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ORIGINAL ARTICLE

Acute continuous moderate-intensity exercise, but not low-volume high-intensity interval exercise, attenuates postprandial suppression of circulating osteocalcin in young overweight and obese adults

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Abstract

Summary Bone remodeling markers (BRMs) are suppressed following the consumption of a meal. Our findings indicate that a single session of continuous moderate-intensity exercise, but not low-volume high-intensity interval exercise, performed 1 h after a meal attenuates the postprandial suppression of BRMs.

Introduction Acute exercise transiently increases BRMs including osteocalcin (tOC) and the undercarboxylated form of osteocalcin (ucOC), a hormone that is implicated in glucose regulation. The effects of acute exercise and exercise-intensity on postprandial levels of tOC and ucOC are unknown.

Methods Twenty-seven adults that were overweight or obese (age 30 ± 1 years; BMI 30 ± 1 kg·m^{-2}; mean ± SEM) were randomly allocated to perform a single session of low-volume high-intensity interval exercise (LV-HIIE; nine females, five males) or continuous moderate-intensity exercise (CMIE; eightfemales, five males) 1 h after consumption of a standard breakfast. Serum tOC, ucOC, and ucOC/tOC were measured at baseline, 1 h, and 3 h after breakfast consumption on a rest day (no exercise) and the exercise day (exercise 1 h after breakfast).

Results Compared to baseline, serum tOC and ucOC were suppressed 3 h after breakfast on the rest day (− 10 ± 1% and − 6 ± 2%, respectively; $p < 0.05$), whereas ucOC/tOC was elevated (2.5 ± 1%; $p = 0.08$). Compared to the rest day, CMIE attenuated the postprandial-induced suppression of tOC (rest day − 10 ± 2% versus CMIE − 5 ± 2%, $p < 0.05$) and ucOC (rest day − 6 ± 4% versus CMIE 11 ± 2%, $p < 0.05$), and increased postprandial ucOC/tOC (rest day 3 ± 2% versus CMIE 15 ± 1%, $p < 0.05$). In contrast, LV-HIIE did not alter postprandial tOC, ucOC, or ucOC/tOC (all $p > 0.1$).

Conclusions Acute CMIE, but not LV-HIIE, attenuates the postprandial-induced suppression of tOC and ucOC. CMIE may be an effective tool to control the circulating levels of BRMs following meal consumption in overweight/obese adults.

Keywords Bone metabolism · Glycemic control · HIIT · Osteoblast

Nigel K. Stepto and Itamar Levinger are co-senior author.

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Introduction

Osteoblasts are bone forming cells which are primarily involved in the synthesis, deposition, and mineralization of type 1 collagen to form bone during growth [1]. During adulthood, osteoblasts and osteoclasts (bone resorbing cells) participate in bone remodeling. This renewal process maintains the quality of bone's material composition by removing and replacing damaged bone with newly synthesized type 1 collagen which undergoes mineralization to form bone [1].

Osteoblasts also synthesize and secrete paracrine proteins including osteocalcin (tOC), and the undercarboxylated form (ucOC), which participate not only in bone mineralization but also in glucose homeostasis [2, 3]. Indeed, higher circulating levels of osteocalcin (tOC and ucOC) correlate with increased insulin sensitivity and improved glycemic control in both human and mice models [3, 4]. In contrast, lower circulating tOC and ucOC is commonly reported in obese individuals and patients with type 2 diabetes and correlates with increased insulin resistance and elevated basal and postprandial hyperglycemia [4–7]. Furthermore, ucOC treatment improves glycemic control in animal models, suggesting a role for ucOC in the maintenance of insulin sensitivity and euglycemia [8]. However, previous studies have established a potential bidirectional osteocalcin/glucose feed-forward loop [9]. For example, tOC and ucOC decrease following nutrient intake such as an oral glucose tolerance test or consumption of a mixed meal [9–11]. Furthermore, postprandial hyperglycemia during a 2 h glucose tolerance test is associated with lower circulating concentrations of bone remodeling markers (BRMs) and decreased circulating tOC [12], supporting a bidirectional feedforward loop between osteocalcin and glucose. The effect of hyperglycemia-induced suppression of BRMs is unclear but may lead to impaired bone remodeling and/or bone quality which may be involved in the increased fracture risk observed in obese people and those with type 2 diabetes [13]. As such, finding strategies to minimize or prevent the effects of postprandial suppression of BRMs in populations at high risk of type 2 diabetes (e.g., obesity) may be beneficial for the improvement and maintenance of bone health and/or glucose regulation.

In contrast to meal consumption, acute exercise transiently increases circulating levels of ucOC, and perhaps also tOC, which correlates with improved glycemic control and increased post-exercise insulin sensitivity in humans [14–16]. However, the capacity of exercise to prevent postprandial suppression of osteocalcin has only recently been investigated [11, 17]. It was reported that continuous moderate-intensity cycling exercise (CMIE) and high-intensity interval cycling exercise (HIIE) had little effect on the postprandial suppression of tOC, ucOC, and other BRMs including beta C-terminal telopeptide (β-CTx) and procollagen type 1 N-terminal propeptide (P1NP) [11, 17]. However, exercise was performed in the hours prior to insulin/glucose infusion or oral glucose tolerance test. Low-volume high-intensity interval exercise (LV-HIIE) has previously been shown to elicit similar and in some cases greater training adaptations when compared to higher-volume CMIE [18]. Whether acute LV-HIIE, or CMIE, performed during the postprandial period can minimize or prevent the meal-induced suppression of tOC and/or ucOC is unknown. We hypothesized that acute bouts of either HIIE or CMIE, performed 1 h after meal consumption, would attenuate the meal-induced suppression of serum tOC and ucOC.

Material and methods

Participants

Additional analysis was conducted on serum that was collected from 27 males and females who were classified as overweight or obese and participated in a previously published study [19]. Participants were sedentary and had not participated in any regular moderate to high levels of physical activity within the past 3 months. Body composition analysis was conducted via a 3D whole body light scanner (Textile/Clothing Technology Corp. $[TC]^2$, Cary, NC) [20]. Participants were randomized (block randomization) into LV-HIIE (five males, nine females) and CMIE (five males, eight females) groups, and matched for body mass (LV-HIIE 84.1 ± 5.1; CMIE 83.3 ± 5.6 kg, $p = 0.92$), body fat percentage (LV-HIIE 33.7 ± 3.0; CMIE 35.7 ± 3.1, $p = 0.50$), age (LV-HIIE 30 ± 1; CMIE 30 ± 2 years, $p = 0.96$), and VO_{2peak} (LV-HIIE 28.7 ± 2.2; CMIE 28.8 ± 1.9 ml kg^{-1} min^{-1}, $p = 0.98$). Females were tested in the early follicular phase of the menstrual cycle. All participants provided written informed consent. This study was approved by the Victoria University Human Research Ethics Committee.

Study design (Fig. 1)

A detailed description of the study protocol has previously been described [19]. In brief, after initial screening, participants underwent a graded exercise test (GXT) on a cycle ergometer (Lode Excalibur Sport) to determine peak aerobic capacity (VO_{2peak}) and maximal sustained power output (W_{max}) [19]. The W_{max} obtained during the exercise test was used to calculate the workload for the LV-HIIE or CMIE in the subsequent main testing sessions. Participants were instructed to abstain from physical activity (48 h) and alcohol and caffeine consumption (24 h) prior to each testing session.

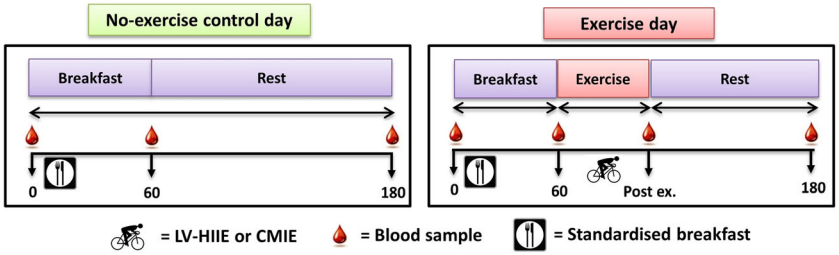

Fig. 1 Study overview. Participants ingested a standardized breakfast on a no-exercise control day and on a separate day when exercise was performed 1 h after breakfast consumption. LV-HIIE low-volume high-intensity interval exercise, CMIE continuous moderate-intensity exercise

Rest control day—postprandial response to a standard breakfast

A minimum of 1 week after completing the GXT session, participants arrived fasted in the lab (~ 10 h overnight fast) and ingested a standardized breakfast. Daily energy and macronutrient intake for the standardized breakfast—which consisted of Kellogg's® Corn Flakes and Kellogg's® All-Bran®, honey, and full cream milk—were based on sex, height, and weight using the Mifflin St. Jeor equation [21]. The diet adhered to the Australian and New Zealand dietary targets for healthy eating and the energy content consisted of approximately 55% carbohydrate, 30% fat, and 15% protein [22]. Immediately after breakfast ingestion, participants rested in the laboratory for a total of 3 h. Blood samples were taken at baseline (prior to breakfast), 1 h, and 3 h after breakfast.

Exercise day—postprandial response when exercise is performed 1 h after a standard breakfast

On the next day, participants again reported to the laboratory in the morning after an overnight fast. Participants then consumed the same standardized breakfast as per the control day. One hour after breakfast consumption, participants performed a single session of either randomly allocated LV-HIIE or CMIE. The LV-HIIE protocol consisted of 8 × 1-min cycling bouts at 100% of W_{max} interspersed with 1-min active recovery periods at 50 W and included a 5-min warm-up and 3-min cool-down at 50% of W_{max}. The CMIE session consisted of 38 ± 1 min of continuous cycling at 50% of W_{max}. The total work performed in the exercise session was significantly greater for CMIE compared to LV-HIIE (CMIE 191 ± 15 kJ; LV-HIIE 147 ± 13 kJ, $p < 0.05$). After completing the exercise session, participants immediately rested on a hospital bed for the remainder of the trial. Venous blood samples were taken at baseline, pre-exercise (1 h after breakfast), immediately after exercise, and 1.5 h after exercise (3 h after breakfast).

Blood sampling

Venous blood was collected from an antecubital vein via an intravenous cannula and kept patent with 0.9% sterile saline. Blood collected in serum-separating tubes were left at room temperature for 10 min to allow clotting, and then immediately centrifuged at 3500 rpm for 15 min at 4 °C. Serum was collected, aliquoted, and stored at − 80 °C until analyzed.

Biochemical analysis

Serum tOC was measured using an automated immunoassay (Elecsys 170; Roche Diagnostics). Serum ucOC was measured by the same immunoassay after adsorption of carboxylated OC on 5 mg/mL hydroxyl-apatite slurry, following the method described by Gundberg et al. [23]. The proportion (percentage) of ucOC among whole OC molecules in serum was calculated and is reflected by the serum ucOC/tOC ratio.

Statistical analysis

Data were checked for normality and analyzed using Predictive Analytics Software (PASW v20, SPSS Inc.). Comparison of multiple means for serum tOC and ucOC and the ucOC/tOC ratio on the rest day were analyzed using a two-factor (group × timepoint) repeated measures ANOVA with time (baseline, 1 h, and 3 h postprandial) as the within-subjects factor and group (LV-HIIE and CMIE) as the between-subjects factor. Comparison of multiple means for serum tOC and ucOC, and the ucOC/tOC ratio on the exercise day were analyzed using a two-factor (group × timepoint) repeated measures ANOVA with time (baseline, post-breakfast, post-exercise, and 3 h postprandial [1.5 h post-exercise]) as the within-subjects factor and group (LV-HIIE and CMIE) as the between-subjects factor. The percent change in serum measurements (percent change from baseline and/or pre-exercise) was analyzed using a two-factor (group × day) repeated measures ANOVA with day (rest and exercise day) as the within-subjects factor and group (LV-HIIE and CMIE) as the between-subjects factor. Significant interactions and main effects were explored using Fisher's LSD post hoc analysis test. Pearson's correlation coefficient was used to identify significant associations between postprandial serum tOC, ucOC, tOC/ucOC, glucose, and insulin. All data are reported as mean ± standard error of mean (SEM), and all statistical analyses

were conducted at the 95% level of significance ($p \leq 0.05$). Trends are reported when $p > 0.05$ and < 0.1.

Results

The effects of meal consumption on osteocalcin

On the resting day, a significant main effect of time was detected for serum tOC, ucOC, and ucOC/tOC (all $p < 0.05$). Serum tOC decreased 1 and 3 h after breakfast, ucOC decreased 3 h after breakfast, and ucOC/tOC increased 1 h after breakfast which remained elevated 3 h after breakfast (Fig. 2a–f).

The effects of acute exercise on postprandial-induced suppression of osteocalcin

Significant interaction effects were detected for serum tOC, ucOC, and ucOC/tOC (all $p < 0.05$). Despite a transient increase in serum tOC and ucOC after LV-HIIE, serum tOC and ucOC at 3 h after breakfast was suppressed to a similar extent on both the resting day and LV-HIIE day (Fig. 2a, c). In contrast, CMIE performed 1 h after breakfast attenuated the 3 h postprandial suppression of tOC and completely restored the postprandial suppression of ucOC (Fig. 2b, d). Furthermore, elevated postprandial ucOC/tOC measured 3 h after breakfast on the rest day was elevated to a greater extent after CMIE, with no effect of LV-HIIE (Fig. 2e, f).

The effects of exercise on 3 h postprandial serum osteocalcin (percent change from baseline)

Comparing the percent change from baseline to 3 h after breakfast confirmed that the postprandial-induced suppression of serum ucOC and tOC, and postprandial increase in the ucOC/tOC ratio, was similar on the rest day and the exercise day when LV-HIIE was performed 1 h after breakfast (Fig. 3a–c). In contrast, when CMIE was performed 1 h after breakfast, the postprandial suppression of tOC and ucOC was attenuated, and the postprandial increase in the ucOC/tOC ratio was enhanced (Fig. 3a–c).

Post-exercise insulin and glucose

Significant interaction effects were detected for post-exercise glucose ($p < 0.01$) and insulin ($p < 0.01$). Blood glucose decreased after CMIE (percent change from baseline) and was significantly lower compared to LV-HIIE (Fig. 4a). In contrast, serum insulin increased after LV-HIIE and was significantly elevated compared to CMIE (Fig. 4b).

Correlations

Lower average postprandial tOC levels measured during the exercise trial were correlated with higher average postprandial glucose ($r = -0.53$, $p < 0.01$) and insulin levels ($r = -0.48$, $p < 0.05$). Likewise, lower 3 h postprandial tOC correlated with higher post-exercise glucose ($r = 0.50$, $p < 0.01$) and insulin ($r = -0.46$, $p < 0.05$).

Sample size

A post hoc power calculation (G*Power 3.1.9.2; two-tailed independent *t* test between LV-HIIE and CMIE groups, alpha = 0.05) using the serum 3 h postprandial ucOC percent change data on the exercise day (LV-HIIE $-6.4 \pm 11.4\%$ [mean ± SD]; CMIE $11.4 \pm 8.2\%$; effect size of 1.75) determined that the study was adequately powered (99%) for the primary outcome.

Discussion

We report that a single session of CMIE performed 1 h after consuming a standard carbohydrate-rich breakfast attenuates the postprandial suppression of tOC, prevents the postprandial suppression of ucOC, and enhances the postprandial increase in the ucOC/tOC ratio in overweight/obese adults. In contrast, LV-HIIE had minimal effect on postprandial osteocalcin levels possibly due, at least in part, to a transient elevation in post-exercise blood glucose.

BRMs decrease in the hours after the ingestion of nutrients such as a glucose challenge or mixed meal [9–11, 24]. In support, we report suppressed serum tOC and ucOC and an increase in the ucOC/tOC ratio 3 h after consumption of a standard breakfast in overweight/obese adults. It is possible that suppressed BRMs following meal consumption may contribute to poorer bone health, and increased fracture risk often reported in populations with elevated fasting or postprandial hyperglycemia [13]. As such, exploring effective strategies that can minimize the postprandial suppression of circulating BRMs may be of importance for the improvement and/or maintenance of bone health and glycemic homeostasis in these populations.

In contrast to meal consumption, acute exercise transiently increases tOC and ucOC in obese males [14, 15]. However, the use of acute exercise as a potential strategy for the prevention of postprandial-induced suppression of BRMs, including tOC and ucOC, is less clear [11, 17]. It was previously reported that both acute cycling HIIE and CMIE had no effect on preventing the postprandial suppression of BMRs, tOC, and ucOC, when exercise is performed prior to ingestion of a glucose challenge or glucose/insulin infusion [11, 17]. Due to the rapid and transient increase in circulating osteocalcin after exercise, it is possible that the benefits of exercise are only experienced when exercise

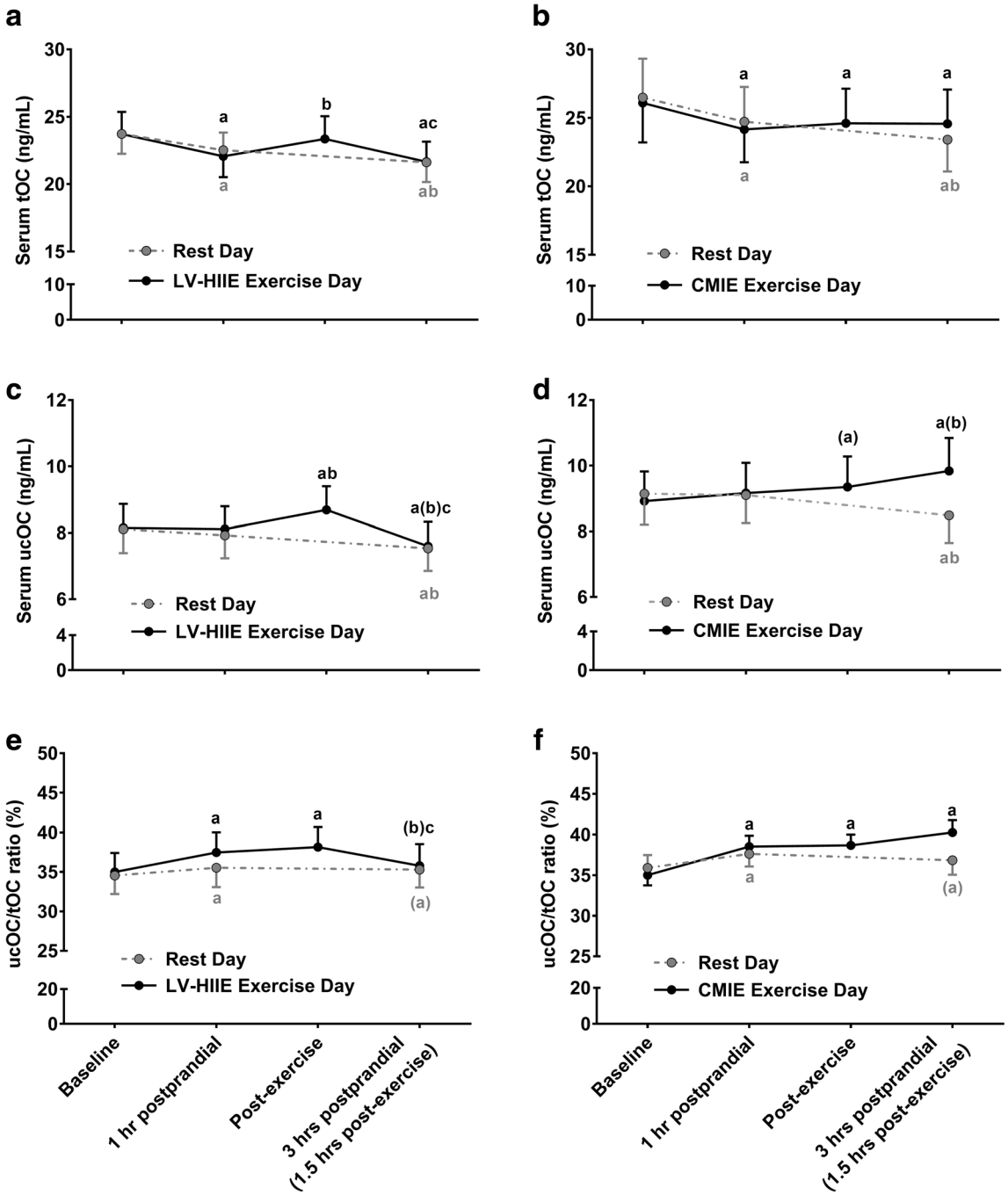

Fig. 2 The effects of acute high-intensity and moderate-intensity exercise on postprandial serum osteocalcin. LV-HIIE low-volume high-intensity interval exercise, CMIE continuous moderate-intensity exercise. **a** Significantly different to baseline ($p < 0.05$). **b** Significantly different to 1 h postprandial ($p < 0.05$). **c** Significantly different to post-exercise ($p < 0.05$). Letters in parenthesis indicate $p < 0.1$. Values are mean ± SEM. $N = 27$

is performed during the postprandial period (i.e., soon after meal ingestion). In support, we provide evidence that acute CMIE performed 1 h after breakfast ingestion, but not LV-HIIE, attenuates the postprandial suppression of tOC and ucOC, and increases the postprandial ucOC/tOC ratio. Taken together, our data indicates that the effects of acute exercise on postprandial osteocalcin are complicated, and is likely mediated by both the timing of exercise relative to meal ingestion and the intensity and/or mode of exercise.

The reason why LV-HIIE did not prevent postprandial suppression of osteocalcin is unclear but may stem from increased counter-regulatory hepatic glucose production often reported after higher-intensity exercise [25]. We have previously reported that LV-HIIE, and other forms of HIIE, leads to

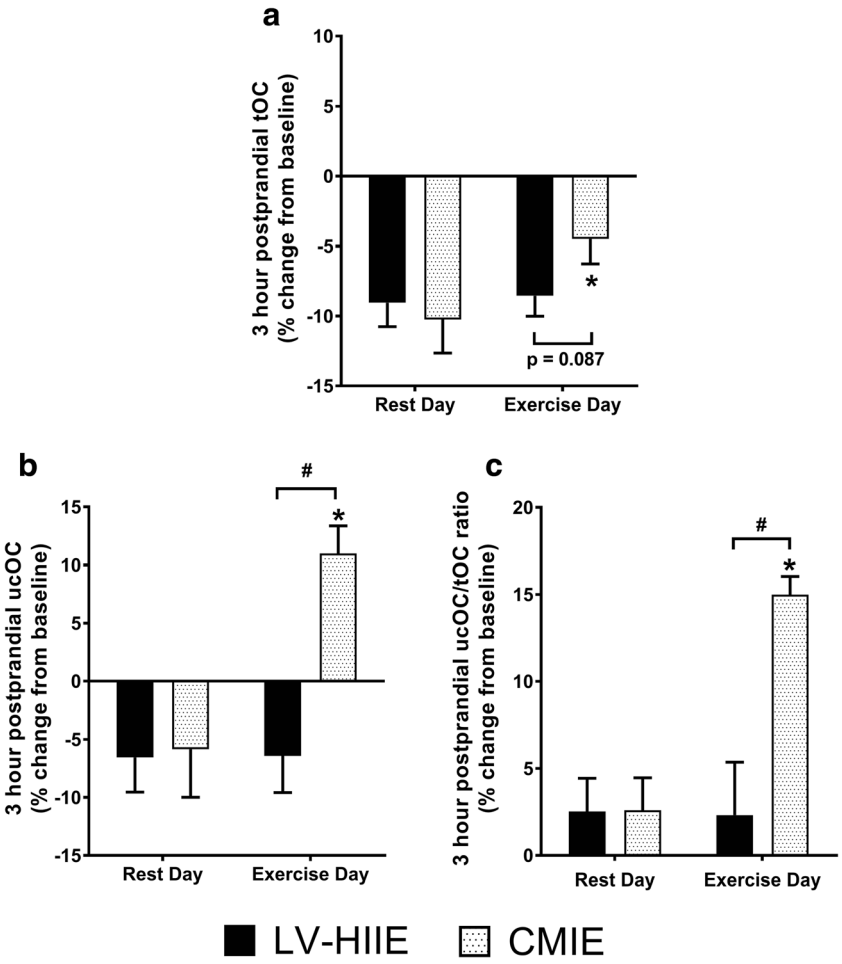

Fig. 3 The effects of exercise on 3 h postprandial serum osteocalcin (percent change from baseline). LV-HIIE low-volume high-intensity interval exercise, CMIE continuous moderate-intensity exercise. #Significantly different to LV-HIIE ($p < 0.05$). *Significantly different to rest day ($p < 0.05$). Values are mean ± SEM. $N = 27$

significantly elevated post-exercise insulin and glucose levels compared to CMIE [19, 26]. The postprandial suppression of BRMs is said to originate largely from elevated glucose levels [27]. In the current study, post-exercise glucose was significantly higher after LV-HIIE compared to CMIE and this correlated with lower circulating tOC. As such, the transient increase in glucose after acute LV-HIIE may in part explain the lack of change in circulating serum osteocalcin. Taken together, our findings support the bidirectional feed-forward loop of osteocalcin and glucose regulation, where higher glucose levels suppress osteocalcin levels, and lower circulating osteocalcin leads to increased hyperglycemia [11, 27].

A potential limitation of the study is the inclusion of both male and females, as the levels of BRMs can be different between sexes. However, the sample size and participant characteristics (including height and weight) of males and females were well

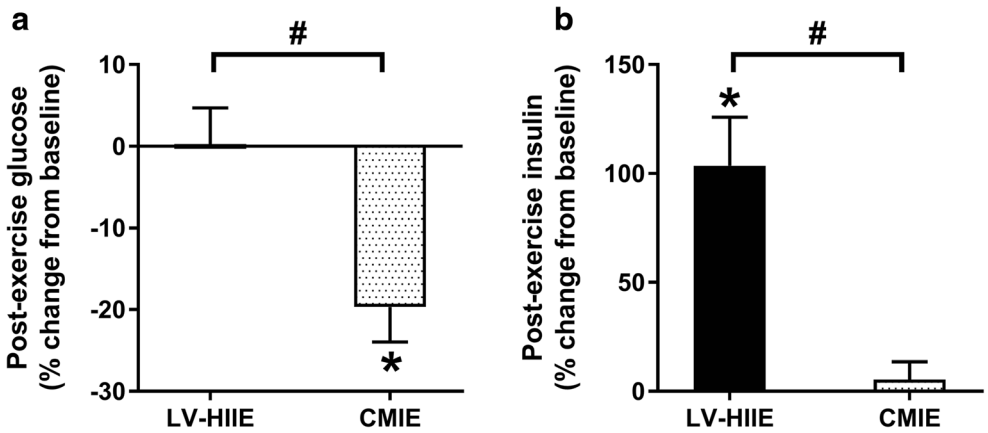

Fig. 4 The effects of acute exercise on peak post-exercise glucose and insulin. LV-HIIE low-volume high-intensity interval exercise, CMIE continuous moderate-intensity exercise. #significant difference between LV-HIIE and CMIE ($p < 0.05$). *Significant difference to baseline ($p < 0.05$). Values are mean ± SEM. $N = 27$

matched between LV-HIIE and CMIE exercise groups, and we used a randomized, control, repeated-measures design. In addition, all females were tested in the early follicular phase of the menstrual cycle to minimize any potential confounding effects of hormonal fluctuations on the outcome measures. We assessed aerobic capacity by assessing VO_{2peak}, defined as the highest VO_2 value recorded during an incremental exercise test. However, we acknowledge that this protocol alone may not reflect true VO_{2max}, as has been recently discussed [28]. Another limitation is that we did not measure other BMRs such as β-CTx and P1NP, as the main focus on this paper was to explore tOC and ucOC which are proposed to play a larger role in whole-body glucose metabolism [4]. Another consideration is that the two exercise bouts differed in both exercise intensity and volume; as such, we cannot ascertain whether the observed differences between LV-HIIE and CMIE are a function of exercise intensity, volume, or a combination of both. Future research will be required to explore the independent manipulation of exercise intensity and volume on postprandial BRMs. Finally, this study used a parallel design. Despite the appropriate matching of participant demographics between groups and the inclusion of a no-exercise control day, future research may benefit from adopting a randomized cross-over design (with appropriate washout) to confirm these findings.

In conclusion, we report that acute CMIE, but not LV-HIIE, attenuates postprandial suppression of tOC, prevents postprandial suppression of ucOC, and increases the ucOC/tOC ratio when performed 1 h after breakfast consumption in overweight/obese adults. As such, CMIE may be a useful tool to control circulating levels of BRMs following a meal.

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Author's contribution LP, CSS, NKS, and IL contributed to the study design and acquirement of ethical approval. LP and CSS contributed to data collection. EB performed biochemical analysis of serum osteocalcin and uncarboxylated osteocalcin. LP statistically analyzed the data and drafted the initial manuscript. The remaining authors critically revised the manuscript. All authors approved the final version of the manuscript. LP is the guarantor of the manuscript and takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

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Compliance with ethical standards

Conflicts of interest None.

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