± 2.1*	17.6 ± 2.4†
% Suppression $HGP_{fasting}$	62.3 ± 6.7	69.0 ± 8.6	73.5 ± 7.3
FATox (mg/min)			
Fasting	95.6 ± 10.7	86.4 ± 10.9	98.9 ± 9.6
Glucose infusion	60.8 ± 10.1	57.8 ± 8.1	71.0 ± 9.7

Differences in outcome variables between groups were assessed by linear-mixed effects models using condition as a fixed-effect covariate and subject as a random effect. Values in a row with different superscript symbols are significantly different, $P < 0.05$.

[a] All values are means ± SEMs. Glucose R_d, glucose rate of disappearance; FFM, fat-free mass; NonOx Glucose Rd, non-oxidative glucose disposal during the continuous infusion of [6,6-^{2}H]-glucose ; Ox Glucose Rd, oxidative glucose disposal during the continuous infusion of [6,6-^{2}H]-glucose; HGP, hepatic glucose production; FATox, fat oxidation.

[b] The difference in insulin concentrations between NO-SIT and SIT-BAL during the [6,6-^{2}H]-glucose infusion was not significant ($P = 0.08$).

[c] The difference in glucose R_d between NO-SIT and SIT-BAL was not significant ($P = .08$).

To determine whether sex (male/female), use of contraceptives or menstrual cycle phase in women, or energy balance on day −1 influenced the insulin action response, we performed a separate analysis incorporating these factors (ie sex, contraceptive use and menstrual cycle phase in women, energy balance on day −1) into the linear mixed-effects model. Insulin action was significantly higher in women than in men (data not shown). However, there was no sex by condition interaction, suggesting that the response to the intervention was the same regardless of sex. Menstrual cycle phase and use of contraceptives in women did not affect model outcomes (data not shown), suggesting that variations in ovarian hormones did not significantly impact insulin action responses in women. There was also no significant effect of energy balance on day −1 on insulin action (data not shown). Therefore, differences in insulin action among the 3 conditions cannot be explained by energy balance on day −1.

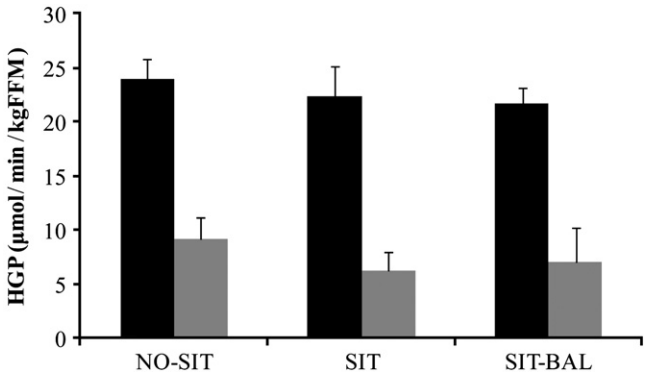

Fig. 2. Mean (± SEM) HGP before (fasting = black bars) and during the [6,6-^{2}H]-glucose infusion (gray bars) across the 3 conditions. A linear mixed-effects model including condition and time (fasting, infusion) as fixed-effect covariates and their interaction was used to determine the effect of intervention on HGP. There was a significant effect of time, where HGP was significantly lower during the [6,6-^{2}H]-glucose infusion compared with fasting HGP in all conditions ($P < .05$). However, there was no significant effect of condition; nor was there a significant condition × time interaction ($P > .05$).

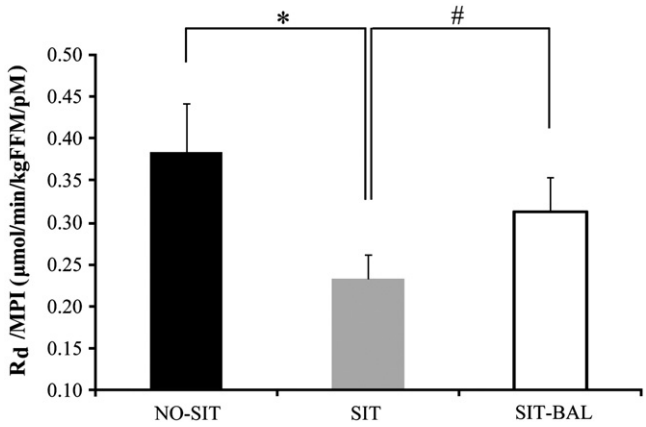

Fig. 3. Whole-body insulin action (R_d/MPI) assessed during the continuous infusion of glucose across the 3 conditions. Values are mean ± SEM. MPI, mean of 140-, 145-, and 150-minute plasma insulin concentrations. Differences in insulin action between groups were assessed by linear-mixed effects models using condition as a fixed-effect covariate and subject as a random effect. Insulin action was 39% lower in SIT compared with NO-SIT (*$P < .001$). Insulin action was 26% lower in SIT compared with SIT-BAL ($^{\#}P = .04$). Insulin action was 18% lower in SIT-BAL compared with NO-SIT ($P = .07$).

The energy content and composition of the evening meal on day 1 were the same for each subject across all 3 conditions (1030 kcal). Therefore, the percent contribution of the evening meal to total daily energy intake (% contribution) varied among subjects (ie, higher % contribution in subjects with low total daily energy intake and vice versa) and among conditions (ie, higher % contribution in SIT-BAL compared with NO-SIT and SIT). To assess whether % contribution of the evening meal had a significant impact on insulin action, we included % contribution of the evening meal into the linear mixed-effects model. There was no significant effect of % contribution of the evening meal on R_d/MPI, nor did its inclusion in the model affect model outcomes (data not shown).

3.4. Substrate metabolism

Oxidative glucose disposal (in micromoles per kilogram FFM per minute) in SIT-BAL was 22% lower compared with NO-SIT ($P < .05$) and 19% lower compared with SIT ($P < .05$) (Table 4). Although nonoxidative glucose disposal during SIT-

BAL was numerically greater compared with both NO-SIT and SIT, these differences were not statistically different ($P = .32$ and $P = .10$, respectively; Table 4). There were no differences in fasting or insulin-suppressed fat oxidation (FATox; in milligrams per minute) among conditions, although FATox during the [6,6-^{2}H]-glucose infusion was slightly greater in SIT-BAL compared with SIT ($P = .08$) (Table 4).

4. Discussion

We determined the metabolic response to 1 day of prolonged sitting, with and without a change in dietary macronutrient intake. The main findings of this study were as follows: (1) compared with the minimal sitting condition (NO-SIT), 1 day of sitting, with energy surplus (SIT), significantly reduced whole-body insulin action; and (2) reducing energy intake by approximately 1000 kcal/d to approximately match low energy expenditure during prolonged sitting (sit, energy balance = SIT-BAL) significantly attenuated, but did not completely prevent, the decline in insulin action. Thus, there are factors other than energy surplus that are also involved in the detrimental impact of sitting on insulin action.

It is well documented that exercise cessation in both humans and rodents leads to a rapid decline in insulin action [23-25]. In the current study, however, we used a distinctly different model (prolonged sitting) to examine the specific metabolic effects of minimizing low-intensity muscle activity, as distinct from exercise deficiency [26,27]. This inactivity paradigm has relevance to many real-world situations because prolonged sitting is a common behavior while at work or traveling etc. Several studies in animals and humans have reported potentially harmful consequences of reduced standing and nonexercise ambulation on metabolic processes including insulin action [5-7,9,11,12,27-30]. How quickly these changes occur has not been systematically investigated. Studies in rodents show significant declines in insulin-stimulated glucose uptake in as little as 24 to 48 hours following hind limb immobilization [5,6]. In humans, the earliest detectable reduction in insulin action was observed following 3 days of complete bed rest [7,10], although changes to insulin action in response to shorter interventions have not been evaluated. The present study is the first to document considerable reductions in whole-body insulin action following just 1 day of sitting in humans. Although we used a different method of evaluation compared with other studies, the magnitude of the reduced insulin action within this 1-day model is similar to changes (~17%-30%) reported after longer periods (ie, days-weeks) of bed rest [7,9,10] or large reductions in ambulation [12]. Therefore, reduced insulin action appears to be an early metabolic response to prolonged sitting in humans that may be sustained by several weeks of continued low muscle activity.

Results of the present study emphasize the importance of a previously unexplored factor (ie, energy surplus) on the metabolic response to sitting. Holding macronutrient composition constant, reducing energy intake reduced approximately one half of the harmful effects of sitting on insulin action. These results suggest that energy surplus plays a key role in the acute metabolic response to sitting. Focusing exclusively on individuals who perform no exercise during the day, those who spend more of their daily time sitting (+2.5 h/d) have more body fat and a propensity to gain weight compared with their sedentary counterparts who sit less [27,31]. Furthermore, data from Stubbs et al [32] suggest that there is no compensatory decline in ad libitum food intake in response to large reductions in energy expenditure. Thus, it is reasonable to conclude that energy surplus and inactivity may often coexist. The lack of significant change in fasting glucose R_a, hepatic insulin action, or fatty acid oxidation in contrast to much larger changes in whole-body insulin action suggests that most of the effects of inactivity, with or without energy surplus, are mediated in skeletal muscle. Data from other studies using bed rest as a model of inactivity support the notion that the metabolic effects of inactivity are manifested predominantly in skeletal muscle. For instance, in men, 7 days of bed rest had no impact on hepatic insulin action despite significant reductions in peripheral and whole-body insulin sensitivity [9,11,28]. In a recent study by Krogh-Madsen [12], both glucose clearance and insulin signaling were reduced during a hyperinsulinemic-euglycemic clamp following 2 weeks of reduced ambulation (from ~10 000 to ~1300 steps per day), but HGP was unchanged. Taken together, these data suggest that reduced skeletal muscle insulin sensitivity is the primary factor underlying lower whole-body insulin action following inactivity. However, because insulin concentrations during the [6,6-^{2}H]-glucose infusion were elevated during SIT and SIT-BAL, we cannot rule out the possibility that effects on insulin secretion, for example via changes in β-cell function, also affect insulin action in response to 1 day of prolonged sitting.

Potential mechanisms to explain the effects of sitting on insulin action could be related to energy surplus, to specific actions of sitting, or to both. Overabundance of fatty acids, glucose, and/or amino acids inhibits key components of the insulin signaling pathway as reviewed in detail by Patti [33] and Krebs and Roden [34]. Although not examined in the current study, the greater reduction in insulin action in SIT compared with SIT-BAL may be related to nutrient-induced impairments to insulin signaling. Reducing energy intake during prolonged sitting, designed to separate the effects of inactivity from energy surplus, did not completely eliminate the impairment to insulin action. Thus, just as the metabolic effects of exercise cannot be fully ascribed to the energy deficit caused by expending hundreds of kilocalories [18,35], in the present study, the detrimental effects of inactivity are not solely attributable to energy surplus. Other factors specific to low muscle activity must play a role in the metabolic response to prolonged sitting. Although still unclear, potential factors may involve (1) greater circulating

levels of counterregulatory hormones (eg, glucagon, epinephrine, cortisol) [36] or (2) hemodynamic changes, including decreased insulin-mediated muscle blood flow [9] and capillary recruitment. In sum, complex and redundant mechanisms are likely responsible for reduced insulin action in response to prolonged sitting. With no compensatory change to diet, mechanisms related to nutrient oversupply are strong candidates to explain part of the reduced sensitivity to insulin. When energy intake is reduced to match low expenditure, mechanisms specific to low muscle activity, which are not yet clear, must be playing a key role.

4.1. Effect of periodic changes to energy balance and activity on insulin action

Throughout the typical course of a day, humans are alternately exposed to periods of calorie surplus (ie, after meals) and deficit (ie, after an overnight fast) and periods of sedentary and active behavior that interact to modulate intermediary metabolism. It is plausible that the magnitude and duration of these events and the time interval between them shape the overall metabolic responses. Data from the current study suggest that the net sum of these "exposures" (at least in terms of energy balance) over 24 hours contribute to the overall metabolic effects observed. For example, we standardized the energy content of the evening meal (ie, 1030 kcal) for all 3 conditions, which could have "washed out" any differences among conditions if only that last meal was important. However, there were clear differences in insulin action between the 2 sitting conditions, implying that the metabolic effect of an energy surplus at breakfast and lunch was sustained. Each "bout" of sitting or calorically dense meal may elicit harmful effects that may accumulate with repeated "exposures" unless appropriate compensations are made to reverse or attenuate these effects.

4.2. Limitations and control for confounding variables

The use of young, healthy, nonobese subjects may limit the generalizability of the study results to other populations. We chose to study the metabolic response to sitting in recreationally active, but not highly trained, individuals to eliminate a potential confounding impact of detraining on these responses. To further minimize the effects of a "day off" from training, subjects were restricted from exercise for 48 hours before each experimental condition and performed no more than 30 minutes of exercise 3 days before testing. This population was also selected to minimize potential confounding effects of disease processes often observed in overweight individuals who have low levels of physical activity. Future studies are required to determine if factors such as aging, obesity, or chronically low levels of activity impact the response to inactivity.

Given the large differences in energy intake and similar sitting and standing time between SIT and SIT-BAL, we are confident that, as designed, these 2 groups were in different energy states. Estimated energy balance was slightly greater in NO-SIT vs SIT-BAL (~200 kcal), which may have underestimated the impact of sitting on insulin action. Thus, the true difference in insulin action between these 2 conditions may be even larger than what we observed.

In summary, results of this study show that a single day of prolonged sitting can dramatically reduce insulin action in healthy young adults. The detrimental effects of prolonged sitting on insulin action were attenuated when subjects were in energy balance. Still, approximately half of the decline in insulin action was not attributable to energy surplus, suggesting that other factors besides energy surplus are involved in the process. Additional research is necessary to determine the cellular and molecular mechanisms mediating the direct effect of sitting on metabolism. Future studies should carefully control energy balance or consider the results in light of acute energy balance.

Results from our study provide evidence for a strong cause-and-effect relationship between time spent sitting and impaired metabolic health as proposed in the tenets describing the inactivity physiology paradigm [27]. The dramatic reduction in insulin action within just 1 day of prolonged sitting suggests the importance of maintaining at least daily low-intensity activity to minimize the harmful effects of physical inactivity on metabolic health. Limiting sitting may be especially important for people who do not perform regular structured exercise. In this regard, it may be prudent to develop public health strategies aimed at limiting sitting and increasing daily low-intensity activity to improve metabolic health [26].

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