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RESEARCH ARTICLE

Physical Activity, Mitochondria, and Disease

Acute effects of daily step count on postprandial metabolism and resting fat oxidation: a randomized controlled trial

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Abstract

To examine the effects of daily step count on same-day fat oxidation and postprandial metabolic responses to an evening high-fat mixed meal (HFMM). Ten healthy participants (5 females, 30 ± 7 yr) completed four different daily step counts—2,000 (2 K), 5,000 (5 K), 10,000 (10 K), and 15,000 (15 K) steps—on separate days in randomized order. On experimental days, participants ate the same meals and walked all steps on an indoor track at a pace of 100 steps/min in three roughly equal bouts throughout the day. After the final walking bout, participants' resting energy expenditure (REE), respiratory exchange ratio (RER), and fat oxidation rate (FAT_{OX}) were measured. Blood samples were obtained before (BL) and 30-, 60-, 90-, 120-, and 240-min following consumption of an HFMM (960 kcal; 48% fat) to measure triglycerides (i.e., postprandial lipemia; PPL), nonesterified fatty acids (NEFAs), insulin, and glucose. Two-way ANOVAs indicated condition effects where PPL was significantly higher after 2 K versus 10 K (+23 ± 8 mg/dL, $P = 0.027$), and NEFAs were significantly higher after 15 K versus 2 K (+86 ± 23 μmol/L; $P = 0.006$). No differences were found for insulin, glucose, or REE among conditions (all $P > 0.124$). Similarly, RER ($P = 0.054$; $\eta_p^2 = 0.24$) and FAT_{OX} ($P = 0.071$; $\eta_p^2 = 0.23$) were not significantly different among conditions. In young adults, 10 K steps elicited the greatest decrease in PPL, an established cardiovascular disease risk factor. NEFA levels were highest after the 15 K condition, likely due to alterations in adipose tissue lipolysis or lipoprotein lipase activity with increased activity.

NEW & NOTEWORTHY This randomized controlled trial demonstrated that walking 10,000, compared with 2,000, steps/day significantly reduced postprandial lipemia (PPL), an independent predictor of cardiovascular disease (CVD) following same-day evening meal consumption. These experimental data support walking 10,000 steps/day to lower CVD risk.

cardiovascular health; exercise; physical activity; postprandial lipemia; sedentary behavior

INTRODUCTION

Physical inactivity is a strong independent risk factor for cardiovascular disease (CVD) (1), yet a staggering 75% of Americans do not meet the physical activity (PA) guidelines (2). The resulting annual healthcare costs associated with inadequate PA are estimated at ~$117 billion in the United States alone (3). Walking is the most popular form of PA (4) and daily step counts are a simple and effective target for improving PA levels in adults (5). The most common target recommended for optimal cardiometabolic health (CMH) is 10,000 steps/day. In line with this, epidemiological evidence shows significant and steady declines in all-cause mortality with increasing daily step counts up to 10,000 steps/day (6). This epidemiological evidence is crucially important as it highlights that both disease (7) and mortality (6) risk are negatively associated with daily step count in a dose-dependent manner, even at levels below the commonly recommended 10,000 steps/day target. Considering the ease with which daily step count could be altered as a method for increasing PA, it is surprising that the direct effects of daily step count on CMH health outcomes have been minimally explored in the context of randomized, controlled experimental studies. As such, addressing this gap in the literature could be of great practical value for the development of daily step-count recommendations for public health.

Hypertriglyceridemia and hyperglycemia are commonly observed after consumption of high-fat and carbohydrate meals and promote atherogenesis (8, 9). As humans spend the vast majority of their day in the fed (postprandial) state, decreasing circulating triglycerides (TG; i.e., PPL) and glucose (PPG) may present an effective approach to limit vascular damage, atherosclerosis, and thus the development of CVD. Accordingly, the metabolic response to a standardized meal serves as a powerful tool that can be used to predict cardiometabolic disease (CMD) risk. In fact, elevated PPL and

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PPG are both independent predictors of CVD and type II diabetes (T2D) and are stronger predictors of future CVD than traditional fasting values (10–14).

It is well-known that a single aerobic exercise bout acutely reduces PPL (15). The importance of background daily step count has also been highlighted for its ability to moderate the reduction in PPL in response to an acute exercise bout, apparently by influencing fat oxidation rates (16–19). The effects of exercise on PPG (20) and insulin sensitivity are dependent on participant health status, exercise timing (e.g., postprandial vs. pre-prandial/postabsorptive), exercise volume, and/or exercise intensity. However, in healthy participants, a reduction in daily steps has been shown to reduce insulin sensitivity (21), whereas pre-prandial walking at durations of 30, 60, or 90 min had no acute effect on insulin sensitivity (22). It is still unclear how varying daily step counts, in and of themselves, influence same-day postprandial fat metabolism soon after walking and whether step counts greater than 10 K present any additional benefit.

Provided that the majority of Americans consume ~45% of total daily caloric intake in the evening (23–25), most individuals will experience the greatest metabolic challenge after completing the lion's share of their daily PA. However, almost all of the studies examining the effects of exercise and PA on postprandial metabolism do so in the morning following completion of the exercise/PA and an overnight fast. Therefore, although these studies are important, they do not always accurately recreate the daily dietary and PA behaviors of most Americans, which may limit their ecological validity. Examining the effects of daily step count on postprandial metabolic responses to an evening meal is a more accurate approach to better replicate the true daily physiological experiences of people in the Western World in response to differing PA doses (7).

The purpose of this study was to examine the effect of daily step-count dose—2,000 (2 K), 5,000 (5 K), 10,000 (10 K), and 15,000 (15 K) steps—on same-day resting fat oxidation, as well as the PPL, PPG, nonesterified fatty acids (NEFAs), and insulin responses to an evening high-fat mixed meal (HFMM). Secondary outcomes included hunger, satiety, and palatability. We hypothesized that as steps/day increased, *1*) resting fat oxidation and baseline NEFA would increase, *2*) postprandial TG and insulin would decrease, and *3*) glucose would not change.

METHODS

Participants

Eleven healthy young adults (5 female, 6 male) were enrolled in and completed this study. Prior to enrollment, volunteers were provided a written and verbal description detailing the study procedures. Individuals who were interested in participation then completed and signed an informed consent form, followed by health history and physical activity readiness (PAR-Q+) questionnaires. To be eligible, participants must have been between the ages of 19 and 45 yr and have been cleared to exercise based on their responses to the PAR-Q+. Therefore, all participants were free from CMD and were cleared for PA with no additional need for medical clearance. Participant characteristics are displayed in Table 1. Data from one male participant was not included in analyses as he was taking medication that interfered with fat and glucose metabolism. Therefore, data from 10 (5 male, 5 female) participants were used in data analysis. Recruitment was carried out via campus-wide email and word of mouth. This study was conducted in accordance with the Declaration of Helsinki and was approved by, and carried out in accordance with, the University's Institutional Review Board for the protection of human subjects (IRB Approval No. 202008287, Approval Date: 10/22/2020).

Experimental Design

A randomized, counterbalanced, crossover design was used in this study, where each participant completed four different conditions separated by a 3- to 10-day washout period in random order. Each of the four experimental visits was identical, with only the step count differing between conditions. The four differently assigned daily step counts were 2 K, 5 K, 10 K, and 15 K. All steps were completed in three approximately equal bouts throughout the day. All walking was completed at a pace of 100 steps/min and controlled using a metronome application on each participant's personal mobile phone. For reference, this pace equates to an absolute intensity of 3.15 metabolic equivalent tasks (METs) (26), which would be defined as moderate-intensity exercise. Participants were instructed to limit background walking to only that necessary before arrival at the laboratory. Steps taken before arrival to the laboratory were tracked by accelerometry and were included in the daily step-count dose to ensure the validity of the step-count dose for each condition. Consequently, if a participant completed 500 steps before arrival on the day of the 15 K step condition, then the participant completed 14.5 K steps in three equal bouts in the laboratory (e.g., ~4,833 steps). To the greatest degree possible (e.g., during the first 3 conditions), participants were blinded to the assigned step-count condition until the morning of their experimental visit to reduce the chance that they altered their behavior in the days leading up to their experimental visits in anticipation of the assigned step dosage.

On the morning of each experimental visit, participants arrived at the laboratory between 0500 and 0700 after completing a 10-h overnight fast. Immediately upon arrival, participants were provided with breakfast before being informed of how many steps they would be completing that day. One hour later, participants began their first walking bout on an indoor track. Three hours after arrival, participants ate lunch, which was followed 1 h later by their second walking bout.

Table 1. *Participant characteristics*

Characteristics	Means ± SD
Sex, M/F	5/5
Age, yr	30 ± 7
Height, m	1.74 ± 0.13
Weight, kg	84 ± 24
BMI, kg/m^2	28 ± 9
SBP, mmHg	116 ± 9
DBP, mmHg	71 ± 9
Habitual steps/day	7,690 ± 3,559

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Six hours after arrival, participants ate a snack. Finally, participants completed their final walking bout such that all final bouts ended 15 min before experimental testing procedures began (i.e., start time of the last bout differed between conditions). All meals were standardized across visits and participants, and details are provided below in the *Standardized Meals* section.

Following the achievement of their target step counts and a quiet resting period, participants' resting, whole body metabolic, and nonprotein substrate oxidation rates were assessed using indirect calorimetry. A forearm intravenous catheter was then placed in an antecubital vein and a baseline blood draw was performed. Participants then completed a hunger questionnaire immediately before and immediately after consuming an HFMM (960 kcals, 36% carbohydrate, and 48% fat). The HFMM was consumed ~2 h after the last walking bout was complete. A palatability questionnaire was completed after consuming the first quarter of this meal. Following completion of the meal, blood was drawn periodically from 30 min through 4 h postmeal consumption. Before their final blood draw, participants completed a final hunger questionnaire. An overview of the experimental design is provided in Fig. 1.

Step-Count Dosing

Step-count doses of 2 K, 5 K, 10 K, and 15 K steps were chosen based on prevailing epidemiological data on step-count dose and cardiovascular health. First, up to ~10,000 steps, there appears to be a direct dose-response relationship (6). Accordingly, the hazard ratio associated with a habitual step-count dose of 5,000 steps (HR = −0.35) is approximately one-half of that at 10,000 steps (HR = −0.6) for CVD incidence (6). The 2 K step dose was chosen as an approximation of the lowest number of steps one may minimally complete (as if completing only minimal activities of daily living) per normative data (27). Finally, whereas the epidemiological data generally suggest a dose threshold of ~10,000 steps for health, statistical uncertainty also increases dramatically beyond 10,000 step doses potentially obscuring the true association between higher doses (i.e., 15 K) and cardiovascular risk.

Lifestyle Controls

Diet.

Participants were asked to consume a normal dietary intake on the day before the first experimental visit and to record all food and drink consumed on a dietary food log. Participants were then asked to replicate their diet on the day before the next three experimental visits and were again provided with a dietary food log to record all food and drink consumed to validate dietary compliance. Finally, participants were instructed to begin an overnight fast 10 h before the start of their experimental visit the next day but were allowed to consume water ad libitum during this period. Dietary information was entered into the ESHA's Food Processor nutrition analysis Software (https://www.esha.com, ESHA Research, Oak Brook, IL), which provided calculations of total calories consumed (kcals) for each day. In addition, participants were asked to refrain from caffeine consumption in the 24 h leading up to each experimental visit, except that participants were allowed to consume a single cup of coffee on the morning of each experimental visit. If participants consumed coffee on the day of the first experimental visit, they were asked to replicate this consumption for the remaining visits.

Activity.

Participants wore a Fitbit (Fitbit Inspire 2, Fitbit Inc, San Francisco, CA), which has been validated for measuring step counts in a range of populations (28–31), on their wrists during the days before each experimental visit so that all activity on the pre-experimental days was recorded. Two days before each experimental visit, participants were provided a reminder text to put on the Fitbit that night before bed to increase compliance. Participants were asked to walk a number of steps that were typical of their behavior, and to complete the same number of steps on each pre-experimental day. The step counts recorded during the first pre-experimental day were then told to the participants by the researchers, and participants were instructed to aim to achieve a similar step count during the day before the final three visits. Participants were also asked to refrain from

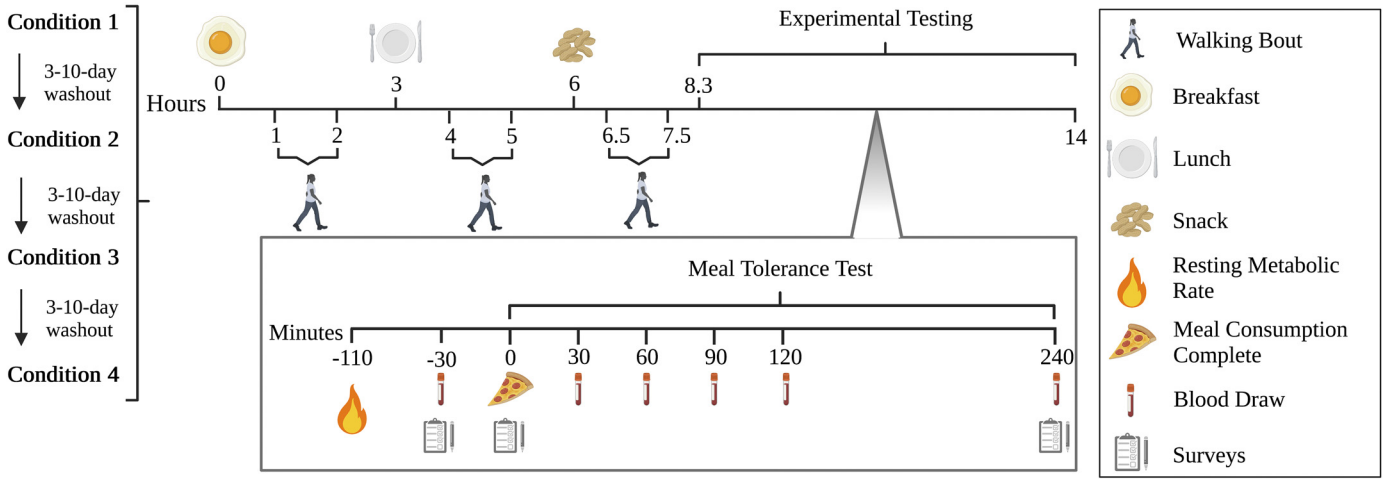

Figure 1. Timeline of events during experimental testing visits. The timeline for experimental visits were identical, with only the daily step count changing between conditions. Final walking bouts ended at the same time for all conditions, thus start time differed between conditions. Created using BioRender.com with permission.

completing planned exercises in the 24 h leading up to their experimental visit start times. In addition, self-reported PA levels were assessed using an International Physical Activity Questionnaire Long Last 7-days Format (IPAQ) at all four visits. Metabolic equivalent task (MET) minutes were calculated from this questionnaire and examined to ensure similar PA levels for the days leading up to each visit (32).

Standardized Meals

Each participant ate the same meals during all four experimental visits, which consisted of breakfast, lunch, a snack, and dinner. The calories and macronutrients in the meals provided in the experimental visits were designed to roughly match the average caloric and macronutrient intake for adults in the United States (2,203 kcal, 245 g carbohydrates, 87 g protein, and 98 g fat) (33). The final participant reported having nonceliac gluten sensitivity. To accommodate this participant, a nongluten alternative diet was provided that was closely matched to the nutritional content of the meals consumed by all other participants and standardized across all visits. The final meal (i.e., dinner), which served as the HFMM, was a pizza (Margherita Pizza, California Pizza Kitchen, Los Angeles, CA) that provided 960 kcal and 51 g of fat (i.e., 48% fat) (34). For the participant with nonceliac gluten sensitivity, a gluten-free pizza (Three Cheese Pizza, Against the Grain Gourmet, Battleboro, VT) was provided that contained 960 kcal and 54 g of fat (i.e., 51% fat).

Blood Sampling and Analyses

A forearm intravenous catheter was inserted into the participants' antecubital vein and blood draws were performed before, as well as 30-, 60-, 90-, 120-, and 240-min postmeal consumption. At each time point, whole blood was used to measure circulating TG, glucose, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) using a point-of-care metabolic analyzer (Cholestech LDX Analyzer, Cholestech Corporation, Hayward, CA). Blood was also collected into a serum separator tube (BD Vacutainer, Franklin Lakes, NJ) at each time point. These tubes were inverted, allowed to clot for 40 min, and then centrifuged at 1,000 rpm to separate serum. Serum was then aliquoted in 1.5-mL microcentrifuge tubes and promptly placed in storage in a −80°C freezer (SU780XLE, Stirling Ultracold, Athens, OH) for later analysis. NEFA concentrations were measured with a commercially available calorimetric assay kit using the acyl-CoA synthetase–acyl-CoA oxidase (i.e., ACS-ACOD) method (LabAssay NEFA, FUJIFILM Wako Shibayagi Corporation, Shibukaya, Japan). The cross-reaction of the kit is <15%. Serum insulin was analyzed using a commercially available, sandwich enzyme-linked immunosorbent assay kit (Human Insulin ELISA, BioVender, Karasek, Czech Republic). The detection range of the kit was 5.1–250 μIU/mL, the sensitivity was 0.17 μIU/mL, and the interassay coefficient of variation was <10%. All assays were performed in accordance with the manufacturer's instructions and read using a microplate photometer (Multiskan FC Microplate Photometer, ThermoFisher Scientific, Waltham, MA).

Resting Metabolic Rate and Substrate Oxidation

Indirect calorimetry was used to assess resting energy expenditure (REE), respiratory exchange ratio (RER), and fat oxidation rate (FAT_{OX}) with a Parvo Medics metabolic cart (TrueOne 2400, Parvo Medics, Sandy, UT) as previously described (35). Briefly, the participant was supine in a quiet, temperature-controlled (20°C–22°C), dimly lit room with a canopy placed and secured tightly over their head, upper chest, and shoulders to capture expired air for this assessment. Participants laid quietly under the canopy for 40 min, with data collected and analyzed during the final 20 min (50–70 min following the final walking bout). Expired air was sampled breath-by-breath and data were averaged over each 60 s period. Measured $\dot{V}O_2$ and $\dot{V}CO_2$ data were then exported in a .csv file, which was analyzed using a custom-written LabVIEW program (v. 21, National Instruments Corp, Austin, TX) to calculate REE (kcal/day), RER, FAT_{OX}, and CHO_{OX} (g/min). Specifically measured $\dot{V}O_2$ and $\dot{V}CO_2$ were used to determine the RER, and fat and carbohydrate oxidation values were determined using the Frayn stoichiometric equation (36), as previously described (16). REE was calculated using the formula described by Weir (37).

Total Energy Consumption and Expenditure

The Moore et al. (38) equation was used to calculate walking energy expenditure (WEE; kcals) on experimental days as previously described. WEE and REE were summed to estimate total energy expenditure (TEE) on experimental days and energy balance was calculated as the difference between total energy intake (i.e., from breakfast, lunch, snack, and dinner) and TEE.

Hunger and Palatability

Hunger and palatability were assessed using 0- to 100-mm visual analog scales, as described and validated by Flint et al. (39). Hunger was assessed immediately before and immediately after pizza consumption, as well as 4 h after pizza consumption in accordance with the methods of Hengist et al. (40). Palatability was assessed after consumption of the second slice of pizza during each condition.

Statistical Analyses

One-way repeated-measures (RM) ANOVAs were used to assess the effects of step-count condition on REE, RER, FAT_{OX}, TG total trapezoidal area under the curve (tAUC), TG incremental AUC (iAUC), glucose tAUC, glucose iAUC, palatability of the test meal, as well as to confirm that no differences in pre-experimental day kcal consumption, steps walked, or MET minutes performed existed among conditions. Two-way ANOVAs were used to assess the effects of step count on postprandial TG, glucose, insulin, NEFAs, subjective hunger, satiety, and desire to consume specific foods. Tukey's or Holm–Sidak's post hoc comparisons were used to explore mean differences when significant interactions or main effects were observed, and descriptive statistics for these post hoc tests (e.g., mean differences) are reported as means ± SE. Mixed-effects analyses were used instead of one- and two-way ANOVAs wherever data were missing, and descriptive statistics for these post hocs were reported as predicted (LS) means ± SE. Partial eta squared (η_p^2) was calculated to show effect size where appropriate. Simple linear regression and Pearson correlations were performed between baseline NEFA levels and glucose at each time point (averaged across conditions) to explore

the potential effects of NEFA on postprandial glucose uptake. Finally, RM ANOVAs with sex as a covariate were used to examine the effect of the condition on RER and FAT_{OX} while covarying for sex. Post hoc analyses for RER and FAT_{OX} included Bonferroni post hoc comparisons. Statistical analyses were performed using GraphPad Prism for macOS (v. 8.4.3), JASP (v. 0.16), and R for MacOS (v. 4.2.2), and significance was set at $P \leq 0.05$. All figures were made using GraphPad Prism for macOS (v. 8.4.3) and BioRender.com.

Sample Size Calculation

The required sample size was estimated using the average TG and TG AUC responses to an HFMM in a prior study examining the effect (f = 1–1.1) of step count on exercise-induced reductions in PPL, which included 10 participants (16). Power, type I error rate, and repeated-measures correlation were set at 95%, 0.05%, and 0.5%, respectively. The analysis indicated that four total participants were needed for this study. Given our more acute design, desire to include equal representation of men and women and the potential for attrition due to the demands of the study protocol, we aimed to recruit and enroll 12 adults to end the study with 10 total participants (50% male, 50% female).

RESULTS

Lifestyle Controls

Participants' PA levels were consistent before each condition as measured by MET minutes during the 7 days leading up to each experimental visit ($P = 0.623$) and the number of steps taken on each pre-experimental day ($P = 0.329$). Similarly, no differences existed in the kilocalories consumed during the pre-experimental days ($P = 0.821$). Data for these variables are displayed in Supplemental Table S1.

Triglycerides and Cholesterol

No significant condition × time interaction was observed for TG ($P = 0.461$). However, there were significant main effects for both condition ($P = 0.045$; $\eta_p^2 = 0.25$) and time ($P = 0.004$; $\eta_p^2 = 0.30$). Post hoc analyses revealed that TG was lower after 10 K versus 2 K (means ± SE; −23 ± 7 mg/dL, $P = 0.027$, Fig. 2A), but no other differences were observed among conditions (all $P \geq 0.280$). Post hoc analyses for the main effect of time indicated that TG increased from BL to 90- (+33 ± 9 mg/dL, $P = 0.005$) and 120-min (+27 ± 9 mg/dL, $P = 0.035$) postmeal. There were no differences in TG tAUC ($P = 0.066$; $\eta_p^2 = 0.23$) or TG iAUC ($P = 0.930$; $\eta_p^2 = 0.02$) between conditions (Table 2).

No significant condition × time interactions were observed for HDL-C ($P = 0.885$) or LDL-C ($P = 0.994$). However, significant main effects of time were observed for both HDL-C ($P < 0.001$; $\eta_p^2 = 0.39$) and LDL-C ($P < 0.0001$; $\eta_p^2 = 0.62$). Post hoc analyses revealed that HDL-C decreased from BL to 30- (−4 ± 1 mg/dL, $P = 0.043$), 60- (−6 ± 1 mg/dL, $P < 0.001$), and 240-min (−5 ± 1 mg/dL, $P = 0.002$) postmeal (Fig. 2B). LDL-C also decreased from BL to 30- (−9 ± 2 mg/dL, $P = 0.001$), 60- (−16 ± 2 mg/dL, $P < 0.0001$), 90- (−9 ± 2 mg/dL, $P = 0.001$), 120- (−13 ± 2 mg/dL, $P < 0.0001$), and 240-min (−11 ± 2 mg/dL, $P < 0.0001$) postmeal, and from 30- to 60-min (−7 ± 2 mg/dL, $P = 0.014$) postmeal (Fig. 2C).

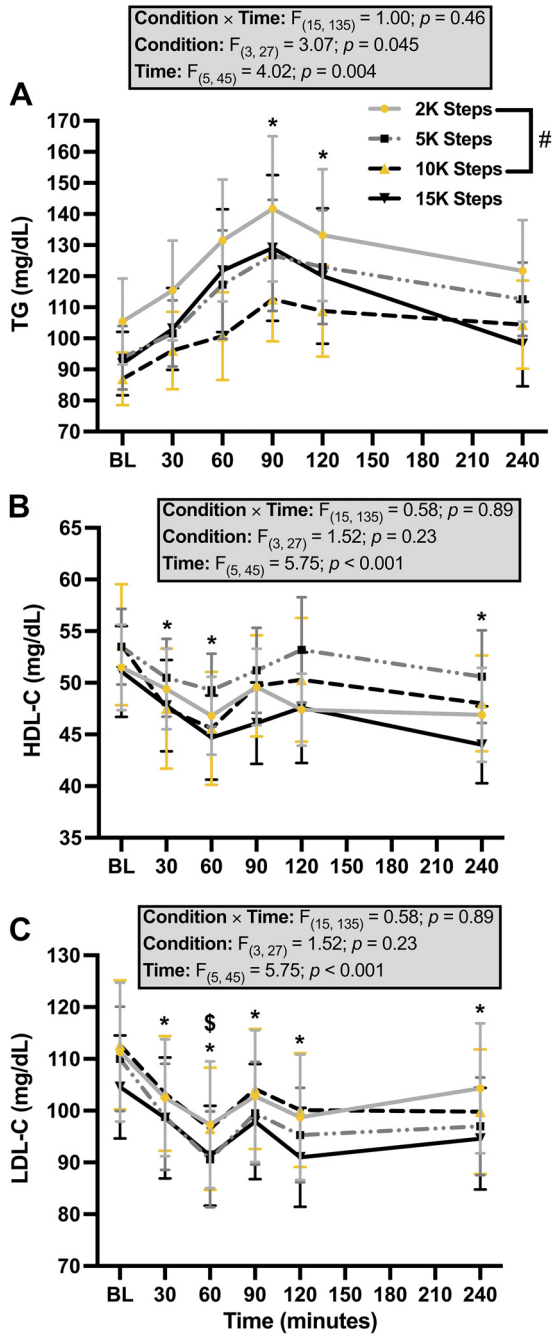

Figure 2. Triglycerides (TG; *A*), HDL-C (*B*), and LDL-C (*C*) before and after a high-fat mixed meal (HFMM) after walking 2 K, 5 K, 10 K, and 15 K steps throughout the day. Two-way repeated measures (Condition × Time) ANOVAs with follow-up lower order ANOVAs and/or adjusted, post hoc comparisons were used to analyze TG, HDL-C, and LDL-C responses; n = 10 (5 female). BL, baseline (i.e., immediately before the HFMM). Time effects denoted by * (different from BL, $P < 0.05$) and $ (different from 30 min, $P < 0.05$). Condition effect is denoted by # (TG were lower at 10 K compared with 2 K; $P = 0.027$). No other between-condition differences were observed for any outcome ($P > 0.05$). Data are expressed as means ± SE.

Nonesterified Fatty Acids

No significant condition × time interaction was observed for NEFA ($P = 0.744$). However, there were significant main effects for both time ($P < 0.0001$; $\eta_p^2 = 0.64$) and condition

Table 2. *Triglyceride and glucose AUC and resting energy expenditure*

	2 K	5 K	10 K	15 K	*P* Value
TG tAUC, mg/dL	509 ± 233	462 ± 177	417 ± 164	448 ± 214	0.066
TG iAUC, mg/dL	87 ± 76	87 ± 128	69 ± 98	81 ± 163	0.930
GLU tAUC, mg/dL	392 ± 59	404 ± 72	389 ± 52	411 ± 50	0.475
GLU iAUC, mg/dL	46 ± 71	51 ± 71	34 ± 56	59 ± 65	0.629
REE, kcal/day	1,726 ± 304	1,765 ± 307	1,748 ± 317	1,740 ± 307	0.552
REE, kcal/day/kg	20.8 ± 2.89	21.27 ± 2.90	21.05 ± 2.88	21.0 ± 2.96	0.501

Using a randomized crossover design, participants walked 2,000 (2 K), 5,000 (5 K), 10,000 (10 K), and 15,000 (15 K) steps throughout the day on 4 separate days ($n = 10$; 5 females). AUC were calculated using the pre- and 30-, 60-, 90-, 120-, and 240-min postmeal time points. AUC, area under the curve; GLU, glucose; iAUC, incremental AUC; REE, resting energy expenditure; TG, triglyceride; tAUC, total AUC. All data are presented here as means ± SD. *P* values are from one-way ANOVA analyses.

($P = 0.010$; $\eta_p^2 = 0.37$). Post hoc analyses for the main effect of the condition indicated that NEFA were elevated significantly after 15 K versus 2 K [predicted (LS) means ± SE difference; +86 ± 23 μmol/L; $P = 0.006$]. NEFAs were also 59 ± 23 μmol/L greater in 15 K than in 10 K, but this difference was not statistically significant after adjustment for multiple comparisons ($P = 0.078$). Post hoc analyses for the main effect of time indicated that NEFAs significantly decreased from BL to 60- (−220 ± 43 μmol/L; $P < 0.0001$), 90- (−268 ± 43 μmol/L; $P < 0.0001$), 120- (−264 ± 43 μmol/L; $P < 0.0001$), and 240-min (-312 ± 43 μmol/L; $P < 0.0001$) postmeal (Fig. 3). NEFA also decreased from 30- to 60- (−147 ± 43 μmol/L; $P = 0.015$), 90- (−195 ± 43 μmol/L; $P < 0.001$), 120- (−191 ± 43 μmol/L; $P = 0.001$), and 240-min (−239 ± 43 μmol/L; $P < 0.0001$) postmeal (Fig. 3).

Insulin and Glucose

No significant condition × time interaction ($P = 0.740$) or main effect of condition ($P = 0.924$) was observed for insulin. However, there was a significant main effect for time ($P < 0.0001$; $\eta_p^2 = 0.70$). Insulin significantly increased from BL to 30- [predicted (LS) means ± SE difference; +281 ± 40 pmol/L, $P < 0.0001$], 60- (+329 ± 41 pmol/L, $P < 0.0001$), 90- (+252 ± 41 pmol/L, $P < 0.0001$), 120- (+290 ± 40 pmol/L, $P < 0.0001$), and 240-min (+144 ± 40 pmol/L, $P = 0.011$) postmeal. Insulin was also significantly lower at 240- versus 30- (−137 ± 40 pmol/L, $P = 0.018$), 60- (−185 ± 40 pmol/L, $P < 0.001$), and 120-min (−146 ± 40 pmol/L, $P = 0.010$) postmeal (Fig. 4*A*).

No significant condition × time interaction ($P = 0.124$) or main effect of condition ($P = 0.394$) was observed in the PPG response. However, there was a significant main effect of time ($P < 0.0001$; $\eta_p^2 = 0.62$). Post hoc analyses revealed that glucose increased from BL to 30- (+24 ± 4 mg/dL, $P < 0.0001$) and 60-min (+29 ± 4 mg/dL, $P < 0.0001$) and from 30- to 90- (+14 ± 4 mg/dL, $P = 0.033$), 120- (+14 ± 4 mg/dL, $P = 0.041$), and 240-min (+22 ± 4 mg/dL, $P < 0.0001$) postmeal, and then decreased from 60- to 90- (−18 ± 4 mg/dL, $P = 0.002$), 120- (−18 ± 4 mg/dL, $P = 0.002$), and 240-min (−26 ± 4 mg/dL, $P < 0.0001$) postmeal (Fig. 4*B*). There were no differences in glucose tAUC ($P = 0.475$) or glucose iAUC ($P = 0.629$) between conditions (Table 2).

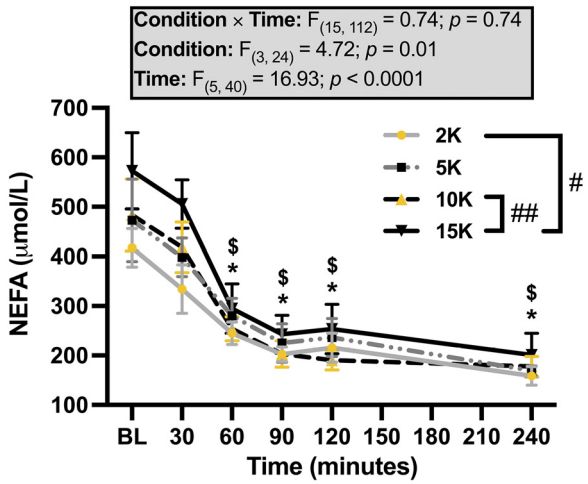

Figure 3. Nonesterified fatty acids (NEFAs) before and after a high-fat mixed meal (HFMM) after walking 2 K, 5 K, 10 K, and 15 K steps throughout the day. Two-way repeated measures (Condition × Time) ANOVAs with follow-up lower order ANOVAs and/or adjusted, post hoc comparisons were used to analyze NEFA responses; $n = 9$ (4 female). BL, baseline (i.e., immediately before the HFMM); Time effects denoted by * (different from BL, $P < 0.05$) and $ (different from 30 min, $P < 0.05$). Condition effect is denoted by # (NEFA were lower at 2 K compared with 15 K; $P = 0.006$) and marginal difference is denoted by ## (10 K vs. 15 K, $P = 0.078$). No other between-condition differences were observed ($P > 0.05$). Data are expressed as means ± SE.

Energy Balance

By design, energy balance, daily steps, TEE, and WEE were significantly different among conditions. Post hoc analysis confirmed that there were significant differences among conditions for each of these variables (all $P < 0.0001$; Fig. 5).

Substrate Oxidation

No differences in absolute REE or relative REE (scaled to body weight) were observed among conditions ($P = 0.575$ and $P = 0.527$, respectively; Table 2). Similarly, RER ($P = 0.055$; $\eta_p^2 = 0.24$) and FAT_{OX} ($P = 0.070$; $\eta_p^2 = 0.23$) were not significantly different among conditions (Fig. 6, *A* and *B*). Based on the observation that RER and FAT_{OX} responses to step-count dose appeared to be more prominent in men, we conducted an exploratory repeated-measures ANOVA analysis with sex included as a covariate. These analyses indicated that the effect of step-count dose on RER ($F_{3,24} = 3.7$; $P = 0.025$; $\eta_p^2 = 0.32$) and FAT_{OX} ($F_{3,24} = 3.1$; $P = 0.047$; $\eta_p^2 = 0.28$) were significant after accounting for sex, an effect driven by the men (*top right* corners of Fig. 6, *A* and *B*). Whereas the omnibus test was significant for RER after covarying for sex, there were no significant post hoc comparisons despite moderate to large-effect size differences observed between 2 K versus 5 K ($P = 0.38$, $d = 0.61$), 10 K ($P = 0.18$, $d = 0.72$), and 15 K ($P = 0.069$, $d = 0.86$). Similarly, while the omnibus test was significant for FAT_{OX} after covarying for

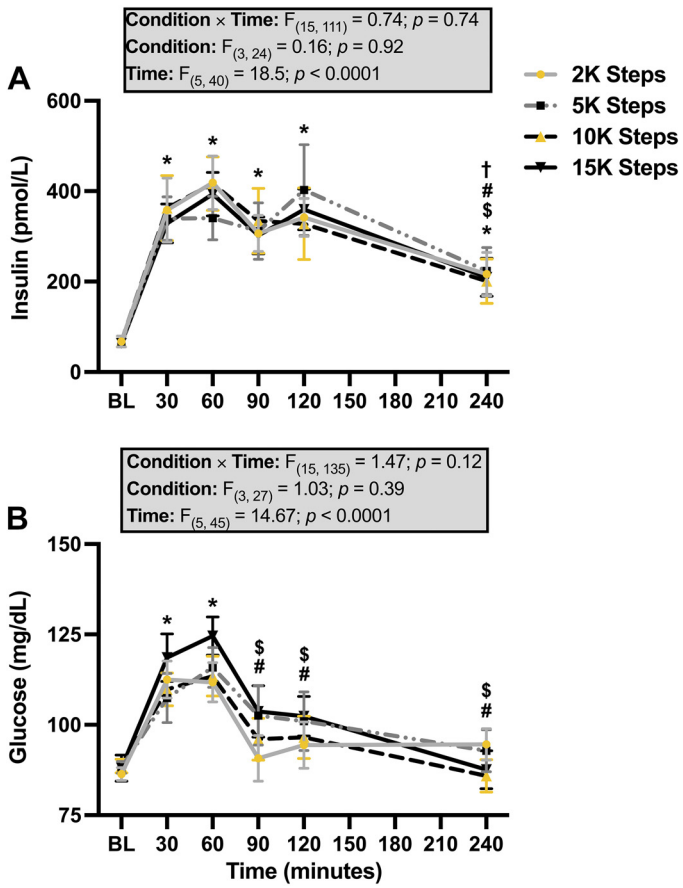

Figure 4. Insulin (*A*) and glucose (*B*) before and after a high-fat mixed meal (HFMM) after walking 2 K, 5 K, 10 K, and 15 K steps throughout the day. Two-way repeated measures (Condition × Time) ANOVAs with follow-up lower order ANOVAs and/or adjusted, post hoc comparisons were used to analyze insulin and glucose responses; $n = 9$ (4 female). BL, baseline (i.e., immediately before the HFMM). Time effects denoted by * (different from BL, $P < 0.05$), $ (different from 30 min, $P < 0.05$), # (different from 60 min, $P < 0.05$), and † (different from 120 min, $P < 0.05$). Data are expressed as means ± SE.

sex, there were also no significant post hoc comparisons despite moderate to large-effect size differences observed between 2 K versus 5 K ($P = 0.30$, $d = -0.65$), 10 K ($P = 0.25$, $d = -0.68$), and 15 K ($P = 0.12$, $d = -0.79$).

Correlations between Baseline NEFAs and Postprandial Glucose

BL NEFA levels were significantly correlated with glucose levels averaged across condition at 60- ($r = 0.419$, $R^2 = 0.176$, $P = 0.014$), 90- ($r = 0.507$, $R^2 = 0.257$, $P = 0.002$), and 120-min ($r = 0.553$, $R^2 = 0.306$, $P = 0.001$) postmeal consumption. No significant correlations between BL NEFA and glucose at BL, 30-, or 240-min postmeal consumption were observed (all $P \geq 0.075$).

Hunger, Satiety, and Palatability

No significant condition × time interactions were observed for subjective hunger, satiety, fullness, or prospective food consumption or subjective desire for salty, sweet, savory, or fatty food ($P \geq 0.375$). There were also no main effects of condition for any of these outcomes ($P \geq 0.283$). However, there were significant main effects of time for all of these outcomes ($P \leq 0.0006$), except for the desire for sweet food ($P = 0.169$). No effect of condition was observed for any of the palatability outcomes ($P \geq 0.303$). Data can be seen in Supplemental Table S2.

DISCUSSION

To our knowledge, the current study is the first to examine the effects of acute daily step count on same-day postprandial metabolism in response to an evening HFMM. The main finding from the present study was that walking 10 K steps throughout the day promoted the greatest reduction in PPL. While walking 15 K steps did not reduce PPL significantly compared with an inactive condition (i.e., 2 K), it did cause a significant elevation in NEFA levels, which may have impacted PPL as discussed below in paragraph 4 of the DISCUSSION. Furthermore, there were nonsignificant, but large effects ($P = 0.054$–0.071; $\eta_p^2 = 0.23$–0.24) of step count on resting (i.e., pre-HFMM) RER and FAT_{OX}, which were significant following adjustment for sex. In general, RER decreased and FAT_{OX} increased as step count increased, and this effect was driven by the men in the current study. However, there were no effects of step count on PPG, insulin, or hunger and satiety measures.

Our data showed that walking 10 K steps/day elicited the lowest PPL response to an evening HFMM. Specifically, after averaging the TG responses across time, we observed a decrease in PPL from 2 K to 10 K ($P = 0.027$), followed by a slight, but nonsignificant, increase from 10 K to 15 K. To our knowledge, the only other study that has examined the effects of daily step count alone (i.e., without additional exercise bouts) on PPL observed increased TG tAUC in response to a HFMM after decreasing daily steps from ~10,500 down to ~1,300 for 2 wk (21). Another study assessed the effects of a single 30-, 60-, or 90-min walking bout on same-day TG responses to two meals and found a slight, but insignificant ($P = 0.06$), decrease in TG as walking duration increased (22). Several studies have assessed the effects of daily background PA, in the form of walking, in combination with an acute moderate exercise bout on next-day PPL (16–18). Collectively, these studies have demonstrated that low daily step counts (e.g., ~2,500–5,000 steps/day) impair subsequent exercise-induced reductions in PPL when compared with higher step-count conditions (~8,500–17,000 steps/day) (16–19), a phenomenon termed "exercise resistance" (17). Thus, our study builds on this prior work and is the first to directly examine the effect of daily step count alone on postprandial metabolism utilizing a practical range of steps (i.e., 2,000–15,000) and an experimental design with high ecological validity. Overall, our results agree, in part, with the prior body of evidence suggesting that increasing daily step counts from 2 K to 10 K elicits a decrease in PPL. Notably, however, increasing steps beyond 10 K did not promote further improvements in PPL using the current study design.

One proposed mechanism for the TG-lowering effects of exercise has been the energy deficit created by exercise. Specifically, several studies have shown that aerobic exercise-induced decreases in next-day PPL are abolished when exercise calories are replaced, suggesting that negative energy balance may be a primary factor promoting reduced

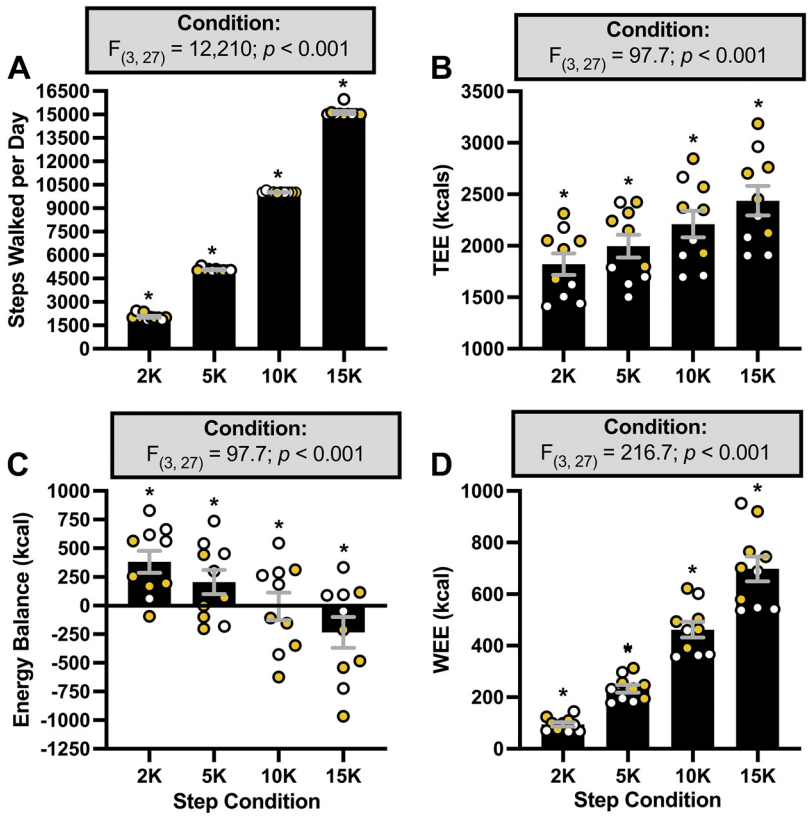

Figure 5. Steps walked in each condition (*A*) and estimated total energy expenditure (TEE; *B*), walking energy expenditure (WEE; *C*), and energy balance (*D*). $n = 10$ (5 female). One-way repeated measures ANOVAs with adjusted, post hoc comparisons were used for analyses of all dependent variables shown. *Significantly different from every other condition. Males and females are designated by yellow and white circles, respectively. Data are expressed as means ± SE.

PPL following exercise (18, 41–43). However, Maraki et al. (43) directly examined the effects of a diet- versus exercise-induced energy deficit on next-day PPL. Although both conditions reduced next-day PPL, the effect was strongest in the exercise versus the diet condition, indicating that exercise has unique effects on PPL that cannot be attributed to energy balance alone (43). By design, our study created different energy balances, whereby the energy consumed was the same in all conditions, but energy expenditure increased with step count. Accordingly, energy balance decreased linearly from +382 kcals in the 2 K to −234 kcals in the 15 K condition (Fig. 5*D*), yet the PPL response decreased linearly only from 2 K to 10 K, and then slightly, but nonsignificantly, increased from 10 K to 15 K. Thus, our data appear to support that the effects of PA (or exercise) on PPL are not solely attributed to the energy deficit created, at least when a HFMM is consumed within ~2 h after physical activity has been completed.

Although it is possible that the metabolic benefits of PA simply became saturated at 10 K, we propose an alternative hypothesis that mild, transient elevations in NEFAs after 15 K may be responsible for this observation (44). Indeed, we observed significantly greater NEFA in the 15 K condition before, and in the early stages after, meal consumption. This could be due to the higher step volume eliciting a greater

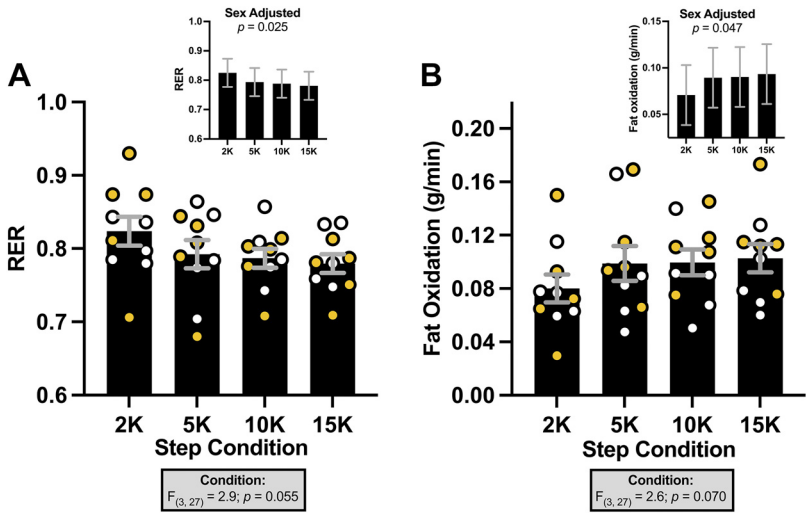

Figure 6. Respiratory exchange ratio (RER; *A*) and fat oxidation (*B*) (g/min), respectively, after walking 2 K, 5 K, 10 K, and 15 K steps throughout the day. $n = 10$ (5 female). One-way repeated measures ANOVAs with adjusted, post hoc comparisons were used for analyses of RER and fat oxidation in *A* and *B*. Sex-adjusted ANOVA results are presented in the *top right corner* of the main figures (*top right corners* of *A* and *B*). Males and females are designated by yellow and white circles, respectively. Data are expressed as means ± SE.

increase in neurally stimulated adipose lipolysis (45) and/or a greater increase in lipoprotein lipase activity, which would promote the release of fatty acids into the NEFA pool (46). NEFA supply to the liver is a primary determinant of hepatic VLDL-TG secretion and glucose production, and NEFAs compete with glucose for utilization in insulin-sensitive peripheral tissues such as skeletal muscle (47). As previously discussed (48), the effect of elevated NEFAs is most apparent postprandially, where plasma NEFA causes increased hepatic glucose production and VLDL-TG secretion despite meal-induced insulin release (49). Notably, elevated NEFAs appear to induce these hepatic effects ~1–2 h following their initial increase (50). While NEFAs were elevated across the 15 K condition independent of time, the elevations were most pronounced at BL and 30 min (Fig. 3*A*). Interestingly, glucose levels were greatest over the first 90–120 min of the HFMM in the 15 K condition, which may reflect elevated gluconeogenesis during this period. Notably, we also observed that baseline NEFA levels were significantly related to glucose levels at 60- ($r = 0.42$), 90- ($r = 0.51$), and 120-min ($r = 0.55$) postmeal, supporting the idea that elevated NEFAs may have impaired insulin-mediated suppression of endogenous glucose production (i.e., hepatic gluconeogenesis) 1–2 h later (49) in this study. Furthermore, around the time that the effects of NEFAs should be diminishing (50), we saw the sharpest decline in glucose, and importantly, TG levels during the 15 K condition. Considering the timing of NEFA-induced changes, it is possible that, had we measured PPL and PPG at 5- and 6-h postmeal, we may have observed additional metabolic benefits in the 15 K condition. However, given that most individuals are only 4–5 h postprandial before consuming their next meal, such a finding would be limited practically. Nevertheless, future studies may wish to examine the effects of evening meal timing, in relation to completion of daily steps, on PPL to more fully explore the possibility of transient, postwalking, NEFA-induced insulin resistance as suggested in this study.

We did not observe significant differences among conditions for either RER ($P = 0.054$) or FAT_{OX} ($P = 0.071$). Several of the aforementioned studies reported that decreased PPL in response to higher background daily step counts was accompanied by increased fat oxidation after HFMM consumption (16, 17, 19). As we measured substrate oxidation before HFMM consumption, while the prior studies measured it afterward, our differing findings are not surprising. Future studies assessing the effects of daily step count should consider measuring substrate oxidation both before and after HFMM consumption. Of note, RER tended to decrease and FAT_{OX} tended to increase from 2 K to 15 K in the current study, and the effect sizes ($\eta_p^2 = 0.24$ and 0.23, respectively) were large. Substrate oxidation was not our primary outcome, and it is likely that we were underpowered to detect differences in resting RER and FAT_{OX}. Furthermore, although we did not plan to—and were thus not powered to—detect sex differences for RER or FAT_{OX} in our sample, we noticed that the changes in RER and FAT_{OX} across conditions appeared to be most dramatic in men. Thus, we conducted a secondary exploratory analysis adjusted for sex, in which both RER and FAT_{OX} were statistically significant ($P = 0.020$ and $P = 0.049$, respectively). Thus, our interpretation is that increasing step count tends to increase resting fat oxidation, an effect that may be strongest in young men. Future studies with larger sample sizes will be needed to better understand potential sex differences in these responses.

In agreement with the aforementioned studies, which assessed the effects of differing background daily steps combined with acute exercise on postprandial responses (16, 19), the current study found no differences in PPG among conditions. We also found no differences in insulin levels among conditions. No prior studies have examined the acute effects of daily step count alone on insulin sensitivity. However, a study that had participants decrease their daily steps from 10,000 or 6,500 per day down to 1,300 steps/day found that decreasing steps decreased insulin sensitivity after 2–3 wk (21). Rohling et al. (51, 52) previously indicated that intensity seems to be the primary determinant influencing the degree of improvement in glucose metabolism following exercise. In addition, Pfeiffer et al. (22) reported no differences between postprandial insulin levels after intensity-matched walking bouts of different durations (i.e., 30, 60, and 90 min). As such, we would not expect to see differences in the insulin response to the meal tolerance test as our walking intensity was consistent across conditions (i.e., 100 steps/minute) and only volume was manipulated. Furthermore, as previously described, acute elevations in circulating NEFA may have obscured any benefits of walking on PPG and insulin by promoting acute decreases in hepatic insulin sensitivity in the present study.

Our study was not without limitations. First, we only assessed whole body substrate oxidation and energy expenditure at rest and not postprandially, a decision that was based on prior studies indicating that fat oxidation is increased similarly postexercise both before and after a high-fat meal (i.e., pre- and postprandially) (53, 54). However, repeated measurement would have certainly increased statistical power to detect between-condition effects. Several other outcomes not observed in the current study could also help to explain our findings with regard to step-count dose on PPL. For example, exercise dose could have influenced gastric emptying time (55) and thus the rate of appearance of postprandial TG and glucose in circulation. Furthermore, it is possible that there is a dose-response association between step count and catecholamine release, which promote lipolysis and reduce insulin secretion but were not measured in this study. We also did not observe glycerol as an indicator of exercise-induced fat oxidation (56) or c-peptide as an indicator of insulin secretion. Finally, future studies may wish to examine whether cardiorespiratory fitness may explain variability in the effect of step-count dose on PPL, whereby perhaps those with higher fitness are protected against impairments in lipid metabolism in response to lower activity and thus display a lesser effect of step-count dose on PPL.

In conclusion, our randomized controlled study examining the effect of step count on postprandial metabolism in young adults used a novel design that maximizes ecological validity. Our data indicate that walking 10,000 steps/day is an effective dose for reducing the PPL response to an evening meal, compared with a sedentary condition (2 K). NEFA levels were highest after the 15 K versus the 2 K conditions, which may have contributed to our observation that increasing steps to 15 K does not promote further improvements in PPL. Furthermore, increasing step dose increased resting FAT_{OX} following sex adjustment, an effect that was driven by the men in the current study. No changes in PPG, insulin, or hunger and satiety measures were observed among step

doses. As PPL is a strong independent predictor of CVD (13), the findings from the current study hold great practical value and may be used to guide daily step-count recommendations to reduce CMD risk in young adults. The present findings also complement the epidemiological evidence surrounding the dose-response relationship between daily step count and CVD mortality and morbidity up to ~10,000 steps/day (6, 7, 57). Further investigation of the effects of daily step count on metabolic health in clinical and older populations is warranted, as are future studies powered to examine the potential sex-related differences observed herein.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL DATA

Supplemental Table S1: https://www.doi.org/10.6084/m9.figshare.24076233.

Supplemental Table S2: https://www.doi.org/10.6084/m9.figshare.24076242.

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DISCLAIMERS

The current study did not receive any external funding. All costs were covered using N.D.M.J.'s start-up funds. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

DISCLOSURES

Within the past 2 years, E.M.R. and N.F.B. have received graduate assistant stipend funding from Woodbolt, LLC. E.M.R. has received grant funding from the American College of Sports Medicine. N.F.B. and N.D.M.J. have received grant funding from the National Strength and Conditioning Association. N.D.M.J. has received grant funding from the American Heart Association, the Center for Integrative Research on Childhood Adversity (Award P20GM109097 through the NIGMS), the Injury Prevention Research Center (Award R49 CE003095 through the NCIPC/CDC), Woodbolt, LLC, and Applied Food Sciences, Inc., and has been the recipient of an NIH Clinical Research Loan Repayment Award. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

E.M.R., N.F.B., and N.D.M.J. conceived and designed research; E.M.R., N.F.B., and N.D.M.J. performed experiments; E.M.R., N.F.B., and N.D.M.J. analyzed data; E.M.R., N.F.B., and N.D.M.J. interpreted results of experiments; E.M.R. prepared figures; E.M.R. drafted manuscript; E.M.R., N.F.B., and N.D.M.J. edited and revised manuscript; E.M.R., N.F.B., and N.D.M.J. approved final version of manuscript.

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