Published in final edited form as:

Med Sci Sports Exerc. 2019 May ; 51(5): 995–1005. doi:10.1249/MSS.0000000000001892.

Metabolic Implications of Diet and Energy Intake during Physical Inactivity

Nathan C. Winn¹, Ryan Pettit-Mee¹, Lauren K. Walsh¹, Robert M. Restaino², Sean T. Ready¹, Jaime Padilla^{1,3,4}, and Jill A. Kanaley¹

¹Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO

²Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, MO

³Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO

⁴Department of Child Health, University of Missouri, Columbia, MO

Abstract

Purpose.—Physical inactivity is associated with disruptions in glucose metabolism and energy balance, whereas energy restriction may blunt these adverse manifestations. During hypocaloric feeding, higher-protein intake maintains lean mass which is an important component of metabolic health. This study determined whether mild energy restriction preserves glycemic control during physical inactivity and whether this preservation is more effectively achieved with a higher-protein diet.

Methods.—Ten adults (24±1 year) consumed a control (64% carbohydrate, 20% fat, 16% protein) and higher-protein diet (50% carbohydrate, 20% fat, 30% protein) during two ten-day inactivity periods (>10,000→~5,000 steps/day) in a randomized cross-over design. Energy intake was decreased by ~400 kcal/d to account for reduced energy expenditure associated with inactivity. A subset of subjects (n=5) completed ten days of inactivity while consuming 35% excess of their basal energy requirements, which served as a positive control condition (overfeeding+inactivity).

Results.—Daily steps were decreased from 12,154±308 to 4,275±269 steps/day ($P<0.05$) which was accompanied by reduced VO_2max (-1.8 ± 0.7 ml/kg/min, $P<0.05$), independent of diet conditions. No disruptions in fasting or postprandial glucose, insulin, and nonesterified fatty acids in response to 75-g of oral glucose were observed following inactivity for both diet conditions ($P>0.05$). Overfeeding+inactivity increased body weight, body fat, HOMA-IR, and 2-hour postprandial glucose and insulin concentrations ($P<0.05$), despite no changes in lipid concentrations.

Corresponding Author: Name: Jill A. Kanaley, PhD, Address: 204 Gwynn Hall, Columbia, MO 65211, Phone: 573.882.2519, Fax #: 573.884.4885, kanaleyj@missouri.edu.

Author contributions:

NCW, RPM, LKW, RMR, SR, JP. Data collection, data analysis, edited manuscript.

NCW, JAK, and JP. Study conception, study design, data interpretation, and edited manuscript.

NCW, drafted manuscript. All authors approved the final version of the manuscript.

This study was registered as a clinical trial (NCT03013764, clinicaltrials.gov).

Conclusions.—We show that independent of diet (normal vs. higher-protein), mild energy restriction preserves metabolic function during short-term inactivity in healthy subjects. That is, metabolic deterioration with inactivity only manifests in the setting of energy surplus.

Keywords

Insulin resistance; energy homeostasis; vascular function; high-protein diet

INTRODUCTION

Physical inactivity is a contributor to multiple pathological conditions [reviewed in (1)] and has recently been highlighted as a leading cause of death in Western societies (2). Among the chronic diseases most affected by physical inactivity are cardiovascular and metabolic diseases. Indeed, prior evidence indicates that physical inactivity contributes to multiple features of metabolic dysfunction including large postprandial glucose excursions and hyperinsulinemia, which have been directly linked to alterations in lipid metabolism, increased oxidative stress, and long-term micro- and macrovascular complications (1).

Understanding the role of physical inactivity as an instigator of metabolic and cardiovascular diseases has been an area of increasing interest in recent years. In this regard, the classic ‘bed rest’ model is utilized for investigating the cardiometabolic impact and mechanisms associated with extreme physical inactivity (3–8). However, bed rest does not mimic the typical ‘free-living’ environment characterized by prolonged periods of inactive behavior (i.e., excessive sitting) with interspersed episodes of low-to-moderate physical activity. Although experimental studies adopting a prolonged sitting approach (i.e., sitting recumbent >5 hours with no movement) (9–11) may have clinical translation, a more representative model emulating physical inactivity is reducing ambulatory activity via step reduction (12, 13). In this model, daily steps are typically reduced from >10,000 to 1,500–5,000 steps per day for several days to weeks. Indeed, 7–14 days of decreased ambulatory activity reduces insulin sensitivity and enhances visceral adiposity in non-obese healthy adult men (14, 15), which is likely related to positive energy balance. Importantly, increased visceral or central adiposity is tightly linked to insulin resistance and increased risk for cardiovascular disease (16). While decreased ambulatory activity provokes insulin resistance and may augment central adiposity, it remains unknown whether a dietary intervention can minimize metabolic dysfunction caused by physical inactivity. Notably, decreased ambulation does not produce compensatory reductions in energy intake (17, 18), which if left unchanged contributes to positive energy balance and increased insulin resistance (13). Thus, reducing energy intake during physical inactivity may attenuate metabolic deterioration associated with decreased ambulation.

Some research suggests that physical inactivity-induced insulin resistance may be related to decreases in fat free mass (13), with skeletal muscle being the principal site of glucose disposal. In this regard, it has been shown that during energy restriction, preventing loss of lean mass via high protein intake exerts greater systemic insulin sensitizing effects compared to an energy-matched high carbohydrate diet (19). Thus, it is possible that consumption of a higher-protein diet and associated maintenance of fat free mass during physical inactivity

would alleviate metabolic dysfunction caused by reduced ambulation. Furthermore, short-term administration of high protein breakfast has been reported to attenuate glycemic excursions throughout the day in adolescents (20) and adults (21) compared to normal protein breakfast. Since physical inactivity is associated with worsened glycemic control, consumption of a higher protein diet may assist in maintaining euglycemia during decreased ambulation.

In this context, we hypothesized that reduction of energy intake to counteract physical inactivity-induced weight gain preserves glycemic control in young healthy subjects and that this preservation is more effectively attained using a higher-protein diet by mitigation of lean mass loss. In addition, since physical inactivity is typically accompanied by overconsumption of energy, we also reasoned that glycemic dysregulation particularly manifests when inactivity is coupled with overfeeding.

MATERIALS AND METHODS

Protocol 1

Participants and study design—This study was approved by the University of Missouri Institutional Review Board and all data collection was conducted on-site at the University of Missouri-Columbia. Written informed consent was obtained from all participants, who were then screened to determine if they qualified. The following inclusion criteria were implemented: 1) males and females between 18 to 45 years of age, 2) body mass index (BMI) $<28 \text{ kg/m}^2$, 3) no known cardiovascular, kidney, or liver disease, 4) no history of surgery for weight loss and weight stable for prior 3 months (weight change $< 3 \text{ kg}$), and 5) physically active individuals (90 minutes of primarily whole body aerobic physical activity >3 days per week and taking greater than 10,000 steps per day) assessed via accelerometers. Accordingly, ten physically active male and female (males, $n=6$; females, $n=4$; mean age, 24 ± 1 year; 100% Caucasian) completed an unblinded randomized cross-over study involving a control (64% carbohydrate, 20% fat, 16% protein) and higher-protein diet (50% carbohydrate, 20% fat, 30% protein) during ten days of reduced ambulatory activity (Figure 1). Accelerometers were worn during the active phase and during the entire inactivity period to track physical activity levels throughout conditions. Diet interventions were randomized prior to the study using a random number generator (www.randomizer.org). Female subjects initiated the study interventions (i.e., Day 0) between days 1–10 of their menstrual cycle.

Physical activity levels and energy expenditure—Subjects were fitted with an accelerometer (ActiGraph GTX3, ActiGraph, Pensacola, FL) on the nondominant hip and a pedometer (Walk4Life™ Elite 3D) on the contralateral hip to document physical activity levels and daily steps, respectively. Baseline activity levels were collected over 3–5 days including at least one weekend day. Participants were instructed to wear accelerometers and pedometers upon waking until just prior to sleep (i.e., ~15–18 hours of wear time per day), with the goal to accumulate no more than 5,000 steps per day during inactivity. The pedometer provided unblinded feedback to subjects regarding their daily step counts to increase adherence to inactivity, whereas both step counts and physical activity energy expenditure were determined via accelerometers (ActiGraph software v 6.13.3, Pensacola,

FL), in a blinded fashion. Estimated energy expenditure using ActiGraph GTX3 has been shown to correlate (Partial Spearman $r=0.5$, $P<0.05$) with energy expenditure assessed via doubly-labelled water (22). Resting metabolic rate (RMR) and non-protein respiratory quotient (RQ) were measured at baseline and post-physical inactivity via indirect calorimetry by using a ventilated hood and an open-circuit system (TrueOne 2400 Metabolic Measurement Cart; ParvoMedics, Sandy, UT). Subjects were instructed to refrain from vigorous physical activity 24 hours prior to RMR testing. Briefly, RMR was performed following an overnight fast (10–12 hours), with subjects fully recumbent during analysis. Respiratory gasses were collected over a 30-minute period, with the last 15 minutes used in data analysis. Total daily energy expenditure was computed as the sum of RMR + activity measured energy expenditure. Accelerometers were also used to provide estimates of time spent in sedentary, light, moderate, and vigorous activities, which were computed over the period of accelerometer wear time per day for each subject using the following Freedson Adult (1998) cut points (23) (i.e., counts per minute (CPM)): sedentary, 0–99 CPM; light, 100–1951 CPM; moderate, 1952 – 5724 CPM; and vigorous >5724 CPM. All subjects wore accelerometers for a minimum 12 hours per day.

Meals and dietary composition—Subjects were fed a control diet (16% kcal from protein, 64% kcal from CHO, 20% kcal from fat) and a higher-protein diet (30% kcal from protein, 50% kcal from CHO, and 20% kcal from fat) in a randomized fashion for 10 days during physical inactivity (Figure 1). Fat percentage of total energy intake was held constant between diets so that the putative effects of protein and carbohydrate manipulation were more easily isolated. In addition, these diets were selected since they are within the Institute of Medicine guidelines for macronutrient compositions for adults (24). Diets were prepared by the University of Missouri Nutritional Center for Health (MUNCH). Subjects met with a member of the research staff every 2–3 days to pick up meals for the subsequent 2–3 days. Meals were packaged in vacuum-sealed containers and given to subjects in rolling coolers. Subjects were given instructions on meal preparation and a checklist to ensure all components of each respective meal were consumed together. Subjects returned all containers to the research staff. Diets were administered as 3 meals per day (e.g., breakfast, lunch, and dinner) and 1–5 snacks were supplemented based on daily energy requirements that were estimated from RMR. Pilot experiments (i.e., $n=3$ recreationally active subjects) revealed that physical activity-associated energy expenditure accounted for ~25% of total daily energy expenditure. Thus, to account for some light activities of daily living, participants were fed ~80% of their total baseline energy expenditure during reduced physical activity. In other words, energy intake was decreased by 15–20% of total active phase energy expenditure which translated on average to an energy restriction of ~400 kcal/d. Dietary characteristics are summarized in Supplementary Table 1 (see Table, Supplemental Digital Content 1, Dietary characteristics of breakfast, lunch, dinner, and snacks).

Aerobic capacity—Maximal oxygen consumption (VO_{2max}) was assessed through indirect calorimetry during the active and inactive phases (Figure 1), respectively, using a continuous treadmill protocol as previously described (25, 26). On each testing day, exercise tests were performed following metabolic and vascular assessments (Figure 1). Briefly, the

treadmill protocol consisted of 2-minute stages, with an initial speed of 3.5 mph, and grade at 0%. Treadmill speed increased to 6.5–9.0 mph after which the grade increased 2.5% every 2 minutes thereafter until volitional fatigue. During the last minute of every stage heart rate and rating of perceived exertion were obtained and recorded. If subjects met three of the four following criteria then they were considered to achieve $\text{VO}_{2\text{max}}$: plateau in VO_2 despite increased workload (15 second average), within 10 beats of age-predicted maximal heart rate, rating of perceived exertion >17 (i.e., Borg Scale), and respiratory exchange ratio >1.15 (27).

Body composition—Fat free mass, fat mass, and percent body fat (BF%) were assessed by dual energy X-ray absorptiometry (Hologic QDR 4500A, Waltham, MA), per manufacturers guidelines. Each scan was performed following a standardized three-hour glucose tolerance test (described below) at approximately 11:00 h. Subjects were supine for each scan and positioned according to anatomical maps provided by the manufacturer. These maps were used to ensure correct positioning upon subsequent scans to enhance reproducibility. All jewelry/metal items were removed prior to scans and participants wore light clothing or were provided gowns during scans. The intra-machine coefficient of variation (CV) for fat mass and lean mass are 0.7% and 1.9%, respectively. Dual energy X-ray absorptiometry was also used to estimate abdominal fat mass from a selected region of interest that spanned from L2 to the iliac crest, whereas hip adiposity was measured by the scanner from a region of interest that comprised the midpoint of the ilium to the femoral neck, as previously described (28). These measurements are reported in kilograms. Assessment of abdominal fat via dual-energy x-ray absorptiometry shows strong agreement ($r=0.9$, $P<0.05$) with computed tomography (29), with a CV for intrasubject reproducibility $<3\%$ (30).

Oral glucose tolerance test—To determine systemic metabolic function, an oral glucose tolerance test (OGTT) was performed before and following 10 days of physical inactivity (Figure 1) to measure postprandial responses of glucose, insulin, lactate, c-peptide, and nonesterified fatty acids (NEFA). Following an overnight fast (~ 10 h), a venous catheter was inserted into the antecubital vein of the forearm of the participant. Baseline blood samples (~ 5 ml) were collected for analysis of fasting insulin, c-peptide, glucose, NEFA, triglycerides, and cholesterol concentrations. After baseline blood draws, a 75-g oral glucose load (Thermo Scientific, Inc.) was ingested within 5 min and blood samples (~ 6 ml) were collected at 10, 20, 30, 60, 90, 120, 150, and 180 minutes after ingestion.

Blood measures and analysis—Blood glucose and lactate concentrations were determined in whole blood using the YSI 2700 SELECT analyzer (Yellow Springs, OH, USA). Plasma insulin and c-peptide concentrations (Cat. #HMHEMAG-34K) were determined using a MILLIPLEX magnetic bead-based quantitative multiplex immunoassay with the MAGPIX instrumentation (Millipore, Darmstadt, Germany), and plasma NEFA concentrations were measured by colorimetric assay (Wako Life Sciences, Inc.). Quantification of plasma cholesterol, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc) and triglycerides were performed by a commercial laboratory (Comparative Clinical Pathology Services, Columbia, MO) on an Olympus

AU680 automated chemistry analyzer (Beckman-Coulter, Brea, CA). Plasma oxidized LDL concentrations were measured using a commercial ELISA kit according to the manufacturer (Oxiselect™ Human Oxidized LDL ELISA, STA-358, Lot# 17022204, Cell Biolabs, Inc.). All measurements were conducted in either duplicates or triplicates, and the coefficient of variation (CV) for each plasma analyte were as follows: intra-assay CV: C-peptide, 2.4%; insulin, 2.9%; NEFA, 0.75%; cholesterol, 0.48%; HDLc, 1.0%; LDLc, 1.4%; triglycerides, 1.3% and interassay CV: C-peptide, 4.1%; insulin, 6.2%; NEFA, 4.9%; cholesterol, 1.3%; HDLc, 0.8%; LDLc, 2.8%; and triglycerides, 1.2%.

Calculations—Glucose, insulin, c-peptide, and NEFAs concentrations were calculated as area under the curve (AUC) during the OGTT (t=0 to 3 h). The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a surrogate measure of hepatic insulin resistance and an index of adipose tissue IR (Adipo-IR) was calculated using the following formula: Adipo-IR=fasting NEFA (mmol/l)*fasting insulin (μU/ml). Basal hepatic insulin extraction was calculated as previously described (31).

Continuous glucose monitoring—Medtronic iPro2® (MedTronic MiniMed, Inc.) continuous glucose monitor (CGM) was inserted in the periumbilical region of the abdomen of each subject and was used to measure interstitial glucose concentrations, as previously described (32). Recent data suggests good agreement between left and right abdominal sensor insertion sites (33). Briefly, during each trial, the CGM was inserted in the same area of the abdomen. The CGM was calibrated according to manufacturer specifications (<http://www.medtronicdiabetes.com/download-library/ipro-2>). Approximately 1 hour after inserting the continuous glucose monitor subjects sampled and recorded a capillary blood glucose measurement using a hand-held glucometer (OneTouch® Ultra®2, LifeScan Inc.). Each subject recorded 4–6 capillary glucose readings per day during glucose monitoring (Figure 1). The CGM measures interstitial glucose concentrations every 5 minutes continuously. The 24-hour average glucose responses were plotted and analyzed for minimum, maximum, 24-hour daily average glucose concentrations, and glucose AUC (e.g. 24-hour AUC, daytime AUC, and nighttime AUC) using the trapezoidal rule. Due to CGM recording failure, complete glycemic data was not available for 3 subjects during the Control diet condition and for 1 subject during the Higher-protein condition.

Brachial artery flow-mediated dilation—For the assessment of endothelial function, brachial artery flow-mediated dilation (FMD) was performed via Doppler ultrasound (GE Logic P5), as previously described (34). The brachial artery was chosen because the purpose was to concentrate on the vascular consequences of systemic metabolic derangements caused by decreased ambulatory activity. That is, by studying the vasculature of the upper limbs, we minimized the negative influence of reduced blood flow-induced shear stress primarily occurring in the lower extremities (i.e., the limbs subjected to reduced activity). Briefly, after 10 minutes of quiet supine rest, two minutes of baseline brachial artery diameter and velocity were recorded using an 11 MHz linear array transducer. A forearm cuff was then inflated to a pressure of 220 mmHg for 5 minutes. Continuous diameter and blood velocity measures were recorded for 30 seconds before and 3 minutes following cuff deflation. FMD percent change was calculated using the following equation: %FMD = (peak

diameter-base diameter) / (base diameter)*100. Shear rate, an estimate of shear stress without blood viscosity, was calculated as 4*mean blood velocity/diameter. Shear rate AUC above baseline up to peak diameter was calculated as a stimulus for FMD.

24-hour ambulatory blood pressure—Each subject was fitted with a portable automated blood pressure cuff (Mobil-O-Graph NG, I.E.M., Stolberg, Germany) on the upper arm for 24 hours during their baseline condition (Active) and the 9th day of physical inactivity (Figure 1). Systolic and diastolic blood pressure and heart rate readings were automatically collected every 30 minutes for 24 hours. Data were extracted and generated using Mobil-O-Graph software from the manufacturer (HMS version 4.8). Data are presented as average daily (00:00 h to 23:30 h) values to the nearest whole number.

Sample size—Sample size requirements for this study were based on a Type I error rate of 0.05, two-tailed testing, and a minimal power level of 0.80. Using G*Power (v3.1.9.2, Dusseldorf, Germany), n=10 subjects (paired design) yielded 83% power to detect a 30% change in postprandial insulin AUC with an effect size of 1.0. Cohen's d was based on a previous study examining changes in postprandial insulin excursions following decreased ambulatory activity in young healthy subjects (12).

Protocol 2

To mimic the typical Western lifestyle, a subset of male subjects (n=5) whom completed Protocol 1 underwent 10 days of physical inactivity while being overfed by 35–40% of total daily energy requirement for weight maintenance. Due to the high subject burden associated with this study, only 5 subjects whom completed Protocol 1 agreed to complete Protocol 2. This overfeeding arm served as a positive control condition. Baseline measurements were collected while subjects were active and post-inactivity measurements were collected after 10 days of inactivity. Overfeeding began the first day of physical inactivity and was continued throughout the 10-day inactivity period. The extra calories were primarily comprised of dessert-like foods that were provided by research staff. An extra ~880 kcal/d were provided to participants normal self-elected diet in the form of evening snacks (50% carbohydrate, 43% fat, and 7% protein). This behavior is typical of the Western holiday seasons, which are characterized by periods of physical inactivity and overconsumption of calories creating a net positive energy balance (35). Subjects underwent baseline (active) and post-physically inactive assessments including body composition, fasting and postprandial (e.g., OGTT) biochemistry (e.g., glucose, insulin, NEFA, cholesterol, LDLc, HDLc, triglycerides, and oxidized LDL concentrations), HOMA-IR and brachial artery FMD. The methodological descriptions of these assessments are identical to those performed for Protocol 1.

Statistical analysis—A 2×2 repeated measures analysis of variance (ANOVA) with physical activity (active vs inactive) and diet (control vs high-protein diet) as factors was used to analyze all variables for Protocol 1 unless otherwise stated. Post hoc tests with Tukey correction were used for pairwise comparisons. To control for potential order effects, subjects were randomized to diet treatment sequence (i.e., 4 participants consumed control diet as first treatment and 6 subjects consumed higher protein diet as first treatment). To test

whether treatment order influenced outcome variables ($n=20$ variables), a mixed factorial ANOVA with time (active and inactive) and diet (control diet and higher-protein diet) as repeated measures and order (sequence in which diet was administered) as an independent factor was conducted. Paired samples t tests were run to compare Active and Overfeeding + inactivity for variables associated with Protocol 2. Since fat mass was increased with overfeeding + inactivity, it was covaried using analysis of covariance (ANCOVA) for the following metabolic outcome variables: glucose AUC, insulin AUC, fasting glucose, and fasting insulin concentrations. Statistical analyses were performed using SPSS statistical software, version 20.0 (IBM, Inc.). Significance was accepted if $P<0.05$. Data are presented as means $\pm$ SEM.

RESULTS

Physical activity and energy expenditure

At baseline, subjects were physically active defined as accumulating $>10,000$ steps per day with an average physical activity level (i.e., total energy expenditure/resting energy expenditure) of 1.3 (Figure 1B; see Table, Supplemental Digital Content 2, **Average time spent in physical activity**). Ambulatory physical activity and activity-associated energy expenditure were decreased by 64% and ~62% during the 10-day inactivity phase, which did not differ between diet conditions (Figure 1B-C). Physical inactivity adherence rates (i.e., the percentage of participants that achieved $<5,000$ steps/day) were 90% and 80% during control diet and higher-protein diet conditions, respectively. Paralleling step reduction, a 24% increase in sedentary time was observed during physical inactivity under both diet conditions ($P<0.05$; see Table, Supplemental Digital Content 2, **Average time spent in physical activity**), whereas time spent in moderate-to-vigorous activity was nearly eliminated ($P<0.05$; see Table, Supplemental Digital Content 2, **Average time spent in physical activity**). Furthermore, VO_2max was decreased by 3% and 5% following control- and higher-protein-diet feeding, respectively ($P<0.05$, Table 1).

Body weight and adiposity

By design, energy intake was decreased by 15–20% of total energy expenditure (~400 kcal/d) to account for the reduction in energy expenditure from physical inactivity (Figure 1). Body weight was significantly reduced by 1% after physical inactivity during both diet conditions ($P<0.05$, Table 1). Fat mass and BF% were not altered by physical inactivity; although, there was a tendency for a reduction in fat free mass after physical inactivity (main effect of inactivity, $P=0.06$, Table 1). Despite no reduction in overall fat mass or hip fat mass, abdominal fat mass was decreased by 5% and 7% respectively, following physical inactivity during diet conditions ($P<0.05$, Table 1). Of note, the absolute reduction in abdominal fat mass was less than 0.075 kilograms. Fat free mass showed a 3-way interaction for diet, time, and testing order, where subjects starting with the control diet ($n=4$) had greater reductions in fat free mass in response to inactivity+higher-protein diet; whereas, subjects that started with the higher-protein diet ($n=6$) had greater reductions in fat free mass in response to inactivity+control diet. These responses reveal that subjects who started with the control diet responded to treatments differently than the subjects that started with higher-protein diet.

Fasting blood chemistries

At baseline (Active), there were no differences in blood glucose or plasma insulin concentrations between conditions (Table 2). Neither fasting glucose nor insulin concentrations were elevated following inactivity ($P<0.05$, Table 2) regardless of diet conditions. However, when testing order (i.e., sequence of diet treatments) was considered, a significant 3-way interaction for fasting insulin was observed such that the subjects starting with the control diet condition ($n=4$) had increased insulin concentrations in response to inactivity+control diet while the subjects starting with the high protein diet ($n=6$) had decreased insulin concentrations in response to inactivity+control diet. Total cholesterol and HDLc concentrations were decreased following inactivity ($P<0.05$), despite no changes in LDLc or oxidized LDL concentrations (Table 2). Cholesterol and lipoprotein cholesterol concentrations did not differ between dietary conditions. A diet x activity interaction ($P=0.01$) was found for fasting triglycerides concentrations such that the higher-protein diet caused a $15\pm 9\%$ reduction ($P=0.09$), whereas the control diet increased triglyceride concentrations by $38\pm 18\%$ ($P=0.12$) during the inactivity phase (Table 2).

Oral glucose tolerance and markers of insulin sensitivity/resistance

In response to an oral glucose load, 10 days of physical inactivity had no effect on postprandial glucose, insulin, or NEFA concentrations regardless of diet condition, including the clinical markers, two-hour glucose and two-hour insulin concentrations (Figure 2A-E). Moreover, neither physical inactivity nor diet influenced surrogate markers of insulin sensitivity or insulin resistance (Figure 2 F&G).

Free-living glycemic control

Due to technical difficulties with the CGM, data from $n=3$ and $n=1$ in the Control diet and Higher-protein diet, respectively, were not available. Accordingly, within diet comparisons (i.e., Active vs Inactive) were conducted via paired samples t tests. Average 24-hour interstitial glucose concentrations were decreased following the 10-day reduction in ambulatory activity in the Control diet (see Figure, Supplemental Digital Content 3, **Effect of physical inactivity on free-living glycemic control in response to a Control diet and Higher-protein diet; $P<0.05$**). Similarly, CGM-measured 24-hour glucose AUC and daytime (0700 h to 2200 h) AUC were decreased with inactivity during the Control diet condition (see Figure, Supplemental Digital Content 3, **Effect of physical inactivity on free-living glycemic control in response to a Control diet and Higher-protein diet; $P<0.05$**). Free-living glycemia was not altered by physical inactivity during the Higher-protein condition (see Figure, Supplemental Digital Content 3, **Effect of physical inactivity on free-living glycemic control in response to a Control diet and Higher-protein diet**). To determine whether a diet x inactivity interaction was evident, two-way repeated measures ANOVA was run for CGM data that was available for all repeated time points ($n=6$ /condition). There were no diet x inactivity interactions ($P>0.05$) for average CGM-measured 24-glucose concentrations or glucose AUCs.

Brachial artery flow-mediated dilation and 24-hour ambulatory blood pressure

Brachial artery FMD was not altered by physical inactivity or dietary conditions (see Table, Supplemental Digital Content 4, **Brachial artery FMD and 24-h ambulatory blood pressure**). A reduction in 24-h ambulatory systolic blood pressure, and heart rate was observed with physical inactivity ($P<0.05$; see Table, Supplemental Digital Content 4, **Brachial artery FMD and 24-h ambulatory blood pressure**), despite no change in diastolic blood pressure. No differences in ambulatory blood pressure were noted between diets.

Effects of overfeeding coupled with physical inactivity on metabolic health

Physical inactivity adherence was 100% over the 10-day period with all five subjects achieving $< 5,000$ steps per day. Time spent in sedentary behavior increased by 10%, while the amount of time spent performing moderate-to-vigorous activity was decreased by 85% ($P<0.05$, data not shown). Overfeeding + physical inactivity increased body weight, body fat percentage, fat mass, and tended to enhance abdominal fat mass in 10 days with no effect on fat free mass (Table 3). Fasting blood glucose, plasma insulin and c-peptide concentrations were increased ($P<0.05$, Table 3), whereas hepatic insulin extraction was decreased following the inactivity period, despite no changes in blood lipids (Table 3). In response to an oral glucose challenge, 2-h glucose and 2-h insulin concentrations were significantly elevated by overfeeding + physical inactivity with a tendency towards increased glucose AUC (Figure 3A&B, D&E). The insulin resistance index, HOMA-IR, was elevated after overfeeding + physical inactivity (Figure 3F-G). Neither fasting nor postprandial NEFA concentrations were affected by the combination of excess energy intake and physical inactivity (Figure 3C). Similarly, no changes in brachial artery FMD (Active, $6.7 \pm 1.8\%$; Inactive $6.7 \pm 0.9\%$, $P=0.97$) were detected after 10 days of physical inactivity + overfeeding.

DISCUSSION

Here we report that – independent of diet composition – controlled dietary feeding causing a mild energy deficit preserves metabolic function during physical inactivity in young healthy individuals, whereas physical inactivity coupled with excess energy intake leads to hyperglycemia and hyperinsulinemia. These findings suggest that energy restriction may be an important factor regulating metabolic health in the setting of decreased ambulatory activity.

Adopting a physically inactive lifestyle may increase the susceptibility to weight gain, a negative contributor to numerous diseases. In this regard, physically inactive individuals exhibit less control over energy intake (17, 18), shifting towards a positive energy balance and associated increases in adiposity. Indeed, using whole-body indirect calorimetry, Stubbs et al. (18) reported that a short-term reduction in physical activity led to a positive energy balance (i.e., of which was predominately positive fat balance) in healthy men that was attributed to the lack of a compensatory decrease in energy intake. This concept that a positive energy balance commonly exists during periods of physical inactivity is not new (13) and may be an important determinant of insulin resistance caused by inactivity. On the

other hand, it is possible that consumption of a higher-protein diet, which has been associated with maintenance of fat free mass during energy restriction, may attenuate metabolic dysfunction that is often accompanied by reduced ambulation. To this end, we aimed to feed healthy subjects higher-protein versus normal protein diets that closely corresponded to their amount of energy expended with physical activity and found that a mild energy deficit, regardless of diet composition, preserved metabolic function during physical inactivity.

Although there was no diet x activity interaction for fat free mass, the higher protein diet exhibited nearly 3-fold lower fat free mass loss compared to control diet, albeit these absolute differences were small (Table 1). Fasting triglyceride concentrations tended to decrease during the higher protein condition only. While it is possible that higher protein consumption drove the reduction in triglycerides, it is more likely that the baseline differences between diets (i.e., higher-protein condition having greater triglycerides than control diet at baseline, Table 2) led to this outcome. Thus, overall, metabolic health was not different between diets, dissociating the effects of diet composition and metabolic function in this short-term study. Further research is needed to determine whether a relationship between diet composition and physical activity status is unmasked in the long-term.

Physical inactivity and excess energy intake are often combined in Western lifestyles, contributing to the epidemic of obesity and type 2 diabetes. Indeed, we found that overfeeding coupled with physical inactivity caused metabolic and glycemic dysfunction in 10 days. Our overfeeding paradigm was intended to mimic excess energy consumption and physical inactivity that accompanies holiday seasons in Western societies. In this regard, recent data indicate that within 10 days of the initiation of a holiday season, percent weight change is significantly elevated (35). In addition, our findings agree with several previous studies showing impairments in glucose metabolism and insulin sensitivity after short-term physical inactivity and overfeeding (14, 36, 37). It is likely that metabolic dysfunction caused by combined overfeeding and inactivity, reported herein, was attributed to excess adiposity. In this context, using fat mass as a covariate statistically abolished overfeeding + inactivity-induced hyperglycemia and hyperinsulinemia in the present study. In contrast to our findings, some studies (12, 38) reported that insulin resistance manifests during short-term inactivity in the absence of changes in adiposity. For example, Heath et al. showed in highly fit individuals that cessation from vigorous exercise training for 10 days provoked hyperinsulinemia and hyperglycemia in response to an oral glucose load, despite no changes in body mass or BF% (38). Similarly, Mikus et al. found that three days of reduced ambulatory activity increased insulin concentrations during an OGTT and enhanced free-living glycemic excursions in healthy young adults (12). Nonetheless, these studies did not control for energy intake (i.e., *ad libitum*). In this regard, one prior investigation found that maintaining energy balance via decreased caloric intake during a single day of physical inactivity did not completely prevent reductions in insulin sensitivity (39). Yet, it is important to note that this investigation (39) introduced a model of prolonged sitting (e.g., 16 h), which may represent a significantly greater metabolic insult than step reduction under free-living conditions, as used herein. Additional studies designed to disassociate inactivity from the accompanied weight gain by retaining energy balance are required for our better understanding of the impact of inactivity on metabolic and cardiovascular diseases (40).

We recently showed that five days of reduced physical activity did not alter endothelial function in the brachial artery of healthy, young, recreationally active men (41). Along these lines, findings from the present study indicate that brachial artery endothelial function is also preserved even after ten days of reduced activity in the settings of energy deficit as well as energy surplus, where metabolic derangements were revealed. This preservation of endothelial function in the upper extremities following inactivity is in stark contrast with the vulnerability of the leg vasculature. Indeed, we previously demonstrated that five days of reduced steps markedly impairs endothelial function in the popliteal artery (41, 42). The susceptibility of the arteries of the lower limbs, relative to the upper extremities, is also manifested in response to prolonged sitting [reviewed in (43)]. Taken together, it appears that vascular dysfunction with short-term reduced ambulation principally occurs in arteries perfusing the limbs exposed to the greatest reduction in muscle activity and thus the vascular effects of inactivity are not systemic.

Considerations

Several aspects of this study require consideration. First, it is conceivable that the metabolic insult associated with decreased steps was not as robust as in previous studies using models of bed rest or more extreme reduction in daily steps (i.e., ~1,500 steps per day). However, the present study was designed to achieve a reduction in ambulatory activity that mimicked the activity levels of physically-inactive adults (i.e., ~5,000 steps per day) (44). That is, we enforced a model of inactivity by which active individuals transitioned to the ambulation levels of physically-inactive individuals. It is likely that adoption of a longer period of inactivity and/or more severe levels of inactivity would have produced some degree of metabolic derangements even despite mild energy restriction and consumption of a higher-protein diet. Second, we did not have a true control condition in the present study (i.e., an arm of physical inactivity in the absence of diet intervention) and this can be viewed as a limitation. Our reasoning for not including a control condition was founded on previous compelling literature already demonstrating that short-term reduced physical activity impairs metabolic function in young healthy subjects (12, 41, 42, 45). Despite the lack of a control arm, we incorporated a condition that coupled overfeeding with physical inactivity, two behaviors that typically coexist (35). This condition was employed as a positive control (i.e., a condition known to provoke metabolic deterioration) and provided evidence that our metabolic measures were sensitive to changes when changes existed. Third, an inherent dilemma when isolating the effects of a single macronutrient is the limitation that at least one other macronutrient must be increased or decreased. Here, we were primarily interested in the potential metabolic effects of higher protein; however, one cannot completely attribute a given outcome to a single macronutrient if another (i.e., carbohydrate in the present study) is increased or decreased concomitantly.

In conclusion, we show that in healthy subjects, metabolic deterioration with inactivity only manifests in the setting of energy surplus. These findings support the notion that restricting energy intake may decrease the likelihood of metabolic derangements during short-term periods of physical inactivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We greatly acknowledge the technical assistance from Ying Liu and Jay W. Porter, and meal preparation by Lana Merrick and Nhan Le. In addition, we would like to thank Dr. Heather J. Leidy and Dr. Elizabeth J. Parks for their suggestions regarding diet composition. We would also like to thank the research participants for their dedication to this study. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Disclosures: This study was supported by the American Egg Board. The American Egg Board had no influence on data interpretation or data analysis. The results of the present study do not constitute endorsement by ACSM.

Financial support: American Egg Board (#00050021 to NCW; JAK, sponsor). JAK is supported by National Institutes of Health (NIH) R01 DK101513 and JP is supported by NIH K01 HL125503 and R01 HL137769.

REFERENCES

1. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. *Comprehensive Physiology*. 2012;2(2):1143–211. [PubMed: 23798298]
2. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet (London, England)*. 2012;380(9838):219–29.
3. Nosova EV, Yen P, Chong KC et al. Short-term physical inactivity impairs vascular function. *The Journal of Surgical Research*. 2014;190(2):672–82. [PubMed: 24630521]
4. Reidy PT, Lindsay CC, McKenzie AI et al. Aging-related effects of bed rest followed by eccentric exercise rehabilitation on skeletal muscle macrophages and insulin sensitivity. *Exp Gerontol*. 2018;107:37–49 [PubMed: 28705613]
5. Tanner RE, Brunker LB, Agergaard J et al. Age-related differences in lean mass, protein synthesis and skeletal muscle markers of proteolysis after bed rest and exercise rehabilitation. *J Physiol*. 2015;593(18):4259–73. [PubMed: 26173027]
6. Drummond MJ, Timmerman KL, Markofski MM et al. Short-term bed rest increases TLR4 and IL-6 expression in skeletal muscle of older adults. *Am J Physiol Regul Integr Comp Physiol*. 2013;305(3):R216–23. [PubMed: 23761639]
7. Saltin B, Blomqvist G, Mitchell JH, Johnson RL Jr., Wildenthal K, Chapman CB. Response to exercise after bed rest and after training. *Circulation*. 1968;38(5 Suppl):VIII–78. [PubMed: 5696236]
8. McGuire DK, Levine BD, Williamson JW et al. A 30-year follow-up of the Dallas Bedrest and Training Study: I. Effect of age on the cardiovascular response to exercise. *Circulation*. 2001;104(12):1350–7. [PubMed: 11560849]
9. Dunstan DW, Kingwell BA, Larsen R et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care*. 2012;35(5):976–83. [PubMed: 22374636]
10. Dempsey PC, Larsen RN, Sethi P et al. Benefits for Type 2 Diabetes of Interrupting Prolonged Sitting With Brief Bouts of Light Walking or Simple Resistance Activities. *Diabetes Care*. 2016;39(6):964–72. [PubMed: 27208318]
11. Larsen RN, Kingwell BA, Robinson C et al. Breaking up of prolonged sitting over three days sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in overweight and obese adults. *Clin Sci (Lond)*. 2015;129(2):117–27. [PubMed: 25731923]
12. Mikus CR, Oberlin DJ, Libla JL, Taylor AM, Booth FW, Thyfault JP. Lowering physical activity impairs glycemic control in healthy volunteers. *Med Sci Sports Exerc*. 2012;44(2):225–31. [PubMed: 21716152]

13. Krogh-Madsen R, Thyfault JP, Broholm C et al. A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity. *Journal of Applied Physiology* (Bethesda, Md. : 1985). 2010;108(5):1034–40.
14. Knudsen SH, Hansen LS, Pedersen M et al. Changes in insulin sensitivity precede changes in body composition during 14 days of step reduction combined with overfeeding in healthy young men. *Journal of Applied Physiology* (Bethesda, Md. : 1985). 2012;113(1):7–15.
15. Olsen RH, Krogh-Madsen R, Thomsen C, Booth FW, Pedersen BK. Metabolic responses to reduced daily steps in healthy nonexercising men. *Jama*. 2008;299(11):1261–3. [PubMed: 18349087]
16. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev*. 2013;93(1):359–404. [PubMed: 23303913]
17. Shook RP, Hand GA, Drenowatz C et al. Low levels of physical activity are associated with dysregulation of energy intake and fat mass gain over 1 year. *Am J Clin Nutr*. 2015;102(6):1332–8. [PubMed: 26561620]
18. Stubbs RJ, Hughes DA, Johnstone AM, Horgan GW, King N, Blundell JE. A decrease in physical activity affects appetite, energy, and nutrient balance in lean men feeding ad libitum. *Am J Clin Nutr*. 2004;79(1):62–9. [PubMed: 14684398]
19. Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. *The Journal of Nutrition*. 2003;133(2):405–10. [PubMed: 12566475]
20. Bauer LB, Reynolds LJ, Douglas SM et al. A pilot study examining the effects of consuming a high-protein vs normal-protein breakfast on free-living glycemic control in overweight/obese ‘breakfast skipping’ adolescents. *International Journal of Obesity* (2005). 2015;39(9):1421–4. [PubMed: 26028058]
21. Rains TM, Leidy HJ, Sanoshy KD, Lawless AL, Maki KC. A randomized, controlled, crossover trial to assess the acute appetitive and metabolic effects of sausage and egg-based convenience breakfast meals in overweight premenopausal women. *Nutrition Journal*. 2015;14:17. [PubMed: 25889354]
22. Chomistek AK, Yuan C, Matthews CE et al. Physical Activity Assessment with the ActiGraph GT3X and Doubly Labeled Water. *Med Sci Sports Exerc*. 2017;49(9):1935–44. [PubMed: 28419028]
23. Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. *Med Sci Sports Exerc*. 1998;30(5):777–81. [PubMed: 9588623]
24. Manore MM. Exercise and the Institute of Medicine recommendations for nutrition. *Curr Sports Med Rep*. 2005;4(4):193–8. [PubMed: 16004827]
25. Kanaley JA, Heden TD, Liu Y et al. Short-term aerobic exercise training increases postprandial pancreatic polypeptide but not peptide YY concentrations in obese individuals. *International Journal of Obesity* (2005). 2014;38(2):266–71. [PubMed: 23736355]
26. Winn NC, Liu Y, Rector RS, Parks EJ, Ibdah JA, Kanaley JA. Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity - A randomized trial. *Metabolism*. 2018;78:128–40. [PubMed: 28941598]
27. McArdle WD, Katch FI, Katch VL. *Exercise physiology: Nutrition, Energy, and Human Performance*. Lippincott Williams & Wilkins; 2010.
28. Kaul S, Rothney MP, Peters DM et al. Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity* (Silver Spring). 2012;20(6):1313–8. [PubMed: 22282048]
29. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. *Obesity* (Silver Spring). 2012;20(5):1109–14. [PubMed: 22240726]
30. Hill AM, LaForgia J, Coates AM, Buckley JD, Howe PR. Estimating abdominal adipose tissue with DXA and anthropometry. *Obesity* (Silver Spring). 2007;15(2):504–10. [PubMed: 17299124]
31. Heden TD, Liu Y, Park YM, Nyhoff LM, Winn NC, Kanaley JA. Moderate amounts of fructose- or glucose-sweetened beverages do not differentially alter metabolic health in male and female adolescents. *Am J Clin Nutr*. 2014;100(3):796–805. [PubMed: 25030782]

32. Heden TD, Liu Y, Kanaley JA. A comparison of adipose tissue interstitial glucose and venous blood glucose during postprandial resistance exercise in patients with type 2 diabetes. *Journal of Applied Physiology* (Bethesda, Md. : 1985). 2018;124(4):1054–61.
33. Wu PT, Segovia DE, Lee CC, Nguyen KL. Consistency of Continuous Ambulatory Interstitial Glucose Monitoring Sensors. *Biosensors (Basel)*. 2018;8(2).
34. Padilla J, Johnson BD, Newcomer SC et al. Adjusting flow-mediated dilation for shear stress stimulus allows demonstration of endothelial dysfunction in a population with moderate cardiovascular risk. *J Vasc Res*. 2009;46(6):592–600. [PubMed: 19571579]
35. Helander EE, Wansink B, Chieh A. Weight Gain over the Holidays in Three Countries. *N Engl J Med*. 2016;375(12):1200–2.
36. Hagobian TA, Braun B. Interactions between energy surplus and short-term exercise on glucose and insulin responses in healthy people with induced, mild insulin insensitivity. *Metabolism*. 2006;55(3):402–8. [PubMed: 16483886]
37. Walhin JP, Richardson JD, Betts JA, Thompson D. Exercise counteracts the effects of short-term overfeeding and reduced physical activity independent of energy imbalance in healthy young men. *J Physiol*. 2013;591(24):6231–43. [PubMed: 24167223]
38. Heath GW, Gavin JR 3rd, Hinderliter JM, Hagberg JM, Bloomfield SA, Holloszy JO. Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*. 1983;55(2):512–7.
39. Stephens BR, Granados K, Zderic TW, Hamilton MT, Braun B. Effects of 1 day of inactivity on insulin action in healthy men and women: interaction with energy intake. *Metabolism*. 2011;60(7):941–9. [PubMed: 21067784]
40. Francois ME, Baldi JC, Manning PJ et al. ‘Exercise snacks’ before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. *Diabetologia*. 2014;57(7):1437–45. [PubMed: 24817675]
41. Boyle LJ, Credeur DP, Jenkins NT et al. Impact of reduced daily physical activity on conduit artery flow-mediated dilation and circulating endothelial microparticles. *Journal of Applied Physiology* (Bethesda, Md. : 1985). 2013;115(10):1519–25.
42. Teixeira AL, Padilla J, Vianna LC. Impaired popliteal artery flow-mediated dilation caused by reduced daily physical activity is prevented by increased shear stress. *Journal of Applied Physiology* (Bethesda, Md. : 1985). 2017;123(1):49–54.
43. Padilla J, Fadel PJ. Prolonged sitting leg vasculopathy: contributing factors and clinical implications. *American Journal of Physiology. Heart and Circulatory Physiology*. 2017;313(4):H722–H8. [PubMed: 28733451]
44. Bassett DR Jr., Wyatt HR, Thompson H, Peters JC, Hill JO. Pedometer-measured physical activity and health behaviors in U.S. adults. *Med Sci Sports Exerc*. 2010;42(10):1819–25. [PubMed: 20305579]
45. Reynolds LJ, Credeur DP, Holwerda SW, Leidy HJ, Fadel PJ, Thyfault JP. Acute inactivity impairs glycemic control but not blood flow to glucose ingestion. *Med Sci Sports Exerc*. 2015;47(5):1087–94. [PubMed: 25207931]

Figure 1. Experimental design and physical activity/energy expenditure.

Healthy physically active adults ($n=10$) defined as exceeding 10,000 steps per day, completed A) two periods of physical inactivity while consuming either a Control diet or Higher-protein diet in a randomized cross-over design. Average B) daily steps and C) energy expenditure (total and physical activity). Data are means $\pm$ SEM. Two-way ANOVA with activity and diet as factors was used for statistical comparisons. Post hoc comparisons with Tukey correction were run when a significant main effect was observed. * $P<0.05$ vs Active. Horizontal arrows represent the number of days for a given data assessment, whereas vertical arrows indicate testing on a single day in the laboratory. Days 1–10 were ‘free-living’. White bars reflect the ‘active’ phase and gray bars represent the ‘inactive’ period. OGTT, oral glucose tolerance test; FMD, flow-mediated dilation; CGMS, continuous glucose monitoring system; BP, blood pressure; DEXA, dual x-ray absorptiometry; EE, energy expenditure. $n=10$ /condition.

Figure 2. Effect of physical inactivity on glucose tolerance and indices of insulin sensitivity/resistance in response to a Control diet and Higher-protein diet.

Physically active and physically inactive A) glucose, B) insulin, and C) NEFA curves with corresponding 3-h AUC (inset) following a 75-g oral glucose challenge during the Control diet and Higher-protein diet conditions. D) Two-hour glucose and E) two-hour insulin during the OGTT. F) HOMA-IR. Data are means $\pm$ SEM. * $P < 0.05$ vs Active. Two-way ANOVA with activity and diet as factors was used for statistical comparisons. Post hoc comparisons with Tukey correction were run when a significant main effect was observed. AUC, area under the curve; NEFA, nonesterified fatty acids; HOMA-IR, homeostasis model assessment of insulin resistance. $n = 10$ /condition.

Ad libitum/Overfeeding

Figure 3. Effect of physical inactivity on glucose tolerance and indices of insulin sensitivity/resistance in response to overfeeding.

Physically active and physically inactive A) glucose, B) insulin, and C) NEFA curves with corresponding 3-h AUC (inset) following a 75-g oral glucose challenge during hypercaloric feeding. D) Two-hour glucose and E) two-hour insulin during the OGTT. F) HOMA-IR.

Data are means $\pm$ SEM or presented as individual responses. * P <0.05 vs Active; # P =0.055 vs Active. Paired-samples t tests were run to compare Active and Inactive. White circles are 'active' phase and gray circles are 'inactive' period. AUC, area under the curve; NEFA, nonesterified fatty acids; HOMA-IR, homeostasis model assessment of insulin resistance. n =5/condition.

Table 1.

Body composition, aerobic capacity, and energy expenditure

Parameter	Control diet(n=10)		Higher-protein diet (n=10)		ANOVA
	Active		Active		P value
Age (years)	24 ± 1	-	24 ± 1	-	-
Body weight (kg)	72.1 ± 4.4	-1.0 ± 0.2 *	71.9 ± 4.2	-0.9 ± 0.2 *	D: <i>P</i> =0.80 A: <i>P</i>=0.001 DxA: <i>P</i> =0.71
BMI (kg/m ²)	25.2 ± 1.2	-0.4 ± 0.1	25.1 ± 1.1	-0.3 ± 1.0	D: <i>P</i> =0.41 A: <i>P</i> =0.83 DxA: <i>P</i> =0.92
Body fat (%)	22.1 ± 2.0	-0.1 ± 0.4	22.5 ± 2.0	-0.4 ± 0.2	D: <i>P</i> =0.19 A: <i>P</i> =0.43 DxA: <i>P</i> =0.37
Total FFM (kg)	57.1 ± 4.6	-0.8 ± 0.4	56.5 ± 4.4	-0.3 ± 0.3	D: <i>P</i> =0.22 A: <i>P</i> =0.06 DxA: <i>P</i> =0.28
Trunk FFM (kg)	27.1 ± 2.2	-0.76 ± 0.45	26.4 ± 2.0	-0.16 ± 0.2	D: <i>P</i> =0.23 A: <i>P</i> =0.07 DxA: <i>P</i> =0.26
Arm FFM (kg)	3.1 ± 0.4	0.03 ± 0.09	3.2 ± 0.4	-0.07 ± 0.05	D: <i>P</i> =0.31 A: <i>P</i> =0.62 DxA: <i>P</i> =0.23
Leg FFM (kg)	9.0 ± 0.8	-0.04 ± 0.08	8.9 ± 0.7	-0.02 ± 0.09	D: <i>P</i> =0.43 A: <i>P</i> =0.32 DxA: <i>P</i> =0.89
Fat mass (kg)	15.5 ± 1.02	-0.2 ± 0.3	15.7 ± 0.9	-0.4 ± 0.1	D: <i>P</i> =0.62 A: <i>P</i> =0.10 DxA: <i>P</i> =0.25
Abdominal fat mass (kg)	1.09 ± 0.07	-0.05 ± 0.02 *	1.07 ± 0.07	-0.08 ± 0.04	D: <i>P</i> =0.13 A: <i>P</i>=0.01 DxA: <i>P</i> =0.60
Hip fat mass (kg)	2.99 ± 0.23	-0.06 ± 0.06	3.16 ± 0.22 [#]	-0.13 ± 0.06	D: <i>P</i>=0.04 A: <i>P</i> =0.09 DxA: <i>P</i> =0.24

Parameter	Control diet(n=10)		Higher-protein diet (n=10)		ANOVA
	Active		Active		P value
VO ₂ max (ml/kg/min)	45.8 ± 1.6	-1.1 ± 0.81	47.9 ± 1.9	-2.5 ± 0.7 *	D: <i>P</i> =0.49 A: <i>P</i>=0.004 DxA: <i>P</i> =0.27
REE (kcal/d)	1762 ± 127	-80.7 ± 36.9 *	1660 ± 75	-121.7 ± 61.0 *	D: <i>P</i> =0.19 A: <i>P</i>=0.02 DxA: <i>P</i> =0.60
$\dot{V}RQ$	0.79 ± 0.05	-0.04 ± 0.04	0.86 ± 0.02	-0.1 ± 0.04	D: <i>P</i> =0.42 A: <i>P</i>=0.01 DxA: <i>P</i> =0.44

All values are means ± SEM.

* *P*<0.05 vs Active (within condition)

P<0.05 vs Control diet (Active).

Two-way ANOVA with activity and diet as factors was used for statistical comparisons. Post hoc comparisons with Tukey correction were run when a significant main effect was observed. BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure; RQ, respiratory quotient; D, diet; A, physical activity; DxA, diet x physical activity interaction.

[^] Paired data were available for n=7.

, delta change (Inactive – Active). Bold values indicate statistical significance.

Table 2.

Blood chemistry

Parameter	Control diet (n=10)		Higher-protein diet (n=10)		ANOVA
	Active		Active		<i>P</i> value
Glucose (mg/dl)	78.7 ± 1.7	-6.3 ± 2.5 *	77.7 ± 1.7	- 1.6 ± 1.5	D: <i>P</i> =0.16 A: <i>P</i>=0.03 DxA: <i>P</i> = 0.10
Insulin (μU/ml)	2.5 ± 0.9	0.8 ± 0.8	3.7 ± 1.5	- 1.4 ± 0.7	D: <i>P</i> =0.81 A: <i>P</i> =0.19 DxA: <i>P</i> = 0.18
C-peptide (pmol/l)	229.0 ± 33.3	- 41.3 ± 35.5	222.3 ± 21.3	- 19.5 ± 18.2	D: <i>P</i> =0.81 A: <i>P</i> =0.19 DxA: <i>P</i> = 0.56
Hepatic insulin extraction (%)	83.6 ± 6.5	- 5.3 ± 4.0	78.5 ± 8.8	5.0 ± 1.9	D: <i>P</i> =0.95 A: <i>P</i> =0.95 DxA: <i>P</i> = 0.08
Lactate (mg/dl)	5.0 ± 0.3	- 0.5 ± 0.5	4.8 ± 0.7	- 0.3 ± 0.5	D: <i>P</i> =0.79A: A: <i>P</i> =0.44 DxA: <i>P</i> = 0.67
Adipo-IR	0.8 ± 0.3	0.7 ± 0.4	1.5 ± 0.6	- 0.1 ± 0.2	D: <i>P</i> =0.12 A: <i>P</i> =0.25 DxA: <i>P</i> =0.50
NEFA (mmol/l)	0.42 ± 0.07	0.04 ± 0.07	0.50 ± 0.07	0.1 ± 0.1	D: <i>P</i> =0.23 A: <i>P</i> =0.24 DxA: <i>P</i> =0.12
Triglycerides (mg/dl)	72 ± 8	15 ± 10	103 ± 15 [#]	- 27 ± 14	D: <i>P</i> =0.22 A: <i>P</i> =0.56 DxA: <i>P</i>=0.01
Total cholesterol (mg/dl)	153 ± 4	- 13 ± 7	158 ± 6	- 10 ± 5	D: <i>P</i> =0.15 A: <i>P</i>=0.009 DxA: <i>P</i> =0.77
HDLc (mg/dl)	49 ± 4	- 8 ± 2 *	51 ± 5	- 8 ± 2 *	D: <i>P</i> =0.39 A: <i>P</i>=0.003 DxA: <i>P</i> =0.90
LDLc (mg/dl)	90 ± 5	- 8 ± 5	86 ± 6	4 ± 4	D: <i>P</i> =0.42 A: <i>P</i> =0.49

Parameter	Control diet (n=10)		Higher-protein diet (n=10)		ANOVA
	Active		Active		<i>P</i> value
					DxA: <i>P</i> =0.11
Oxidized LDL (µg/ml)	101.9 ± 9.6	26.5 ± 21.5	132.4 ± 14.4	– 17.2 ± 17.4	D: <i>P</i> =0.50 A: <i>P</i> =0.76 DxA: <i>P</i> =0.12

All values are means ± SEM.

* *P*<0.05 vs Active (within condition)

P<0.05 vs Control diet (Active).

, delta change (Inactive – Active). Two-way ANOVA with activity and diet as factors was used for statistical comparisons. Post hoc comparisons with Tukey correction were run when a significant main effect was observed. BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure; RQ, respiratory quotient; D, diet; A, physical activity; DxA, diet x physical activity interaction. Bold values indicate statistical significance.

Table 3.

Age, body composition, and blood chemistry

Parameter	Overfeeding(n=5)		
	Active		<i>P</i> value
Age (years)	26 ± 1	-	-
Body weight (kg)	80.8 ± 3.2	1.0 ± 0.3	0.04
BMI (kg/m ²)	26.6 ± 1.3	0.3 ± 0.1	0.03
Body fat (%)	17.0 ± 1.9	0.7 ± 0.2	0.01
Total FFM (kg)	65.0 ± 2.9	0.2 ± 0.1	0.12
Trunk FFM (kg)	31.8 ± 1.7	-0.05 ± 0.2	0.82
Arm FFM (kg)	3.8 ± 0.2	0.2 ± 0.1	0.09
Leg FFM (kg)	9.0 ± 2.0	1.8 ± 1.9	0.39
Fat mass (kg)	14.0 ± 2.0	0.8 ± 0.2	0.01
Abdominal fat mass (kg)	1.0 ± 0.2	0.1 ± 0.04	0.06
Hip fat mass (kg)	2.5 ± 0.3	0.1 ± 0.1	0.38
Glucose (mg/dl)	83.2 ± 0.7	3.5 ± 0.9	0.02
Insulin (μU/ml)	4.3 ± 1.1	2.1 ± 0.7	0.04
C-peptide (pmol/ml)	228.7 ± 17.2	59.6 ± 16	0.02
Hepatic insulin extraction (%)	85.9 ± 4.0	-2.1 ± 0.7	0.05
Lactate (mg/dl)	5.1 ± 0.6	0.6 ± 0.3	0.10
Adipo-IR	1.2 ± 0.2	-0.02 ± 0.4	0.96
NEFA (mmol/l)	0.34 ± 0.08	-0.15 ± 0.01	0.22
Triglycerides (mg/dl)	94 ± 11	14 ± 23	0.57
Total cholesterol (mg/dl)	164 ± 5	8 ± 11	0.51
HDLc (mg/dl)	47 ± 5	3 ± 2	0.25
LDLc (mg/dl)	99 ± 5	2 ± 10	0.84
Oxidized LDL (μg/ml)	173 ± 61	30 ± 54	0.60

All values are means ± SEM. BMI, body mass index; FFM, fat free mass. Paired-samples t tests were run to compare Active and Inactive. Bold values indicate statistical significance.