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Nutrition and exercise physiology

Age-Related Differences in the Appetite-Regulating Hormone Response to Exercise

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A B S T R A C T

Background: Acute exercise alters appetite-regulating hormones like peptide tyrosine tyrosine (PYY), glucagon-like peptide-1 (GLP-1), and ghrelin, suppressing appetite and reducing food intake. The effect of exercise on hunger and satiety has been shown to vary by body composition, sex, and habitual physical activity, but the influence of aging is less understood.

Objectives: We aimed to examine age-related differences in the effect of acute exercise on appetite-regulating hormones.

Methods: Participants from 2 age cohorts (younger adults, 19–29 y, $n = 39$; older adults, 65–75 y, $n = 29$) completed 2 45-min study conditions on separate days in randomized order: *1*) an exercise bout (60% $\dot{V}O_{2peak}$) on a bicycle ergometer (*Exercise*), and *2*) a seated rest period (*Rest*). Plasma concentrations of PYY 3–36 (PYY3–36), GLP-1, and acylated ghrelin, as well as subjective perceptions of hunger, fullness, thirst, and nausea (via visual analog scales), were measured before a standardized snack (fasted) and before and after a subsequent exercise/rest condition.

Results: *Exercise* induced a greater increase in PYY3–36 relative to *Rest* in younger adults compared to older adults (difference: 26.6 pg/mL; 95% confidence interval (CI): 3.4, 49.8 pg/mL; $P = 0.025$). GLP-1 concentrations were consistently greater in older adults independent of the study condition (*Exercise/Rest;* all $P < 0.001$), but the GLP-1 response to exercise did not differ by age group ($P = 0.456$). Similarly, exercise responses in acylated ghrelin ($P = 0.114$) and subjective appetite perceptions (all $P \geq 0.288$) did not differ between younger adults and older adults.

Conclusions: The present study showed age-related differences in the appetite-regulating hormone response to 45 min of nonfasted, moderate-intensity exercise in PYY3–36 but not GLP-1 or acylated ghrelin. The age-related variations did not translate into differences in subjective hunger or fullness.

Keywords: aging, aerobic exercise, appetite, hormones, satiety

Introduction

Appetite and ingestive behavior are physiologically regulated by a complex interplay between central neural networks and peripheral sensory signals [1]. Gastrointestinal hormones, such as the anorexigenic (appetite-suppressing) hormones peptide tyrosine tyrosine (PYY) and glucagon-like peptide 1 (GLP-1), and the orexigenic (appetite-stimulating) hormone ghrelin, are important modulators of food intake [2]. Studies also suggest that circulating concentrations of these appetite-regulating hormones are acutely altered by exercise, resulting in suppressed appetite and temporarily reduced food intake postexercise [3–7]. However, these exercise-induced changes in appetite-regulating hormones can be modulated by a variety of factors, such as

Abbreviations: CV, coefficient of variation; *Exercise*, exercise condition (60% $\dot{V}O_{2peak}$) on a bicycle ergometer; GLP-1, glucagon-like peptide-1; PYY, peptide tyrosine tyrosine; *Rest*, control condition (seated rest period); RPE, ratings of perceived exertion.

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exercise mode, duration, and intensity [3,8,9], as well as individual characteristics, including body composition, nutritional status, sex, and habitual physical activity [3,10,11].

Healthy aging is associated with (often unintentional) reductions in appetite and energy intake, which affect 15–30% of independently living older adults and even more in hospital and nursing home settings [12], and the reduced appetite and energy intake are independent risk factors for frailty, disability, and morbidity and mortality [13]. It has further been shown that circulating concentrations of PYY and other hormones with anorectic effects (e.g., insulin, leptin, cholecystokinin) are increased in older adults [14]. Although exercise is commonly recommended for older adults [15], our understanding of the relationship between exercise and appetite in older adults is limited due to the general paucity of aging-specific data and inconsistent findings [3,16,17]. Our recent meta-analysis reported reductions in fasting leptin and glucose in adults aged 60+ [17] following prolonged exercise, suggesting improved satiety sensitivity. However, the age-dependent effects on exercise-induced changes in PYY, GLP-1, or acylated ghrelin remain to date unknown [17]. Examining how exercise-induced changes in appetite-regulating hormones might be altered in older adults will help better understand how exercise impacts hunger and satiety in aging populations with potential implications for longer-term weight maintenance and longevity.

The aim of the current study was to investigate age-related differences in appetite-regulating hormones (PYY, GLP-1, and acylated ghrelin) following acute aerobic exercise. Given the age-related declines in appetite regulation [14,18,19], we hypothesized that exercise-induced hormonal responses would be partially blunted in older adults.

Methods

Study design

The present study is a subproject of a larger project that systematically assessed the impact of exercise on food choices. The general methodology and results from other subprojects have been reported elsewhere [20,21]. Briefly, participants completed 2 45 min study conditions in randomly assigned order (block randomization, block size of 4) on 2 separate days ≥5 d apart: *1*) an exercise bout on a bicycle ergometer (LC6; Monark) at an intensity of 60% of the individual peak oxygen uptake ($\dot{V}O_{2peak}$) (*Exercise*) and *2*) a seated rest period (*Rest*).

Experimental study conditions

Each study condition visit was scheduled 30–60 min after the habitual wake-up time (between 06:30 and 10:00, identical time at each visit), and participants arrived following an overnight (≥8 h) fast. Participants were further instructed to refrain from alcohol, caffeine, and strenuous physical activity during the 24 h before their visits. Participants completed a 24 h diet recall on their first visit, and they were instructed to replicate the diet as closely as possible on the day before their second study condition visit. At each study condition visit, participants received a small, standardized breakfast [commercially available cereal bar (240 kcal; 39 g of carbohydrates, 8 g of fat, 8 g of protein) and 8 oz (237 mL) of bottled water] upon arrival to the laboratory, and they were instructed to rest for 30 min in a seated position before completing each study condition. During *Exercise*, the cycling resistance was set to the workload (W) corresponding to 60% $\dot{V}O_{2peak}$, as determined via an incremental exercise test during a preliminary screening visit [20]. Heart rate and ratings of perceived exertion were monitored by trained laboratory personnel at regular intervals (every 5 min). During *Rest*, participants sat quietly in a chair for 45 min while being allowed to listen to music or watch preapproved television programs that contained no images of or references to food. Upon completion of each condition, participants rested for another 30 min after completing each study condition (Figure 1). Throughout both visits, participants were allowed to listen to music or watch preapproved television programs, free of food cues. The University of Nebraska-Lincoln's institutional review board approved the study, and written informed consent was obtained from all participants before enrolment. All study procedures were conducted in accordance with the Declaration of Helsinki.

Participants

Volunteers for this study were recruited from our university and its surrounding communities via fliers and word-of-mouth advertising. Eligible participants were males and females with a BMI (in kg/m^2) of 18.5–29.9, weight stability (≤2.5 kg weight change during the past 6 mo), and who exercised ≥1 bout/wk. Pregnancy, smoking, current or past eating disorders, or any medical condition or medication use that could affect appetite or present any contraindications to exercise were exclusion criteria. For females not using hormonal contraceptives, exercise sessions were scheduled during the early-to-mid follicular phase [22,23] to limit the effects of the menstrual cycle on exercise-induced changes in appetite-regulating hormones [24]. To compare younger with older adults, we recruited participants from 2 distinct age ranges: *1*) 19–29 y and *2*) 65–75 y. Participants in the older age range had no evidence of dementia or non-normative decline [25].

Appetite-regulating hormones

Plasma concentrations of PYY3–36, total GLP-1 (1–37a), and acylated ghrelin were measured at 3-time points at each study

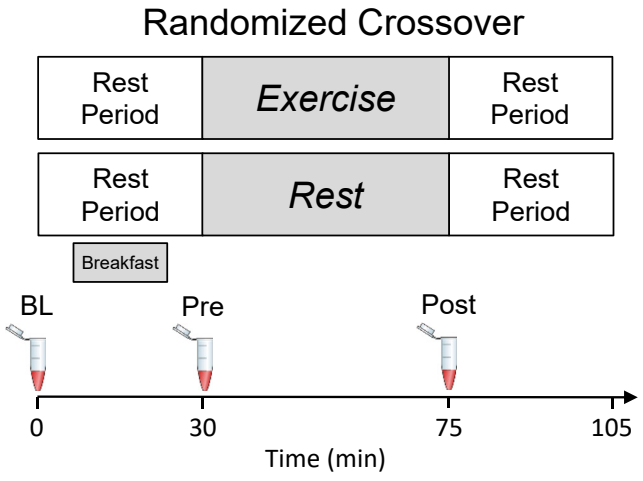

FIGURE 1. Schematic overview of study design, illustrating the time points of blood collection. BL, baseline.

condition visit (Figure 1): *1*) immediately after arrival to the laboratory [fasted, before consuming the standardized breakfast; baseline (BL)], *2*) after completing the initial 30 min rest period (i.e., immediately before the 45 min study condition; pre), and *3*) immediately after completing the study condition (post). Whole-blood samples were collected from participants in a seated position via venipuncture in 10 mL EDTA tubes (K_3 EDTA). A protease inhibitor (100 μL aprotinin; Sigma Aldrich) was added to PYY3–36 and acylated ghrelin samples. Immediately after collection, the EDTA tubes were placed on ice for 15 min and then centrifuged at 1800 × g; 10 min; +4°C. Subsequently, plasma fractions were divided into aliquots and stored at −80°C until analysis. ELISAs were used to measure concentrations (in duplicate) of PYY3–36 [Invitrogen (catalog # EH387RB); ThermoFisher Scientific; interassay coefficient of variation (CV): 6%; intra-assay CV: 7%], total GLP-1 [Invitrogen (catalog # EH221RB); ThermoFisher Scientific; interassay CV: <12%; intra-assay CV: <10%], and acylated ghrelin [Invitrogen (catalog # BMS2192); ThermoFisher Scientific; interassay CV: 8.5%; intra-assay CV: 6%].

Subjective appetite measures

Before each exercise session (pre) and immediately after (post), participants rated their subjective perceptions of hunger, fullness, thirst, and nausea on a visual analog scale from 0 (not at all) to 10 (very) via online Qualtrics panels administered by trained study personnel.

Statistical analyses

The distribution of variables was verified by visual inspection of histograms and quantile-quantile plots of the residuals. Data are presented as the mean and SE of the mean. Differences in hormone concentrations and subjective appetite between younger adults and older adults and between conditions (*Exercise* compared with *Rest*) at each time point were analyzed by analysis of variance. Given our main aim was to investigate differences in appetite-regulating hormones and appetite between age groups following acute aerobic exercise, the isolated effect of exercise on appetite-regulating hormones and subjective perceptions of hunger, fullness, thirst, and nausea was quantified as the difference between the pre-post change during *Exercise* adjusted for the pre-post change during *Rest*:

$$(Post_{Exercise} - Pre_{Exercise}) - (Post_{Rest} - Pre_{Rest})$$

Because older adults had a significantly higher body fat compared to younger adults (Table 1) and greater body fat is associated with lower concentrations of PYY and GLP-1 [26,27], we also assayed our analyses with body fat (kilogram and percentage) as covariates. Because neither body fat mass (all $P \geq 0.460$) nor percent body fat (all $P \geq 0.536$) were significant, and the adjusted results did not differ meaningfully from the original models, we report the models without covariate adjustment. All analyses were performed in SPSS version 29, and significance was accepted as $P < 0.05$ (2-sided).

Results

BL characteristics of younger adults [mean age 22.0 (SD = 2.6) y, mean BMI 23.7 (SD = 2.4), 77% White, 59% female] and older adults [mean age 68.5 (SD = 3.0) y, mean BMI 26.0 (SD = 3.1), 97% White, 55% female] are presented in Table 1. Compared to younger adults, older adults had a higher body weight, BMI, and body fat and lower aerobic fitness (all $P \leq 0.016$). All other demographic and anthropometric characteristics, as well as fasting concentrations of appetite-regulating hormones, did not differ between younger adults and older adults ($P \geq 0.080$; Table 1). Although energy expenditure during *Exercise* was significantly lower in older adults ($P < 0.001$), average relative heart rate and perceived exertion during the exercise session were similar in older and younger adults (Table 1).

Changes in PYY3–36 during *Exercise* and *Rest* in younger adults and older adults

During *Exercise*, PYY3–36 did not differ between younger adults and older adults at any time point (all $P \geq 0.770$; Figure 2A). During *Rest*, PYY3–36 did not differ by age group at

TABLE 1
Participant characteristics.

	Younger adults (n = 39)	Older adults (n = 29)
Sex, *n* (%)		
Female	23 (59.0)	16 (55.2)
Race/ethnicity, *n* (%)		
White	30 (76.9)	28 (96.6)
African American	6 (15.4)	0 (0.0)
Asian	2 (5.1)	1 (3.4)
Other	1 (2.6)	0 (0.0)
	Mean (SD)	Mean (SD)
Anthropometrics		
Age, y	22.0 (2.6)	68.5 (3.0)
Weight, kg	68.5 (9.7)	75.0 (12.1)
BMI, kg/m^2	23.7 (2.4)	26.0 (3.1)
Total body fat, %	14.1 (6.3)	24.7 (3.6)
Fat mass, kg	9.8 (5.0)	18.7 (4.8)
Fat-free mass, kg	58.6 (8.6)	56.3 (8.3)
$\dot{V}O_{2peak}$, mL/kg/min	37.6 (6.5)	24.2 (6.4)
Appetite-regulating hormones (baseline)[1]		
Fasting PYY3–36, pg/mL	110.8 (47.0)	112.4 (58.5)
Fasting GLP-1, pmol/L	10.9 (5.2)	13.2 (5.6)
Fasting acylated ghrelin, pg/mL[2]	868.5 (388.0)	1073.1 (577.2)
Exercise session		
Energy expenditure (exercise session), kcal	344 (92)	252 (72)
Average heart rate, bpm	141 (16)	120 (11)
Average relative heart rate, % of maximum	73 (8)	75 (7)
Average RPE	12.7 (1.7)	13.3 (1.8)

Data are mean (SD) unless stated otherwise. Weight, BMI, body fat (kilogram and percentage), $\dot{V}O_{2peak}$, energy expenditure, and average heart rate (bpm) differed by age (all $P \leq 0.016$); all other characteristics did not differ between younger adults and older adults (all $P \geq 0.080$).

Abbreviations: bpm, beats per min; GLP-1, glucagon-like peptide-1; PYY3–36, peptide tyrosine 3–36; RPE, ratings of perceived exertion; SD, standard deviation; $\dot{V}O_{2peak}$, peak oxygen uptake.

[1] Hormone concentrations are reported as means between pre-exercise and pre-rest. Fasting concentrations before the 2 study conditions did not differ in younger adults (all $P \geq 0.100$) or older adults (all $P \geq 0.150$).

[2] Data available for 37/39 of younger adults and 29/29 of older adults.

pre ($P = 0.189$) but was higher in older adults compared to younger adults at post [+38.0 pg/mL; 95% confidence interval (CI): 10.9, 65.2 pg/mL; $P = 0.007$; Figure 2A]. The isolated exercise effect in PYY3–36 (pre-to-post change during *Exercise* relative to *Rest*) was greater in younger adults compared to older adults (+26.6 pg/mL; 95% CI: 3.4, 49.8 pg/mL; $P = 0.025$; Figure 2B).

Changes in GLP-1 during *Exercise* and *Rest* in younger adults and older adults

During *Exercise*, GLP-1 was higher in older adults relative to younger adults at pre (+7.4 pmol/L; 95% CI: 3.3, 11.4 pmol/L; $P < 0.001$; Figure 2C) and post (+6.2 pmol/L; 95% CI: 3.4, 9.0 pmol/L; $P < 0.001$; Figure 2C). Similarly, during *Rest*, GLP-1 was higher in older adults relative to younger adults at pre (+11.8

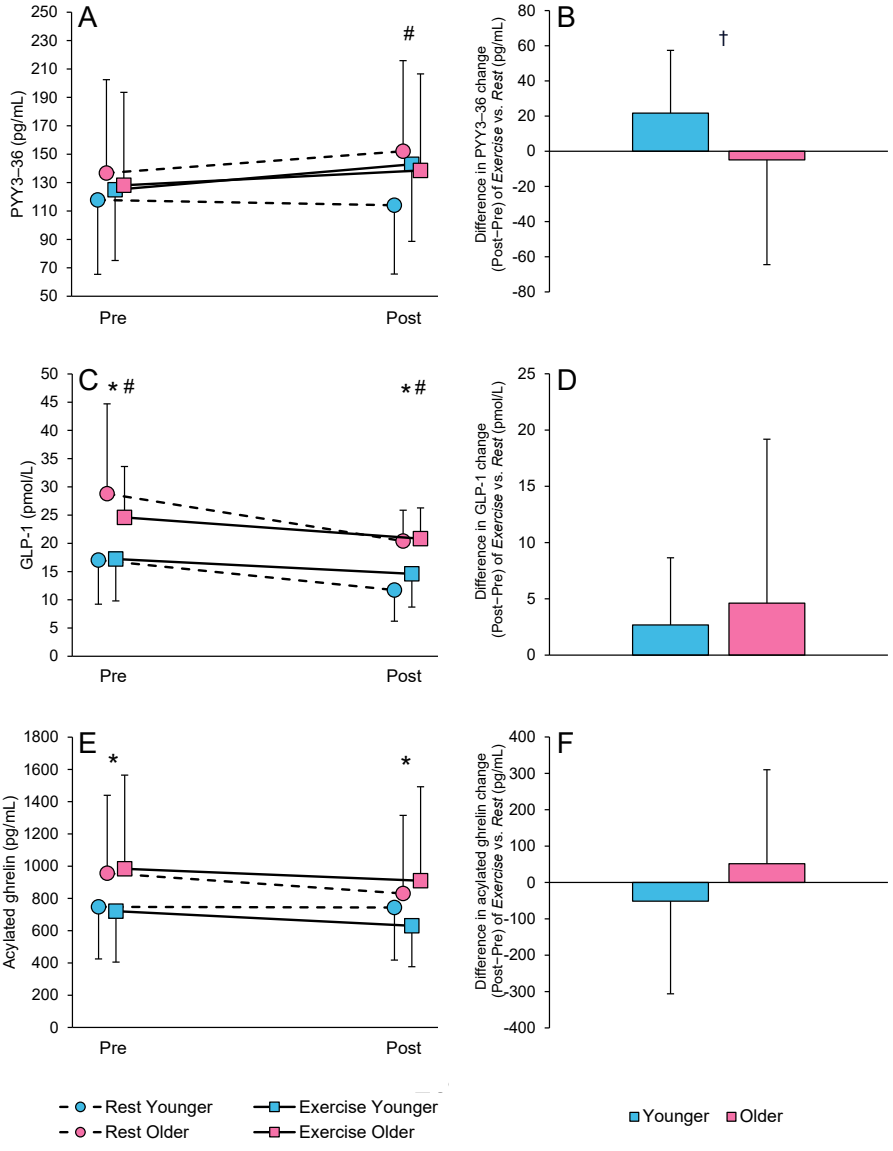

FIGURE 2. Concentrations of PYY3–36 (A), GLP-1 (C), and ghrelin (E) before (pre) and after (post) the study condition (*Exercise* compared with *Rest*) in younger adults and older adults. Panels (B), (D), and (F) show the change in PYY3–36 (B), GLP-1 (D), and ghrelin (F) from pre-to-post (*isolated exercise effect*) during *Exercise* relative to *Rest* in younger and older adults. Values are means ± SE of the mean. Differences in hormone concentrations between younger adults and older adults and between conditions (*Exercise* compared with *Rest*) at each time point were analyzed by analysis of variance. *Significant difference between younger and older adults during *Exercise* ($P < 0.05$). #Significant difference between younger and older adults during *Rest* ($P < 0.05$). †Significant difference in the pre-post change during *Exercise* relative to *Rest* between younger and older adults ($P < 0.05$). *Exercise*, exercise condition (60% V̇ O2peak) on a bicycle ergometer; GLP-1, glucagon-like peptide-1; PYY3–36, peptide tyrosine tyrosine 3–36; *Rest*, control condition (seated rest period).

pmol/L; 95% CI: 5.9, 17.6 pmol/L; $P < 0.001$) and post (+8.7 pmol/L; 95% CI: 6.0, 11.4 pmol/L; $P < 0.001$; Figure 2C). However, pre-to-post changes in GLP-1 during *Exercise* relative to *Rest* (isolated exercise effect) did not differ between younger adults and older adults (−1.9 pmol/L; 95% CI: −7.1, 3.2 pmol/L; $P = 0.456$; Figure 2D).

Changes in acylated ghrelin during *Exercise* and *Rest* in younger adults and older adults

During *Exercise*, acylated ghrelin was increased in older adults relative to younger adults at pre (+263.1 pg/mL; 95% CI: 41.9, 484.4 pg/mL; $P = 0.021$; Figure 2E) and post (+279.5 pg/mL; 95% CI: 66.5, 492.4 pg/mL; $P < 0.001$; Figure 2E). During *Rest*, acylated ghrelin did not differ between younger adults and older adults at any time point (all $P \geq 0.064$). Pre-to-post changes in acylated ghrelin during *Exercise* relative to *Rest* (isolated exercise effect) did not differ between younger adults and older adults (−103.1 pg/mL; 95% CI: −231.7, 25.6 pg/mL; $P = 0.114$; Figure 2F).

Changes in subjective appetite measures during *Exercise* and *Rest* in younger adults and older adults

Hunger and fullness did not differ between younger adults and older adults at any time point during *Exercise* or *Rest* (all $P \geq 0.086$; Figure 3A and B). Subjective thirst was lower in older adults compared to younger adults at pre during *Rest* (−1.3 points; 95% CI: −2.4, −0.3 points; $P = 0.016$; Figure 3C) but did not differ by age group at post or any time point during *Exercise* (all $P \geq 0.082$). Subjective nausea was lower in older adults compared to younger adults at pre during *Exercise* (−0.8 points; 95% CI: −1.6, −0.2 points; $P = 0.024$; Figure 3D) but did not differ by age group at post or any time point during *Rest* (all $P \geq 0.139$). Pre-to-post changes in all subjective appetite measures during *Exercise* relative to *Rest* (isolated exercise effect) did not differ between younger adults and older adults (all $P \geq 0.288$).

Discussion

The present study aimed to assess age-related differences in appetite-regulating hormone changes after acute aerobic exercise following a small pre-exercise breakfast. To our knowledge, this is the first study to do so.

The observed effects of exercise-induced changes in appetite-regulating hormones partially support our original hypothesis. We expected blunted PYY3–36, GLP-1, and acylated ghrelin responses in older adults due to known age-related declines in ghrelin and increases in PYY and GLP-1 [14,18,19]. In line with this, PYY3–36 continuously increased from pre-to-post during

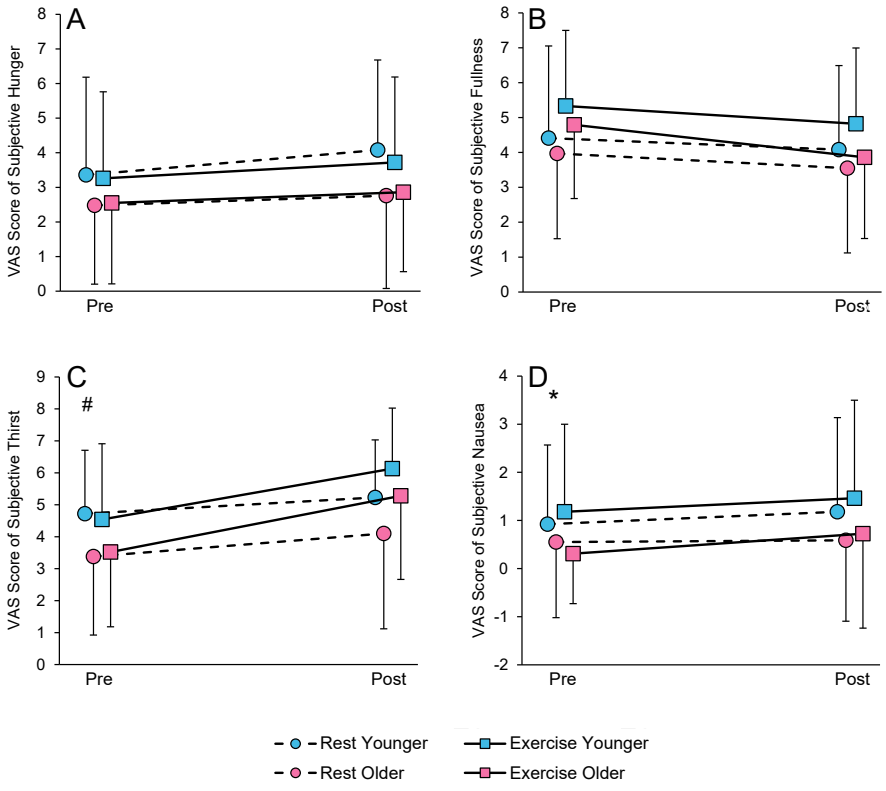

FIGURE 3. Subjective perceptions of hunger (A), fullness (B), thirst (C), and nausea (D) before (pre) and after (post) the study condition (*Exercise* compared with *Rest*) in younger adults and older adults. Items were rated on a visual analog scale (VAS) from 0 (not at all) to 10 (very). Values are means ± SE of the mean. Differences in subjective ratings between younger adults and older adults and between conditions (*Exercise* compared with *Rest*) at each time point were analyzed by analysis of variance. *Significant difference between younger and older adults during *Exercise* ($P < 0.05$). #Significant difference between younger and older adults during *Rest* ($P < 0.05$). *Exercise*, exercise condition (60% $\dot{V}$ O2peak) on a bicycle ergometer; *Rest*, control condition (seated rest period); SE, standard error.

Exercise and *Rest* in older adults. In contrast, in younger adults, PYY3–36 only increased during *Exercise* but remained virtually unchanged during *Rest*. As a result, exercise-induced changes (isolated exercise effect) in PYY3–36 were significantly blunted in older adults compared to younger adults. Although the mechanisms driving exercise-induced elevations in PYY are not well understood, increases in catecholamines during exercise can stimulate PYY release from L cells [28]. Kohrt et al. [29] demonstrated that older adults exhibit an attenuated catecholamine response to acute exercise; thus, a lower exercise-induced catecholamine response may explain the blunted PYY response to exercise in older adults within this study. However, as catecholamine concentrations were not assessed in our study, this remains speculative and warrants further investigation. Further, the breakfast snack that preceded each study condition (>100% greater increase in PYY3–36 in older adults compared with younger adults after the pre-exercise snack; data not shown) may have overshadowed a potential exercise effect on PYY3–36 during *Exercise* in older adults. Generally, greater postprandial PYY concentrations in older adults compared to younger adults [14] and a longer time to reach the peak in these concentrations in older adults (60–79 y) compared to younger adults (20–39 y) [30] have been reported before, potentially explaining the lack of exercise effect seen in our study. Future studies might want to consider a longer time frame (e.g., >1 h) between a preceding meal and the exercise bout when aiming to examine exercise-induced changes in PYY3–36 concentrations in older adults. Moreover, there was a high level of variability in PYY3–36 concentrations in older adults (31–61% CV), which differs from previous reports [19] and potentially affected the present results; hence, this requires additional study.

For GLP-1, we found higher concentrations in older adults compared to younger adults at both time points. Because this effect occurred independent of the study condition (*Exercise*/*Rest*), with a similar change from pre-to-post, the isolated exercise effect did not differ by age group. Although not reported, it is noteworthy that similar to PYY3–36, the pre-exercise breakfast snack had a substantially larger effect on GLP-1 concentrations in older adults (+102% at pre relative to BL) compared to younger adults (+58%), which, again similar to PYY3–36, likely persisted throughout the exercise session and superimposed any exercise effect. These results are at odds with a meta-analysis showing no differences in postprandial GLP-1 between older and younger adults [14]. However, substantial heterogeneity has been noted [14], and trends for higher postprandial concentrations, particularly beyond 30 min, have been described [31]. Possible reasons for these discrepancies could include differences in age range, body composition characteristics, and GLP-1 measurement methods [3]. Additionally, the greater GLP-1 values at BL in older adults, albeit not statistically significant, may have influenced the present results.

Acylated ghrelin concentrations differed by age group at both time points during *Exercise*, with consistently greater values in older adults. However, the isolated exercise effect did not differ by age group. Interestingly, fasted (BL) values in acylated ghrelin were also greater in older adults compared to younger adults, albeit not statistically significant. Although this differs from previous findings describing age-related reductions in fasting and postprandial acylated ghrelin concentrations [14,18,19], recent data support higher fasting concentrations of acylated ghrelin in older adults, particularly in those with low appetite and food intake [31]. Further, when accounting for the greater BL concentrations in older adults, changes in acylated ghrelin following the standardized breakfast (pre) and *Exercise* (post) were comparable between younger adults and older adults, with a continuous decrease throughout the study period and no effect of *Exercise* compared with *Rest*, which aligns with the literature [14].

We found no differences in subjective hunger and fullness between younger adults and older adults at any time point during *Exercise* or *Rest*, indicating that the overall higher GLP-1 concentrations and the blunted exercise response in PYY3-36 in older adults did not translate into altered satiety. When looking at the isolated exercise effect, we likewise did not observe any age-related differences for either of the subjective appetite measures. Collectively, these results suggest that age does not influence exercise-induced changes in these outcomes for our specific exercise condition (45 min, nonfasted bike ergometer exercise at 60% $\dot{V}O_{2peak}$). As reported before [32], a lack of alignment between exercise-induced changes in appetite hormones and subjective appetite is not uncommon, highlighting the complex nature of appetite regulation, which involves integrating a wide range of neuroendocrine and psychological factors. Nevertheless, our findings have important implications. The absence of compensatory appetite changes in older adults suggests that exercise could help create a negative energy balance, potentially counteracting the excess fat accumulation often seen in age-related sarcopenic obesity.

The present study aimed to assess age-related differences in the effect of acute exercise on appetite-regulating hormones. However, certain sample differences and limitations may have accounted for some variation and influenced the findings.

First, older adults had a substantially higher BMI [26.0 compared with 23.7 and body fat (24.7% compared with 14.1%)] compared to younger adults. Although both BMI (23.0–29.9 for adults aged 60–79 y [33]) and percent body fat [34] were in the healthy weight range in both age groups, it has been reported that greater body fat reduces PYY and GLP-1 concentrations [26,27]. In our study, fasting PYY3–36 did not differ between age groups. It is possible that an age-related increase in PYY3–36 concentrations counterbalanced the PYY3–36-lowering effect of the higher body fat in older adults. Importantly, however, when included as a covariate in our models, body fat did not alter results meaningfully. Additionally, the lack of an age effect in appetite-regulating hormones may reflect the overall good health status of our participants. Many participants (and particularly those in the older cohort) reported engaging in daily physical activity, carefully monitoring their diet, and managing health conditions (e.g., hypertension) through medication and routine check-ups. The absent age effect may, therefore, suggest that maintaining an active lifestyle and a healthy diet can help regulate appetite hormones into older adulthood. Future studies should examine whether similar results occur across different BMI levels, health conditions, and activity levels, considering individualized hormone regulation and varying exercise responses.

Second, older adults had a lower $\dot{V}O_{2peak}$ (24 mL/kg/min) compared to younger adults (38 mL/kg/min) and consequently expended less energy (252 kcal compared with 344 kcal) during the exercise session, which might have affected hormone

concentrations. However, all participants exercised at the same relative intensity (60% $\dot{V}O_{2peak}$) duration (45 min), both of which factors can modulate hormone concentrations. The average heart rate (75% compared with 73%) and ratings of perceived exertion (13.3 compared with 12.7) confirmed that the exercise intensity – relative to the participants' individual fitness – was similar across age groups and in a moderate range [35]. Because simultaneously controlling all 3 factors (intensity, duration, and energy expenditure) is impossible, our study maximized standardization through a randomized crossover design and a standardized pre-exercise snack. Responses in appetite-regulating hormones have been reported to be fairly inconsistent at moderate exercise intensities, although tending to be generally greater during higher-intensity exercise [32], potentially explained by intensity-related greater sympathetic activity that has also been shown to stimulate PYY secretion [28]. It is, therefore, possible that a higher exercise intensity in our study would have elicited greater hormone responses per se as well as in potential age-related differences in these outcomes [36]. Additionally, individual exercise ability may have played a role in our study. Although 60% $\dot{V}O_{2peak}$ is considered moderate intensity, this might represent a very different physiological demand for each participant. Some individuals may have found it more challenging, leading to greater stress responses, which could have influenced hormone secretion. Future studies should consider stratifying participants based on fitness levels to better assess how relative intensity influences appetite regulation.

Third, the present study evaluated the acute appetite responses to a single exercise session. Previous research has shown that the acute exercise-induced changes in acylated ghrelin and PYY remain consistent with identical exercise stimuli performed repeatedly [37], suggesting stability of the effects over time. However, it remains unclear if this stability extends to age-related differences in appetite-regulating hormones over time, especially as individuals adapt to exercise training. Previous research indicates that exercise-induced mechanisms of hunger and satiety (including changes in fasting glucose and serum leptin) and compensation differentially affect adults aged 60+ y [17]. Still, more research on chronic exercise-induced effects on PYY, GLP-1, and acylated ghrelin in older adults is needed, investigating the intra-individual and interindividual variability that may be increasingly present with increased age [14]. Given the nutrient sensitivity of GLP-1 and PYY, future research should examine exercise-induced changes in appetite-regulating hormones under fasted conditions to better isolate exercise-specific effects. Although a limitation of the current study is the use of a nonfasted protocol, this approach was chosen to reflect real-world behavior and minimize the risk of hypoglycemia in older adults.

Fourth, time of day could be a factor influencing the present results. All exercise sessions took place in the morning, meaning that potential diurnal variations in appetite hormone responses were not assessed. Previous studies have shown that appetite regulation can fluctuate throughout the day [38–40], which could mean that hormone responses to exercise differ in the morning compared with evening sessions. Future research should investigate whether exercise timing plays a role in appetite hormone regulation, particularly in older adults. Another key consideration is the pre-exercise meal. Participants were provided with a standardized snack, which, for many, deviated from their usual breakfast routine or food preferences. Since food cravings and eating habits vary widely, the pre-exercise meal could have influenced postexercise appetite responses differently across individuals. Further, although participants followed a controlled fasting period before the study visits, real-life eating patterns often involve variable fasting durations. Although our fasting protocol was necessary to standardize hormone activation conditions, it does not reflect everyday meal timing and should be considered in future research.

Finally, it may be necessary to extend the postexercise observation period, as it is not uncommon for divergent responses to appear between groups or conditions 30 min or even 60 min postexercise and for exercise to affect gut hormone responses to subsequent food intake [41]. In our study, the primary objective was to assess the effect of *Exercise* compared with *Rest* on hypothetical postexercise food choices and acute postexercise energy intake (reported previously [20,21]), and a longer postexercise period before food consumption would have affected these results.

In conclusion, the present showed age-related differences in appetite-regulating hormone responses to acute exercise. PYY3–36 increased with exercise only in younger adults, whereas in older adults, increases occurred regardless of condition, blunting the exercise effect. GLP-1 concentrations were overall greater in older adults but unaffected by exercise. These hormonal changes did not translate into changes in subjective hunger or increased fullness, however, suggesting that exercise may help prevent fat mass accumulation in both young and older individuals.

Author contributions

The authors' responsibilities were as follows – KK, JRS, JBB, JAF: acquired funding; KK, JRS: designed the study; SEB, JAF, JBB, HJH, KK: collected data; CH: conducted statistical analyses, drafted the manuscript, and created tables and figures; KK, JLD, JBB, HJH, SEB, JAF, JRS: provided critical manuscript revision for important intellectual content; and all authors: read and approved the final manuscript.

Conflict of interest

KK, JRS, JBB, and JAF report financial support was provided by University of Nebraska Food for Health Collaboration Initiative. The other authors report no conflicts of interest.

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Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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