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ORIGINAL ARTICLE

Ghrelin is not altered after acute exercises at different intensities in overweight middle-aged individuals



La ghréline n'est pas modifiée après des exercices aigus à des différentes intensités chez des personnes en surpoids d'âge moyen

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Acute exercise;
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Growth hormone;
Insulin-like growth factor-1;
Insulin-like growth factor binding protein-3;
Overweight

Summary

Objectives. – The purpose of this study was to investigate the effect of acute moderate (MEx) and heavy (HEX) exercises on ghrelin, growth hormone (GH), insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) in untrained subjects.

Methods. – A pre/post interventional study including seven overweight middle-aged males (mean ± SD: age = 36.4 ± 4.35 years) was designed. Participants performed two sessions of exercise at 7-day interval. Each session consisted in cycling exercise during 20 min at 60% (moderate exercise, MEx) or at 80% (heavy exercise, HEX) of peak aerobic power (PAP). Venous blood samples were obtained before, at the end (0 min) and after 30, 60, 90 and 120 min recovery in each session.

Results. – After exercise and during recovery, ghrelin, IGF-1 and IGFBP-3 concentrations have been unchanged in both groups. However, GH concentration has significantly increased at the end of exercise (0.12 ± 0.09 vs. 1.08 ± 0.73 ng·mL⁻¹; P = 0.004; ES = 1.99) and 30 min after

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MOTS CLÉS

Exercice aigu ;
Ghréline ;
Hormone de
croissance ;
Insulin-like growth
factor-1 ;
Insulin-like growth
factor binding
protein-3 ;
Surpoids

(0.12 ± 0.09 vs. 0.60 ± 0.36 ng·mL⁻¹; $P=0.035$; $ES=1.98$) in HEx session, only. No significant correlation was observed at baseline between ghrelin and the other markers (GH, IGF-1 and IGFBP-3).

Conclusion. – Our results suggested that 20 min moderate or heavy sub-maximal exercises not stimulate the production and the release of ghrelin after 2 hours recovery. In our experimental conditions, it appears that: ghrelin does not participate in the regulation of the GH response to exercise in overweight males and; exercise intensity and duration may be determinant to affect ghrelin secretion.

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Résumé

Objectifs. – Le but de cette étude était d'étudier l'effet d'un exercice aigu modéré (MEx) et intense (HEx) sur la ghréline, l'hormone de croissance (GH), l'insulin-like growth factor-1 (IGF-1) et l'insulin-like growth factor binding protein-3 (IGFBP-3) chez des sujets non entraînés.

Méthodes. – Il s'agit d'une étude interventionnelle de type pré-/post-test qui inclut sept hommes en surpoids d'âge moyen (moyenne \pm écart-type: âge = $36,4 \pm 4,35$ ans). Les participants ont effectué deux sessions d'exercices séparés de 7 jours. Chaque session consiste en un exercice de cyclisme pendant 20 minutes à 60 % (exercice modéré, MEx) ou à 80 % (exercice intense, HEx) aux pics de la puissance aérobie (PAP). Des prélèvements sanguins ont été réalisés avant, à la fin (0 min) et après 30, 60, 90 et 120 minutes de récupération à chaque session.

Résultats. – Après l'exercice et durant la récupération, les concentrations de ghréline, d'IGF-1 et d'IGFBP-3 n'ont pas changé chez les deux groupes. Cependant, la concentration de GH a significativement augmenté à la fin de l'exercice ($0,12 \pm 0,09$ vs $1,08 \pm 0,73$ ng·mL⁻¹; $p=0,004$; $ES=1,99$) et 30 minutes après ($0,12 \pm 0,09$ vs $0,60 \pm 0,36$ ng·mL⁻¹; $p=0,035$; $ES=1,98$) seulement chez HEx. Aucune corrélation significative n'a été démontrée à l'état basal entre la ghréline et les autres marqueurs (GH, IGF-1 et IGFBP-3).

Conclusion. – Nos résultats suggèrent que les exercices sous-maximaux modérés ou intenses de 20 minutes ne stimulent pas la production et la libération de ghréline après 2 heures de récupération. Dans nos conditions expérimentales, il semble que: la ghréline ne participe pas à la régulation de la réponse de GH à l'exercice chez les mâles en surpoids et; l'intensité et la durée de l'exercice peuvent être déterminantes pour affecter la sécrétion de ghréline.

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1. Introduction

Ghrelin is a 28-amino acid peptide hormone secreted primarily from cells within the stomach. It is also synthesized and secreted in many other tissues, including small intestine, pancreas, hypothalamus, cardiomyocytes, chondrocytes, and placenta [1,2]. Plasma ghrelin concentrations raised before meals and decreased following meals, which suggested an orexigenic role (appetite stimulating) of the hormone [3,4]. Ghrelin was discovered as an endogenous ligand of growth hormone secretagogue receptor (GHSR) [5].

Exercise has repeatedly been shown to be a potent stimulus for growth hormone (GH) release [6], but conflicting results have been reported regarding the acute effects of exercise on ghrelin, insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) [7,8]. These hormones have been suggested to have roles associated with energy balance [9], metabolic regulation [10] and changes in body composition [11] and identifying their response to exercise might lead to a better understanding

of their roles in the adaptive processes that were initiated by exercise.

The effect of acute exercise on plasma ghrelin concentrations has been studied extensively. However, the findings were equivocal with studies showing increased [7,12], decreased [13,14] or unchanged [15,16] circulating ghrelin after exercises at different types and durations.

Exercise intensity has been identified as a potential determinant that modulate ghrelin response to exercise [17,18], with suppression occurring after exercise at higher ($\geq 60\%$ of maximal oxygen consumption [VO_{2max}]) [18,19], but not lower ($\leq 50\%$ VO_{2max}) intensities [20,21]. Studies comparing acute moderate versus high intensity exercise suggested exercising at a higher intensity might be more potent for suppressing ghrelin concentrations [22,23]. However, the effect of exercise bouts at different intensities has revealed contrasting findings [24,25].

Considering that data on ghrelin responses to exercise of different intensities and recovery in overweight individuals were currently not available, we hypothesized that only acute heavy exercise will modify circulating ghrelin levels.

Table 1 Physical characteristics of subjects ($n = 7$).

Variables	Mean \pm SD	RangeMin–Max
Age, years	36.4 \pm 4.35	30.0–42.0
Height, m	1.82 \pm 0.05	1.74–1.86
Body mass, kg	93.9 \pm 6.48	86.0–103
Body fat, %	26.6 \pm 2.75	22.0–30.5
BMI, kg·m ⁻²	28.3 \pm 1.66	25.4–30.0
PAP, W	263 \pm 29.4	209–296

Data are expressed as mean \pm SD. BMI: body mass index; PAP: peak aerobic power.

We also tested whether ghrelin interfere with exercise-induced secretion of growth hormone and related factors such as IGF-1 and IGFBP-3.

2. Methods

2.1. Subjects

Seven overweight middle-aged male volunteers participated in this study. Body composition and maximal variable from incremental exercise are presented in [Table 1](#).

Height was determined using standard physician's scales to the nearest 0.1 cm. Body mass and fat percent were measured using bioelectrical impedance analysis (Tanita, Germany). Participants were apparently healthy untrained individuals and none of them was smoker or taking medication. Before the beginning of the protocol, participants were fully informed of the purpose and procedures of the study, and written and verbal consent was obtained. The experimental protocol was approved by the Committee for Human Protection in Biomedical Research of Tunisia and was carried out in compliance with the Declaration of Helsinki.

2.2. Preliminary test

Before exercise sessions, each participant performed a maximal graded test using a calibrated cycle ergometer (Ergoline, Germany) in order to determine the peak aerobic power (PAP). The test consisted of a 3-min warm-up followed by increments in power output of one minute at 60 rpm until exhaustion. The power was considered maximal, at exhaustion, when the following criteria were achieved: inability of the subject to maintain the required pedalling frequency; attainment of age-predicted maximal heart rate ($HR_{max} = 210 - (0.65 \times \text{age}) \pm 5\%$ [26] measured with a heart rate monitor (Polar S810, Finland). The loads during warm-up and increments were individually adjusted by taking into account the age and body mass of each subject [27].

2.3. Exercise sessions

For each participant, two separate exercise sessions were performed with a 7-day interval. The experiment was designed as follows; moderate exercise (MEx); 3 min warm-up at 25% of PAP followed by 20 min cycling exercise at 60% of PAP, heavy exercise (HEX); a similar warm-up followed by 20 min cycling exercise at 80% of PAP. A standardized

breakfast (500 kcal) was eaten (07:30 a.m.) at the laboratory (the last meal taken by the subjects was towards 8:00 p.m. and no other food or drink was taken until breakfast). Then the subjects remained seated until blood sampling (08:30 a.m.) and the exercise started at 09:00 a.m. To maintain optimal hydration, the subjects drank two times 200 mL of water every 30 min; just before and after 10 min recovery post-exercise [28].

2.4. Methods of analysis

Blood samples were collected by venepuncture on 6 occasions:

- before the exercise (at 08:30 a.m, control value [S_1]);
- at the end, 0 min (S_2);
- after 30 min (S_3);
- after 60 min (S_4);
- after 90 min (S_5) and;
- after 120 min (S_6) during recovery.

Serum was separated and frozen at -80°C for later analysis.

Serum ghrelin concentration was measured by an immunoradiometric assay (Linco Research, USA). Serum GH, IGF-1 and GHBP-3 concentrations were measured by radioimmunoassay methods using the corresponding commercial reagents kit (Immunotech, France). The intra-assay coefficients of variation (CVs) were below 6% for all above-mentioned parameters.

2.5. Statistical analysis

Mean \pm standard deviation (SD) were calculated for all variables. Friedman two-way analysis was performed, relating exercises (MEx and HEX) and time of sampling (S_1 to S_6). When this analysis revealed significant differences, a Wilcoxon paired test was used to identify significant changes between S_1 and S_2 to S_6 and a Mann-Whitney test for unpaired data was used to locate where significant differences existed between moderate and heavy exercises. The effect size (ES) was calculated using Cohen's d classification of a small ($0.2 \leq \text{ES} < 0.5$), moderate ($0.5 < \text{ES} < 0.8$) and large ($\text{ES} \geq 0.8$) effect size [29]. Correlation between basal ghrelin, GH, IGF-1, IGFBP-3, percent body fat and BMI was tested using the Spearman method. $P < 0.05$ was considered statistically significant.

3. Results

No differences were found between baseline values for almost measured biochemical parameters after MEx and Hex protocols. Plasma ghrelin, IGF-1 and IGFBP-3 levels were not be significantly modified in either exercise conditions ([Figs. 1–3](#)). GH concentrations increased substantially over baseline values at the end (0.12 ± 0.09 vs. $1.08 \pm 0.73 \text{ ng}\cdot\text{mL}^{-1}$; $P = 0.004$; $\text{ES} = 1.99$) and after 30 min (0.12 ± 0.09 vs. $0.60 \pm 0.36 \text{ ng}\cdot\text{mL}^{-1}$; $P = 0.035$; $\text{ES} = 1.98$) after Hex ([Fig. 4](#)). At rest and during recovery, no significant

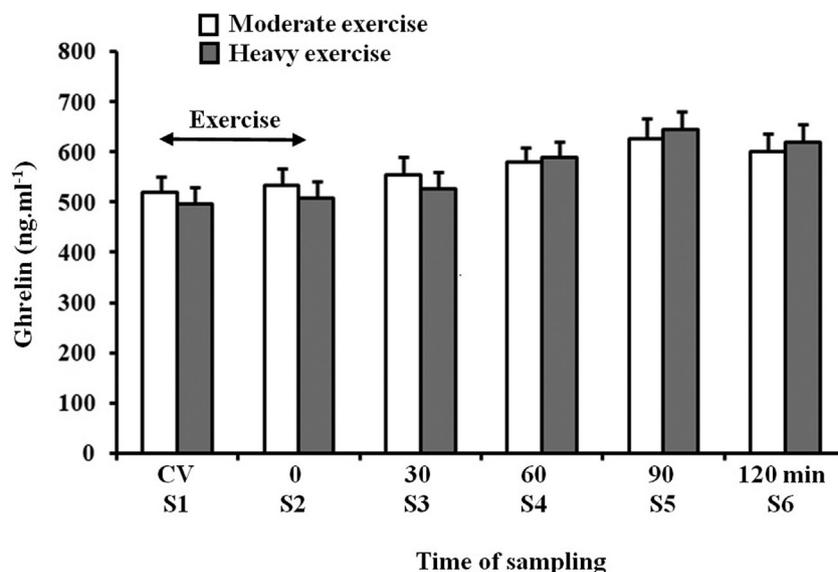


Figure 1 Mean \pm SE plasma concentrations of ghrelin after the moderate exercise (20 min at 60% of PAP) and after the heavy exercise (20 min at 80% of PAP). CV: control value; PAP: peak aerobic power.

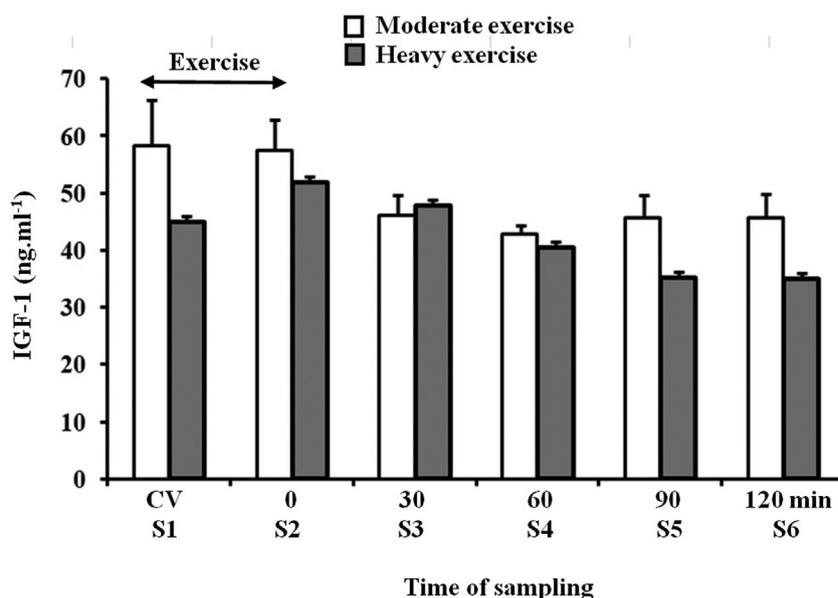


Figure 2 Mean \pm SE plasma concentrations of insulin-like growth factor-1 (IGF-1) after the moderate exercise (20 min at 60% of PAP) and after the heavy exercise (20 min at 80% of PAP). CV: control value; PAP: peak aerobic power.

differences in circulating ghrelin, GH, IGF-1 and IGF-1 were observed between trials.

For both moderate and heavy exercises tests, the baseline values of ghrelin were not significantly correlated to body composition parameters: BMI ($r=0.494$, $P=0.259$ and $r=-0.198$, $P=0.670$, respectively), percent body fat ($r=0.354$, $P=0.437$ and $r=0.153$, $P=0.743$, respectively). In addition there was no significant relationship during the moderate and heavy protocols between basal ghrelin and other blood measured variables: GH ($r=0.111$, $P=0.610$ and $r=0.77$, $P=0.239$, respectively), IGF-1 ($r=-0.716$, $P=0.070$ and $r=-0.477$, $P=0.279$, respectively) and

IGFBP-3 ($r=0.287$, $P=0.533$ and $r=-0.421$, $P=0.347$, respectively).

4. Discussion

The present study showed no significant change in serum ghrelin concentrations after acute sub-maximal exercise and during the recovery period in overweight middle-aged males. Acute high intensity exercise resulted in an increase in serum GH concentrations at the end of exercise and at 30 min post-exercise recovery, but serum IGF-1 and IGF-1 have not changed in both moderate and heavy exercises.

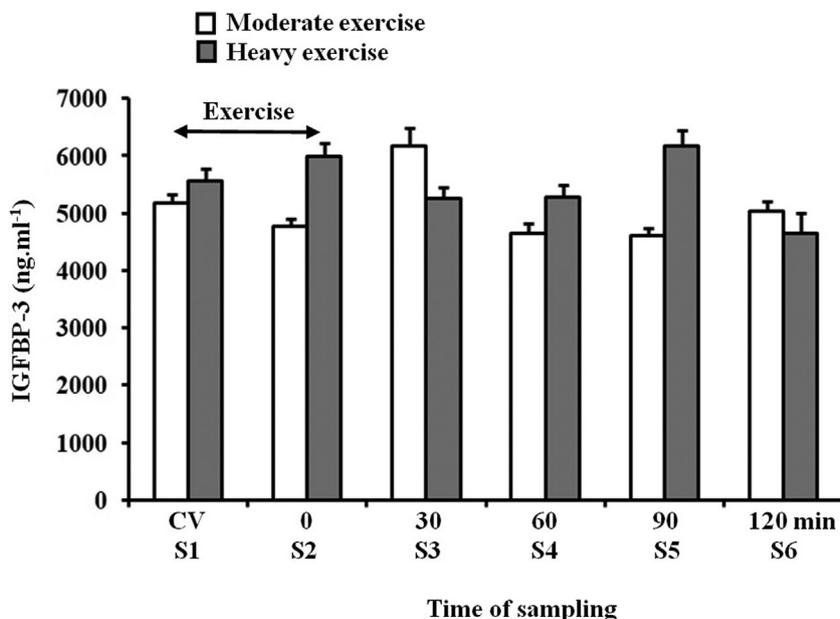


Figure 3 Mean \pm SE plasma concentrations of insulin-like growth factor binding protein-3 (IGFBP-3) after the moderate exercise (20 min at 60% of PAP) and after the heavy exercise (20 min at 80% of PAP). CV: control value; PAP: peak aerobic power.

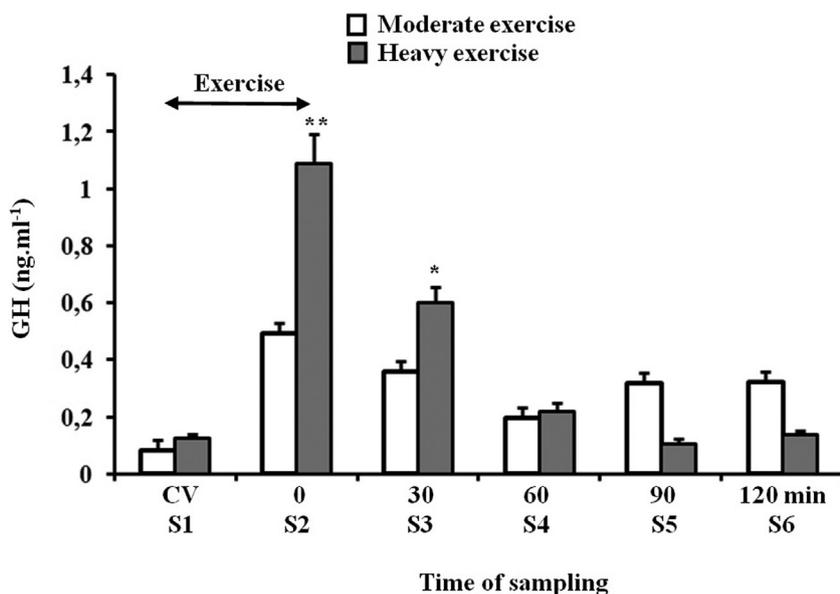


Figure 4 Mean \pm SE plasma concentrations of growth hormone (GH) after the moderate exercise (20 min at 60% of PAP) and after the heavy exercise (20 min at 80% of PAP). CV: control value; PAP: peak aerobic power. * $P < 0.05$; ** $P < 0.01$ CV vs. other sampling.

4.1. Ghrelin response to acute exercise

Our results did not support our first hypothesis and suggested that heavy acute exercise did not modify ghrelin concentrations. The effect of acute exercise on plasma ghrelin concentrations has been studied extensively, but the findings were equivocal. Studies showed either increased circulating ghrelin after sub-maximal exercises at different durations [12] and intensive resistance exercise [7], decreased levels after progressive intensities aerobic exercise [13] and high intensity aerobic cycling [14], or unchanged levels after treadmill running [15,16].

In accordance with our results, Howe et al. [25], Zoladz et al. [30], Schmidt et al. [15], Burns et al. [16] and Kraemer et al. [31] reported no significant changes in ghrelin concentrations after acute exercises. Indeed, ghrelin concentrations did not change after moderate-intensity exercise at 60% of VO_{2max} (500 kcal) [25], incremental exercise, with the increase of power output by 30W every 3 minutes until 70% of VO_{2max} [30], treadmill exercise at different intensities (50%, 70%, and 90% of VO_{2max}) [15], 60 min run at 72% of VO_{2max} [16] and progressively intense intermittent exercise at 60%, 75%, 90%, and 100% of VO_{2max} [31]. In three of these studies ghrelin was accompanied by raised

GH concentrations [15,30,31]. GH concentrations were not measured in the studies of Howe et al. [25] and Burns et al. [16].

The mechanisms underpinning the exercise-induced stability of ghrelin were unclear. We could speculate that the redistribution of blood flow from splanchnic areas might be particularly deficient and could explain partially the stability of ghrelin, it appeared to be dependent on the exercise intensity. Therefore, further research was required to elucidate the stability of ghrelin after acute exercise.

4.2. IGF-1 and IGFBP-3 responses to acute exercise

Exercise influences circulating IGF-1 and IGFBP-3 which were depend on exercise intensity and types. Vigorous running [31] and endurance exercise [32] led to increases in IGF-1 and IGFBP-3 secretion. Other report argued that 30s maximal exercise [33] decreased IGF-1 and IGFBP-3 concentrations. Correct blood liver irrigation might lead to explain the relative stability of IGF-1 and IGFBP-3 levels after our sub-maximal exercises.

4.3. GH response to acute exercise

The second result was a significant increase of GH concentrations at the end and after 30 min of the heavy protocol. It has been shown that acute exercise with sufficient intensity (approximately 70% of VO_{2max}) increased plasma GH concentrations [31], and that GH secretion was related to exercise intensity in a linear dose-response manner [34]. These results were consistent with our findings that showed a rise in GH levels after heavy exercise only.

The relationship between ghrelin, GH and acute exercise seemed to be complex and three models might exist:

- unchanged ghrelin with raised GH [13,35];
- raised ghrelin with raised GH [36,37] and decreased ghrelin and raised GH [38,39].

The mechanisms linking ghrelin to GH were less unclear and further studies were necessary to clarify why GH increased or remained stable in parallel with increased, decreased or unchanged ghrelin. After these three proposed models, it appeared that GH secretion was thought to be controlled mainly by the two neurohormones, growth hormone releasing hormone (GHRH) and somatostatin [40,41]. Ghrelin could be considered as one of the secretagogues of GH.

The present study had several limitations. First, the subjects were middle-aged and untrained, and therefore the findings might not apply to young subjects or trained subjects. Second, the low sample size might have limited the power to detect significant relationships between plasma ghrelin and other variables. Third, we focused only on ghrelin, GH, IGF-1 and IGFBP-3 concentrations, rather than looking at concentrations of other specific hormones that could modulate ghrelin concentrations such as leptin or insulin [36,42].

5. Conclusions

In conclusion, it appeared that acute moderate or heavy sub-maximal exercise does not result in immediate or prolonged changes in ghrelin concentrations in overweight middle-aged men. In contrast, heavy exercise caused significant increase post-exercise values of GH. It seemed that, after acute heavy exercise GH was not related with ghrelin. Exercise intensity and duration might be determinant to affect increase/decrease of ghrelin. Future studies were required to determine factors that determine exercise-induced ghrelin changes as well as signification and potential benefits of these changes.

Disclosure of interest

The authors declare that they have no competing interest.

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