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BRIEF REPORT

The influence of exercise training dose on fasting acylated ghrelin concentration in older women

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Abstract This study investigated if exercise dose affected acylated ghrelin response to exercise training, and how body weight or fat mass changes might affect the responses. Non-obese older women (n = 49) were randomly assigned to 4-month moderate-intensity aerobic exercise of one of two doses (8 or 14 kcal kg^{-1} body weight weekly). Following exercise training, fasting acylated ghrelin concentrations changed differently between the two groups (p for group × time interaction = 0.050). It decreased in the moderate-dose (Cohen's d = 0.52, $p = 0.019$), but did not change in the low-dose exercise group. Adjustment for weight or fat changes did not affect these results. Therefore, exercise training dose can have specific effects on acylated ghrelin that are not dependent on weight or fat loss. However, whether the different acylated ghrelin changes are associated with differing degree of subsequent weight maintenance worth further investigation.

Keywords Ghrelin · Exercise training · Weight loss · Energy balance · Older women

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Introduction

Ghrelin is a peptide hormone that regulates energy balance primarily through the stimulation of appetite and the initiation of food intake (Gil-Campos et al., 2006; Klok et al., 2007). Studies suggest that circulating ghrelin concentration increases with weight loss (Hansen et al., 2002; Leidy et al., 2004; Mason et al., 2015) and decreases with weight gain (Otto et al., 2001). Exercise perturbs the energy balance and can potentially alter body weight and body composition. Interestingly, evidence suggests that the acute effect of exercise on ghrelin are different from dietary restriction even with similar amounts of energy deficit (King et al., 2011). However, a 12-month randomized controlled trial determined that greater weight loss was associated with increased fasting ghrelin concentration, regardless whether the intervention was caloric restriction or exercise alone or combined (Mason et al., 2015). Therefore, body weight change may moderate the effects of exercise training on ghrelin.

However, exercise training studies have shown inconsistent results regarding ghrelin responses even when body weight was reduced. Fasting ghrelin concentrations have been reported to be increased (Kang et al., 2018; Leidy et al., 2004), unchanged (Gibbons et al., 2017; Ravussin et al., 2001), or decreased (Prado et al., 2015). These inconsistent findings may be related to the specific exercise regimen performed. It must be noted that most of these studies employed only one exercise regimen, not testing the effect of exercise intensity or dose, which has been shown to influence ghrelin responses to acute exercise (Broom et al., 2017; Holliday & Blannin, 2017). To date, we are not aware of any exercise training study that has specifically examined how the dose of exercise may influence the ghrelin response after training. Thus, the purpose of this

study was to investigate whether and how the dose of exercise training influenced the ghrelin response in non-obese older women. A secondary purpose was to examine whether the amount of weight or fat mass change played a role in the response. Most previous exercise training studies were conducted in obese participants; however, evidence indicates different ghrelin responses in normal weight and obese individuals after a bout of exercise (Heden et al., 2013). Therefore, we investigated the ghrelin response after a period of training in non-obese older women.

Methods

This study used data collected in the Women's Energy Expenditure in Walking programs (WEWALK study, Clinicaltrials.gov identifier: NCT01722136) (Wang et al., 2017). The research protocol was approved by the University of South Carolina Institutional Review Board. All participants provided a signed written informed consent.

Participants and exercise intervention

Participant recruitment and eligibility criteria were detailed previously (Wang et al., 2017). Briefly, the participants were non-obese (body mass index, BMI, 18–30 kg m^{-2}) older women (60–75 years). They self-reported being weight stable (± 3%) for the past 3 months, physically inactive (less than 20 min × 3 times per week of structured exercise) for the past 3 months, and nonsmoking for the past year. No one was taking medications known to affect exercise performance or metabolism. The participants who completed the study and had ghrelin concentrations at both baseline and post-intervention were included in this analysis (n = 49).

Participants were randomized into one of two 4-month exercise groups: low-dose and moderate-dose. The exercise energy expenditure goal was 8 and 14 kcal kg^{-1} body weight per week, respectively. The target exercise intensity for all women was 50–55% of the participant's heart rate reserve, with resting heart rate and peak heart rate obtained during the graded exercise test (see below). All training sessions were supervised by exercise trainers and occurred at the Clinical Exercise Research Center of the University of South Carolina. Women in the low- and moderate-dose groups walked an average of 105 ± 9 and 160 ± 14 min per week, respectively.

Tests and measurements

Graded exercise test

Participants completed a graded exercise test on a motor-driven treadmill using an incremental protocol. Gas analysis was performed by a metabolic measurement system (TrueOne 2400, Parvo Medics, Salt Lake City, UT). The 12-lead ECG was monitored by a standard system (Quinton Q-Stress; Cardiac Science, Bothell, WA). Peak volume of oxygen consumption (VO_{2peak}) was determined by the maximal 30-s averaged VO_2 value during the test. Peak heart rate was the highest heart rate obtained during the test.

Body composition

Height was measured to the nearest 0.1 cm without shoes, and weight was measured to the nearest 0.1 kg while the participant was wearing lightweight scrubs after an overnight fast after voiding. The dual-energy X-ray absorptiometry (enCORE, GE Healthcare model 8743, Waukesha, WI) full body scan was utilized to measure whole-body lean mass, fat mass, bone mass, and body fat.

Blood sample collection and analysis

Venous blood samples were collected via venipuncture of the forearm after fasting for at least 12 h. Blood was drawn into EDTA tubes, with serine protease inhibitor (Pefabloc SC, Roche, Indianapolis, IN) added, and centrifuged at 3000 rpm for 20 min at 4 °C. Plasma was then aliquoted into Eppendorf tubes and stored at − 80 °C until analysis. Plasma concentration of acylated ghrelin was quantified using a multiplex immunoassay (Human Metabolic Hormone Magnetic Bead Panel, Millipore, Billerica, MA) per manufacturer instructions on a MAGPIX multiplexing system (Luminex, Austin, TX). All samples were run in duplicates. The assay has a minimum detectable concentration of 13 pg/ml. The intra-assay and inter-assay coefficient of variance in our laboratory were both < 10%.

Statistical analysis

Descriptive statistics were first examined. Acylated ghrelin concentrations were found to be non-normally distributed; therefore, natural logarithm was calculated to transform these data. Further analyses were performed using the transformed data. General Linear Model (GLM) with repeated measures were used to determine whether the changes following exercise training were different between the two exercise groups. Covariates including change val-

ues of body weight, fat mass, lean mass, percent body fat, and VO_{2peak} were adjusted in the analyses. Bivariate correlation analyses were used to determine the correlations between acylated ghrelin concentrations and body weight, body composition, and VO_{2peak}. The exercise training effects were examined based on assigned treatment, regardless of adherence or study retention (i.e., intent-to-treat).

Given the implicated role of body weight and fat mass changes in ghrelin changes in the literature, secondary analyses were conducted by stratifying the entire sample by weight change and fat mass change, using classification criteria in consideration of sample size in each category and for ease of comparison with the literature. GLM with repeated measures were used to compare changes in ghrelin concentrations across categories of weight change and fat change. A p value of ≤ 0.05 was used to denote statistical significance. The SAS software (version 9.4, SAS Institute, Cary, NC) was used to perform analyses.

Results

Participant characteristics: at baseline and exercise training effects

The majority of the women were Caucasian (92%) and had 4 years or more of college education (67%). Women in the two exercise groups had similar distributions of races and education levels. They were older (age = 65.1 ± 4.4 years, mean ± SD) and non-obese (BMI = 25.8 ± 3.6 kg m^{-2}). As shown in Table 1, at baseline there were no significant differences between the two groups in age, height, body composition, or VO_{2peak} (all p values > 0.47).

Body composition and VO_{2peak} after the exercise training are also included in Table 1. There were no differences between the two groups in the changes in any body composition measure (p for group × time interaction > 0.31 for all). Body weight, fat mass, and percent body fat decreased in the entire sample (all p values < 0.01), but lean mass did not change ($p = 0.17$). A significant group x time interaction for VO_{2peak} ($p = 0.036$) was found, indicating different changes between groups: VO_{2peak} did not change in the low-dose exercise group ($p = 0.068$), but increased in the moderate-dose group ($p < 0.0001$).

Fasting acylated ghrelin concentration: at baseline and exercise training effects

Fasting acylated ghrelin concentrations at baseline and post-intervention by exercise group are shown in Table 2. At baseline, there were no differences between the two exercise groups ($p = 0.98$ using transformed data). Acylated ghrelin concentration was not associated with body weight, fat mass, lean mass, percent body fat, or VO_{2peak} (all p values ≥ 0.075).

Fasting acylated ghrelin concentrations changed differently following exercise intervention in the two groups (p for group × time interaction = 0.050). Specifically, acylated ghrelin concentrations did not change in the low-dose exercise group, but significantly decreased by 12.2 (− 3.2, 38.9) pg ml^{-1} [median (lower quartile, upper quartile)] or 39.3% (− 14.6%, 66.7%) in the moderate-dose exercise group (Table 2). This corresponds to a medium effect size. With adjustment for change values of body weight, fat mass, lean mass, or percent body fat, the group x time interaction was only slightly changed (p values ranged 0.038–0.053) with the exception of adjustment for changes of VO_{2peak} ($p = 0.084$). In the low-dose exercise group, acylated ghrelin concentrations remain unchanged after adjustment for any of the above covariates (all p values > 0.54). In the moderate-dose exercise group, acylated ghrelin concentrations still decreased after adjustment for changes in body weight, fat mass, lean mass, and percent body fat (all p values < 0.05), but no longer changed after adjustment for changes in VO_{2peak} ($p = 0.12$). Additionally, the changes in acylated ghrelin concentration after exercise training were not associated with body composition measures or VO_{2peak} at baseline or their change values (all p values ≥ 0.05).

To further examine the potential influence of body weight change on ghrelin concentration, the entire sample was stratified into weight change $\leq -3\%$, -3% to ≤ 0, and 0 to $\leq 3\%$ (not including 3 women who gained $> 3\%$ weight). Women in these categories did not have different changes in acylated ghrelin concentration (p for weight change category x time interaction = 0.52), and it did not change in either category (Table 2). Similarly, the potential influence of fat mass change on acylated ghrelin concentration was analyzed by stratifying the entire sample into fat mass change $\leq -10\%$, -10% to $\leq -5\%$, -5% to ≤ 0, and > 0. There were no differences in changes in acylated ghrelin concentration among the categories based on fat mass change (p for fat mass change category x time interaction = 0.64), and it did not change in either category (Table 2). The effect sizes for acylated ghrelin changes in those who gained weight or gained fat mass were close to medium effect size.

Discussion

To our knowledge, how the dose of exercise training affects acylated ghrelin concentrations has not been directly examined. We report that fasting acylated ghrelin concentrations in non-obese older women decreased after a

Table 1 Baseline characteristics of participants by exercise group

	Low-dose (n = 25)	High-dose (n = 24)
Age (years)	65.3 ± 4.7	64.9 ± 4.2
Height (cm)	162.3 ± 6.4	163.6 ± 6.3
Weight (kg)		
Baseline	68.2 ± 9.8	68.1 ± 9.4
Post-intervention	67.5 ± 9.6	66.8 ± 9.6*
Fat mass (kg)		
Baseline	27.2 ± 7.5	26.8 ± 6.5
Post-intervention	26.4 ± 7.2*	25.4 ± 6.7†
Lean mass (kg)		
Baseline	39.8 ± 3.8	40.0 ± 5.0
Post-intervention	39.6 ± 3.6	39.7 ± 5.0
Body fat (%)		
Baseline	38.6 ± 6.3	38.3 ± 6.6
Post-intervention	38.1 ± 6.4	37.2 ± 6.9*
VO_{2peak} (ml kg^{-1} min^{-1})		
Baseline	20.6 ± 3.5	20.1 ± 3.5
Post-intervention	21.7 ± 4.3	23.1 ± 5.2‡
Exercise time (min $week^{-1}$)	105 ± 9	160 ± 14

Data are presented as mean ± SD

*$p < 0.05$ compared to baseline value

†$p < 0.01$ compared to baseline value

‡$p < 0.0001$ compared to baseline value

Table 2 Fasting acylated ghrelin concentrations by group, and by weight and fat change category

Ghrelin (pg ml^{-1})	Baseline	Post-intervention	Cohen's d	*P* values
Exercise group				
Low-dose (n = 25)	32.0 (21.6, 80.0)	39.9 (22.0, 61.0)	0.09	0.66
High-dose (n = 24)	34.8 (21.3, 63.5)	29.6 (16.0, 34.0)	0.52	**0.019**
Weight change category				
Gained ≤ 3% (n = 11)	56.3 (21.6, 149)	34.8 (22.0, 45.2)	0.49	0.14
Lost 0–3% (n = 22)	33.8 (21.7, 62.0)	29.5 (17.0, 53.3)	0.10	0.64
Lost > 3% (n = 13)	26.5 (19.5, 65.0)	32.0 (28.6, 61.0)	0.07	0.80
Fat mass change category				
Gained fat mass (n = 14)	56.3 (21.6, 126)	31.0 (17.0, 54.3)	0.47	0.15
Lost 0–5% (n = 15)	36.0 (13.0, 129)	32.0 (18.0, 45.9)	0.09	0.74
Lost 5–10% (n = 10)	25.0 (21.7, 32.0)	32.3 (12.4, 53.3)	0.01	0.98
Lost > 10% (n = 10)	32.2 (19.5, 65.0)	31.8 (24.3, 61.0)	0.13	0.68

Data are presented as median (lower quartile, upper quartile). Cohen's d values are calculated using natural log transformed data. *P* values are for respective baseline and post-intervention comparisons using natural log transformed data

Bold value indicates significant difference for the respective baseline and post-intervention

moderate-dose, but did not change after a low-dose, moderate-intensity aerobic exercise program of 4 months. Adjusting for weight or fat mass changes did not affect these findings.

Previous studies have suggested weight loss as an important factor influencing ghrelin response to exercise training. Fasting ghrelin concentration was reported to increase two folds in normal-weight women who experienced weight loss (> 1.5 kg) (Leidy et al., 2004) and in

postmenopausal women who lost weight (Foster-Schubert et al., 2005), and larger increases occurred in the participants who lost greater amounts of weight (Foster-Schubert et al., 2005; Mason et al., 2015). Our data did not reveal weight loss or fat loss influenced acylated ghrelin responses after exercise training, and there was a lack of acylated ghrelin concentration change in those who lost weight or fat mass. Interestingly, the effect sizes for the acylated ghrelin changes in those who gained weight or fat mass were close to medium, suggesting it may decrease with gaining weight or fat mass. This would be consistent with previous studies' finding. With the sample size of 11 and 14 in these categories, our power to detect such near medium effect sizes at an α of 0.05 was 45% and 51%, respectively. Thus, it is a limitation of this study that it was underpowered to examine acylated ghrelin responses by weight or fat mass change.

In our study, a 3% of weight loss was approximately an average of 2.2 kg, which was greater than typical short-term fluctuation in body weight of 1–2 kg (Bhutani et al., 2017). This was also greater than the weight loss cutoff of 1.5 kg in the study in normal-weight women (Leidy et al., 2004) where a two-fold increase in ghrelin was observed with $>$ 1.5 kg weight loss. Of note, they provided a diet with carefully calculated caloric content throughout the exercise training intervention, which may have impacted their findings, given that exercise's effect on ghrelin is different from food restriction even with similar amounts of energy deficit (King et al., 2011). However, weight loss $>$ 5% following exercise training has been reported to increase ghrelin concentration in postmenopausal women (Mason et al., 2015). Therefore, it is possible that the weight loss obtained in our study was not large enough to affect acylated ghrelin concentrations.

We found that fasting acylated ghrelin had different changes in women who completed a moderate- versus a low-dose exercise training. Note that the difference between the two doses was only an average of 55 min per week. There was no difference in body weight and body composition between groups at baseline, and there were similar changes in body weight and composition between the two groups after exercise training. These indicate similar degree of chronic energy deficits. Despite this, we found a difference between groups in acylated ghrelin concentration change. Thus, our results suggest that exercise training dose can have specific effects on acylated ghrelin that are not dependent on the amount of energy deficits.

The participants' body weight status must also be considered when examining the effect of exercise on ghrelin. After an acute bout of aerobic exercise, fasting acylated ghrelin concentration reduced by 18% in normal-weight, but did not change in obese individuals (Heden et al., 2013). This may help explain that our participants who were non-obese exhibited decreased or unchanged ghrelin concentrations, whereas increased ghrelin concentrations were found in previous exercise training studies with mainly obese participants (Foster-Schubert et al., 2005; Mason et al., 2015). Further research is certainly warranted to examine how body weight status influence the effects of exercise on ghrelin.

Additionally, we measured acylated ghrelin concentrations whereas total ghrelin was reported in almost all previous exercise training studies. Ghrelin has two major molecular forms: acylated and desacyl ghrelin (Hosoda et al., 2000); their ratio is suggested to remain constant over an array of conditions that affect ghrelin levels (Espelund et al., 2003). The acylated form is generally considered to promote food intake (Asakawa et al., 2005). However, it is unknown whether it is the ratio of the two forms or their relative amounts that is more clinically relevant.

We did not measure appetite in our study; however, this may not be a real limitation of our study given the lack of association between changes in circulating ghrelin and appetite that has been previously reported following exercise or food restriction (Deighton et al., 2013, 2014; Rosenkilde et al., 2013). Another limitation is that we did not measure other hormones related to appetite or energy metabolism, and we did not measure post-prandial acylated ghrelin responses to a standardized meal or total acylated ghrelin. All exercise sessions being monitored by trained staff was a major strength of our study. In conclusion, our results suggest that exercise training dose can affect acylated ghrelin responses that are not dependent on the amount of weight or fat mass change. However, whether the decrease in fasting acylated ghrelin after the moderate-dose exercise is associated with better subsequent weight maintenance than the low-dose exercise worth further investigation.

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Compliance with ethical standards

Conflict of interest Kimberly P. Bowyer, James A. Carson, J. Mark Davis and Xuewen Wang declare that they have no conflict of interest.

Human and animal rights and Informed consent All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all patients for being included in the study.

References

Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., et al. (2005). Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut, 54,* 18–24.

Bhutani, S., Kahn, E., Tasali, E., & Schoeller, D. A. (2017). Composition of two-week change in body weight under unrestricted free-living conditions. *Physiological Reports, 5,* e13336. https://doi.org/10.14814/phy2.13336

Broom, D. R., Miyashita, M., Wasse, L. K., Pulsford, R., King, J. A., Thackray, A. E., et al. (2017). Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *Journal of Endocrinology, 232,* 411–422.

Deighton, K., Barry, R., Connon, C. E., & Stensel, D. J. (2013). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *European Journal of Applied Physiology, 113,* 1147–1156.

Deighton, K., Batterham, R. L., & Stensel, D. J. (2014). Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite, 81,* 52–59.

Espelund, U., Hansen, T. K., Orskov, H., & Frystyk, J. (2003). Assessment of ghrelin. *APMIS Supplementum, 109,* 140–145.

Foster-Schubert, K. E., McTiernan, A., Frayo, R. S., Schwartz, R. S., Rajan, K. B., Yasui, Y., et al. (2005). Human plasma ghrelin levels increase during a one-year exercise program. *Journal of Clinical Endocrinology and Metabolism, 90,* 820–825.

Gibbons, C., Blundell, J. E., Caudwell, P., Webb, D. L., Hellstrom, P. M., Naslund, E., et al. (2017). The role of episodic postprandial peptides in exercise-induced compensatory eating. *Journal of Clinical Endocrinology and Metabolism, 102,* 4051–4059.

Gil-Campos, M., Aguilera, C. M., Cañete, R., & Gil, A. (2006). Ghrelin: A hormone regulating food intake and energy homeostasis. *British Journal of Nutrition, 96,* 201–226.

Hansen, T. K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J. S., et al. (2002). Weight loss increases circulating levels of ghrelin in human obesity. *Clinical Endocrinology, 56,* 203–206.

Heden, T. D., Liu, Y., Park, Y., Dellsperger, K. C., & Kanaley, J. A. (2013). Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology, 115,* 680–687.

Holliday, A., & Blannin, A. (2017). Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration. *Journal of Endocrinology, 235,* 193–205.

Hosoda, H., Kojima, M., Matsuo, H., & Kangawa, K. (2000). Ghrelin and des-acyl ghrelin: Two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochemical and Biophysical Research Communications, 279,* 909–913.

Kang, S. J., Kim, J. H., Gang, Z., Yook, Y. S., Yoon, J. R., Ha, G. C., et al. (2018). Effects of 12-week circuit exercise program on obesity index, appetite regulating hormones, and insulin resistance in middle-aged obese females. *Journal of Physical Therapy Science, 30,* 169–173.

King, J. A., Wasse, L. K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R. L., et al. (2011). Differential acylated ghrelin, peptide $YY_{3–36}$, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *The Journal of Clinical Endocrinology and Metabolism, 96,* 1114–1121.

Klok, M. D., Jakobsdottir, S., & Drent, M. L. (2007). The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obesity Reviews, 8,* 21–34.

Leidy, H. J., Gardner, J. K., Frye, B. R., Snook, M. L., Schuchert, M. K., Richard, E. L., et al. (2004). Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. [Research Support, U S Gov't, P H S]. *The Journal of Clinical Endocrinology and Metabolism, 89,* 2659–2664.

Mason, C., Xiao, L., Imayama, I., Duggan, C. R., Campbell, K. L., Kong, A., et al. (2015). The effects of separate and combined dietary weight loss and exercise on fasting ghrelin concentrations in overweight and obese women: A randomized controlled trial. *Clinical Endocrinology—Oxford, 82,* 369–376.

Otto, B., Cuntz, U., Fruehauf, E., Wawarta, R., Folwaczny, C., Riepl, R. L., et al. (2001). Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *European Journal of Endocrinology, 145,* 669–673.

Prado, W. L., Lofrano-Prado, M. C., Oyama, L. M., Cardel, M., Gomes, P. P., Andrade, M. L., et al. (2015). Effect of a 12-week low vs. high intensity aerobic exercise training on appetite-regulating hormones in obese adolescents: A randomized exercise intervention study. *Pediatric Exercise Science, 27,* 510–517.

Ravussin, E., Tschop, M., Morales, S., Bouchard, C., & Heiman, M. L. (2001). Plasma ghrelin concentration and energy balance: Overfeeding and negative energy balance studies in twins. *Journal of Clinical Endocrinology and Metabolism, 86,* 4547–4551.

Rosenkilde, M., Reichkendler, M. H., Auerbach, P., Torang, S., Gram, A. S., Ploug, T., et al. (2013). Appetite regulation in overweight, sedentary men after different amounts of endurance exercise: A randomized controlled trial. *Journal of Applied Physiology, 115,* 1599–1609.

Wang, X., Bowyer, K. P., Porter, R. R., Breneman, C. B., & Custer, S. S. (2017). Energy expenditure responses to exercise training in older women. *Physiological Reports, 5,* e13360.