Effects of exercise of different intensity on gut peptides, energy intake and appetite in young males

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Abstract

Introduction and research aims: The aim of the work was an evaluation of the impact of physical exertion on the regulating of food intake and digestive system hormone release as well as the partly connected phenomenon of evaluating the subjective sensation of hunger and the amount of food consumed at various time following physical exercise.

Materials and methods: The tests covered 12 young, healthy men, for whom the effects of physical exertion of a moderate and high intensity on the subjective sensation of hunger/satiety, evaluated by means of visual analogue scales, on food intake as well as on the metabolic and hormonal parameters were tested.

Results: Physical exertion resulted in a fall in the subjective sensation of hunger, but only following intensive exertion was this statistically significant. The intake of food was greater after exertion when compared to the control group. Moderate exertion resulted in a statistically significant but short-lived increase in the ghrelin level. This effect was not observed after intensive exertion, while in those tests during the post-meal period there occurred a fall in the concentration of ghrelin in the plasma. After exertion a physical fall was observed in the concentration of insulin in the plasma, for the intake of food resulted in a notable increase in its level.

Conclusions: Physical highly intensive exertion, results in a temporary reduction in the subjective sensation of hunger but leads to an increased food intake. The current research suggests that moderate but not intensive physical exertion stimulates the secretion of ghrelin.

Key words
ghrelin, gastrin, insulin, energy intake, exercise, hunger

INTRODUCTION

Obesity has been recognized as a global epidemic that poses a major risk for metabolic and cardiovascular diseases and its consequences range from increased risk of death to serious reduction of the quality of life. It is now generally accepted that this phenomenon is unquestionably related to a decrease in physical activity, caused by dramatic changes in lifestyle [1].

It is generally recognized that body weight depends on a balance between food intake and energy expenditure. Long-term energy surplus is a possible mechanism of obesity and increased physical activity is a strategy used to fight obesity, mainly because is thought to contribute to a negative energy balance by increasing energy expenditure [2].

Because any energy spent during the exercise could lead to increase the sensation of hunger and drives food consumption, it is appropriate to ask about the effectiveness of exercise in losing weight or in preventing weight gain. Classic physiologic studies have suggested that in the long term energy expenditure leads to increased energy intake to maintain body weight [3]. In some studies however, exercise has been demonstrated to markedly diminish also food intake but this reaction was short-lived, recovering approximately 15 min post-exercise. The acute effect of exercise on hunger/satiety sensations and food intake largely depends on the intensity of exercise [4, 5].

The important factor in control of energy balance is an appetite regulation. Feeding behavior is a complex interaction of various processes controlling the initiation and termination of meals, its size, composition and frequency, and the long-term regulation of food intake in relation to body energy requirements [6]. Our knowledge of the regulation of food intake has increased radically with the discovery of peptide ghrelin that sends signals to the brain regarding the body’s nutritional status [7]. Ghrelin was first discovered as an endogenous ligand for growth hormone secreting receptor [8]. Further studies showed that it strongly stimulated food intake and body mass and that its plasma concentration increases before meals and decreases with feeding, achieving concentrations sufficient to stimulate...
hunger and food intake [9]. Ghrelin was originally isolated from rat and human stomach and has been known to increase the secretion of growth hormone (GH), food intake, and body weight gain. Ghrelin is also known to stimulate the gastric motility and the secretion of gastric acid. Furthermore, synergistic action of gastrin and ghrelin on gastric acid secretion was shown. Although gastrin has important roles in postprandial secretion of gastric acid, ghrelin may be related to acid secretion during fasting period or at night [10] but relationship between both gastric hormones and their actions still to be elucidated.

Glucose, leptin and insulin have suppressive effects on ghrelin secretion [11, 12]. Insulin, is thought to inhibit neuropeptide Y (NPY) / agouti-related protein (AgRP) neurons in the arcuate region of the hypothalamus and thus reduce food intake [13]. Recent findings indicated that insulin is also a physiological modulator of plasma ghrelin and that insulinaemia mediates the effect of nutritional status on its concentration [14].

There are limited number of studies which investigated together the effects of exercise on hunger, food intake and energy regulating hormones and the lot of these studies concentrated on obese patients. The majority of these studies have focused on high intensity exercise [15, 16, 17, 18, 19]. These studies showed that intense (≥ 70% VO_2max) but not low intensity exercise caused a post exercise suppression of hunger and ghrelin [20, 21, 22].

There is still uncertainty regarding the effects of different types of exercise on appetite and food intake. Clear understanding of the changes in gut hormones during exercise could aid the development of optimal measures for the prevention and treatment of obesity [2].

Since fire-fighting requires a high level of fitness both for the safety of the firefighting personnel as well as for the adequate performance of their tasks, a physical fitness test was developed to examine a firefighter’s physical ability (Firefighter Fitness Test). This test which include treadmill test, pull hammer ergometer, bicycle ergometer, ladder ergometer and exercise in smoke chamber engaging multiple muscle groups is considered to be potentially maximal or supramaximal exercise [23].

The aim of the study was an evaluation of the impact of physical exertion on the regulating of food intake and digestive system hormone release as well as the partly connected phenomenon of evaluating the subjective sensation of hunger and the amount of food consumed at various time following physical exercise.

**MATERIALS AND METHODS**

**Participants**

Twelve moderately active men (mean ± SD age – 28.7±4.1; height – 1.78 ± 0.1 m; weight – 87.2 ± 11.7) were recruited from firefighters trainees to participate in this study. The sample size was determined using a meta analysis was designed to assess the influence of meal and exercise timing on postprandial lipemia [24]. None of the subjects had signs or symptoms of acute or chronic disease or was taking any medications. Participants received monetary compensation for participation. The volunteers were fully informed of the study details and gave their written consent prior to participation. The study protocol was approved by the Jagiellonia University Bioethics Committee, and all procedures complied with the Declaration of Helsinki.

All subjects were instructed to consume a stable diet containing 40–50% carbohydrate, 15–25% protein and 30–40% fat (energy percent) at least two weeks prior to and throughout the study period. All subjects were asked to refrain from vigorous exercise and ingesting caffeine or alcohol 24 h prior to the main trials. All experiments started at 8.00 a.m., after a 12 hour overnight fast.

**Preliminary tests**

Subjects attended the laboratory for an initial session during which anthropometric data were collected and they were familiarized with equipment. A submaximal fitness test was used to estimate VO_2max, bearing in mind the considerable limitations of this approach [25]. Test was performed on a Monark cycle ergometer with the seat height adjusted such that the subject’s knee was slightly flexed when the ball of the foot rested on the pedal at the lowest point in a revolution. Heart rates were monitored and recorded using a HR monitor (Polar F1 – Polar Electro Oy, Kempele, Finland). The ambient temperature range was about 23.8°C.

Initial work loads of 120 W at 60 pedal revs per min were used. If the heart rates after 2 minutes was less than 120 bpm, the work load was increased to 180 W and the test was continued. If in 4th minute the heart rates was less than 120 bpm, the work load was increased to 210 W and the test was continued until this criterion was met. The predicted VO_2 max was read from the nomogram or accompanying tables and multiplied by the von Dobeln age correction factors [26, 27].

**Study protocol**

To investigate the effect of exercise intensity or duration on appetite subjective sensation, food intake and hormonal and metabolic parameters the experiments were performed in two variants; one with and one without *ad libitum* test meal after exercise. Each participant took part in following studies:

1. Low-intensity exercise (LIE) bout on a bicycle ergometer for 30 min at 30% predicted VO_2 max which was intended to be below the aerobic–anaerobic threshold.

2. Higher intensity (HIE) study (Firefighter Fitness Test) above the aerobic anaerobic threshold which included:
   a. Horizontal treadmill: 6 min, 9 km/h
   b. Pull hammer ergometer: pull force 25 kG, 20 pulls
   c. Ladder ergometer 30 m, vertical
   d. Bicycle ergometer: 1 min, 200 W
   e. Smoke chamber and training galleries (maze) exercise: about 5 min.

3. Control, sedentary study (SS) – for the control trial participants rested for the entire duration of the trial.

Either exercise or sedentary study was followed by the test meal and by a postprandial observation or in control test, rest time. Subjects rated their subjective feelings of hunger, prospective food consumption and fatigue on 100 mm visual analogue scales (VAS) at baseline, exercise, feeding and during the postprandial period, respectively, as described previously [28, 29, 30]. To determine exercise intensity, a Rating of Perceived Exertion (RPE) using the 6–20 Borg Scale was recorded together with heart rate (HR). During the resting condition, participants remained seated, while allowed to read/write quietly. During test meal the subjects received sandwiches consisting of bread, butter and ham (2.73 kcal/g, energy percent: 44.4% carbohydrate, 16.2% protein...
and 39.4% fat) which they had to eat until reaching satiety. The total amount of food was assessed by weighing food items to the nearest 0.1 g and energy content (kcal) was determined [31]. In all experiments venous blood samples were obtained at baseline, exercise, feeding and during the postprandial period and ghrelin, insulin and gastrin plasma levels were evaluated using specific radioimmunoassay performed in the specialized Hormonal Research Laboratory of Isotopic Diagnostics at the Department of Physiology, Jagiellonian University Medical College, as described previously [32].

Shortly, concentrations of all hormones in the plasma were detected using the unit 1272 Clinigamma, LKB WALLAC, Finland in accordance with the producer’s instructions. The concentration of ghrelin in the test plasma samples was determined using S-2227R1KU4864 radioimmunoassay kits (Peninsula Laboratories, San Carlos, CA, USA). All measurements were performed in duplicate. Assay sensitivity was 3.0 pg/mL, and the specificity of the antibodies for the labeled human ghrelin was 100%. Assessments of gastrin levels were performed using a commercial kit (GAS-PR RIA; CIS Bio-International, France) following the manufacturer’s recommendations. The plasma samples (100 μL) were incubated in duplicate at 37°C for 3 h with 100 μL of tracer (125I-gastrin) and 300 μL of anti G-17 antibody. The antibody equally recognized and had affinity to the gastrins G-17 and G-34. Points of the standard curve at concentrations of 11.2, 28.4, 68.4, 255.8, 651.2 pmol/L were prepared from lyophilized synthetic G-17 and incubated as above. Separation of the free from bound fraction was obtained by immunoprecipitation. Final radioactivity in the samples was assayed, and standard curve points were measured in a gamma counter (1574 Clinigamma, Wallac-LKB, Sweden), using the computer program Spline in order to calculate the concentration of gastrin. Plasma insulin concentration was measured using kit Insulina-RIA-Prop (Polatom, Otwock-Swierk, Poland) in accordance with manufacturer’s instructions. The detection limit was 2.5 mU·L⁻¹ and the intra- and inter-assay variations were 4% and 4.8%.

Plasma lactate concentration ([La]pl) was measured using an automatic analyser (Ektachem XR 700, Kodak, USA).

Statistical analysis
All calculations were made using Statistica 10 (StatSoft) software. The normality of the data distribution was verified by means of Smirnov-Kolmogorov nonparametric test and results are expressed as means ± S.D. Statistical analysis was done using analysis of variance and two way ANOVA test with Tukey post hoc test where appropriate. Also Friedman test and paired Wilcoxon tests were used where appropriate. Differences of p<0.05 were considered significant.

RESULTS
Effects on self-reported hunger and motivation to eat measures and subsequent food intake
As shown on table 1 and 2 initial hunger (‘How hungry do you feel?’) and motivation to eat (‘How much do you feel you can eat?’) ratings did not differ among trials prior to the exercise. Exercise lowered significantly both ratings prior to meal consumption for all the exercise trials and this effect was particularly pronounced after HIE bout but these effects were transient.

In sedentary study when ad libitum test meal was not given, self-reported ratings of hunger and particularly motivation to eat rose steadily to the end of experiment (Tab. 1 and 2). Following the test meal, hunger and motivation to eat ratings decreased during the all trials. Furthermore, hunger and motivation to eat ratings were lower (p<0.05) during the HIE but not LIE comparing to sedentary study and the appetite suppressing effect of food intake remained augmented to the end of experiment (Tab. 1 and 2).

Energy intake
As shown on Fig. 1 energy intake at the test meal was significantly higher after the exercise (both in LIE and HIE bouts) when compared with the control intervention but during first 15 min of feeding effect was significant only in bouts) when compared with the control intervention but significantly higher after the exercise (both in LIE and HIE bouts) when compared with the control intervention but during first 15 min of feeding effect was significant only in studies with HIE intervention.

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<th>Table 1. Subjective feelings of motivation to eat (cm) before and after an exercise intervention (LIE or HIE) and after the test meal/control.</th>
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Values represent means ± SD for 12 subjects.

*P<0.05; LIE or HIE versus sedentary test.

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<th>Table 2. Subjective feelings of hunger (cm) before and after an exercise intervention (LIE or HIE) and after the test meal/control.</th>
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Values represent means ± SD for 12 subjects.

*P<0.05; LIE or HIE versus sedentary test.
Plasma metabolites and hormones

Baseline plasma ghrelin concentrations were not significantly different among trials (Fig. 2). In the sedentary study without test meal plasma ghrelin levels rise steadily from a mean baseline of 143.3±39 pg/ml to a maximum of 172.9±10 pg/ml at end of the experiment. Following exercise, fasting plasma ghrelin concentrations increased significantly (p<0.05) during the LIE trial but during and after HIE intervention plasma ghrelin fell significantly and this effect was observed to the end of experiment.

Following the test meal during the HIE but not intervention ghrelin concentrations were significantly lower comparing to sedentary study. On the other hand in postprandial period (30 min after finishing test meal) after LIE bout ghrelin was significantly higher comparing too sedentary test (Fig. 2).

Plasma insulin levels fell slightly during the exercise period and rose significantly during the postprandial period. During recovery from exercise time plasma insulin level was significantly higher after HIE bout than in sedentary test (Fig. 3).

Despite a tendency for higher gastrin levels during the LIE but not HIE intervention, when compared with a similar period of resting, they did not reach statistical significance. During recovery from exercise time plasma gastrin level was significantly lower than in sedentary test. Test meal caused an significant rise in plasma gastrin concentrations and there was no significant difference between trials (Fig. 4).

The HIE bout caused pronounced increase of plasma lactate from a baseline of 1.43±0.03 mmol/l to 7.53±0.08 mmol/l, and there was no significant changes between with and without test meal groups. and HIE bouts when compared with the control intervention but these effects were not statistically significant.
It was found that acute exercise could affect appetite sensations, endocrine response and EI in response to light exercise on bicycle ergometer and to moderately heavy general exercise during firefighter fitness test which engage larger muscle mass. That trial includes a number of physically demanding activities and the test has used not only cycle ergometer and standard horizontal treadmill but also vertical ladder treadmill, weight lifting and run the obstacle path in training gallery [23].

We demonstrated that hunger was suppressed after both exercise bouts but this response was more pronounced after heavy exercise, but food intake was increased after both light and heavy exercise. In several human studies a considerable decrease in subjective hunger after intensive exercise was observed. This exercise-induced anorexia was short lived and although usually observed only during, and for a short time after intensive (>60% VO2max) exercise [4, 39, 40, 41] has also been reported after moderate intensity exercise ([42]), consistent with our findings. A redistribution of blood flow from the splanchnic circulation, towards the skeletal muscles observed during exercise, could be implicated in this phenomenon [43]. Rowell and colleagues found a 60–70% decrease in splanchnic blood flow in humans exercising at 70% VO2max [44]. At maximal exercise intensity, splanchnic blood flow may be reduced even by about 80% [45].

In our study, on normal weight, young men, 30 min of moderate exercise caused a significant increase in EI at the ad libitum test meal. This is a rather surprising result and it couldn’t be explained by differences in hunger sensations. A significant decrease in hunger scores or motivation to eat immediately before food intake were observed between conditions. However, this apparent uncoupling between subjective feelings of hunger and food intake has been previously reported [28, 46]. The increased EI in response to acute exercise may be the result of cognitive factors including attitudes and beliefs associated with exercise, such as that exercise increases hunger or the common behavior of using food as reward for exercising [34]. There is a controversy regarding the effects of acute exercise on subjective indicators of appetite and energy intake. Some studies have shown no significant effects, few have observes significant reduction of hunger and some even increase in hunger and subsequent EI [47]. There are also varied observations on the acute effects of different intensities of exercise on subsequent energy intake [48], and such contradictory results may be partly caused by subject differences (gender, nutritional state and fitness) and experiment methodology (exercise intensity and duration, interval between exercise and feeding, macronutrient composition of the test meal). Motivation to eat and food intake in response to acute exercise seem to be modulated by gender, body weight and eating behavior. In general, acute exercise has no effect on subsequent EI in men, whereas in women an increase in EI is usually observed, either decreasing or abolishing the effects of exercise on EI [39, 49]. Normal weight women, unlike men, report an increased sensory attractiveness of food with exercise and do not experience the transient suppression of hunger observed immediately after exercise [41, 50]. Obesity can also affect the response to exercise and while it has been shown that normal weight individuals (mainly women) increase their EI in response to a 3-day exercise intervention, compared with a similar period of rest, obese subjects fail to show such a compensatory response [50].

**Figure 4.** Gastrin plasma levels (pg/ml)- basal and after the exercise intervention (LIE or HIE), just after the test meal/control and 30 min after test meal (postprandial)/ control in experiments without meal (top panel) and with meal (down panel). Values represent means ± SD for 12 subjects. *p<0.05; **p<0.01; LIE or HIE versus sedentary test. Statistical significance versus basal values is not shown on figure for sake of the clarity.

**Heart rate**
In all experiments heart rate at baseline was around 66 beats/min. Exercise induced an increase to 115.4±8.84 beats/min in the LIE and to 171±4.3 beats/min in the HIE trial.

**Hemoglobin and hematocrit**
No significant changes in either hemoglobin or hematocrit were observed over time during the exercise or resting sessions. Hemoconcentration was thus unlikely to have occurred during the exercise sessions performed in the present study.

**DISCUSSION**
Physical activity was believed to be an ineffective form of weight control because of the assumed accompanying increase in food intake. Recently some studies have shown however that acute exercise induced a short inhibition of appetite, even if this does not automatically translate into a decrease in following food intake [33, 34, 35]. Also when the physical activity level decreases, food intake does not seem to be down-regulated in the same way [34, 36, 37, 38]. These observations stressed the relatively weak coupling between energy intake and its expenditure.
Ghrelin is the first gastrointestinal hormone which has been shown to stimulate food intake [8]. It is also known to stimulate growth hormone release [8] and this observation has inspired several studies investigating the possible action of ghrelin as a mediator in exercise-induced stimulation of growth hormone. However, in most studies exercise had no effect on plasma ghrelin levels while increased significantly growth hormone [51, 52].

Ghrelin is a possible candidate to explain changes of food intake after exercise. In our study plasma ghrelin levels increased after low-intensity exercise but not after the higher intensity intervention when we observed significant decrease. This rise, however, was not paralleled by any increase of hunger sensations in either experimental condition but contrary we observed the decrease in hunger and motivation to eat. Food intake was however increased and effect was stronger after high-intensity than low-intensity exercise. Since ghrelin levels were increased after the LIE and decreased after HIE bout, it seems rather unlikely that ghrelin is of major importance for augmented food intake. Although there is a close relationship between total and the biologically active peptide stimulated acylated ghrelin [53] it cannot be excluded that after exercise this relationship is somewhat different. In recent study authors observed a significant decrease in acylated ghrelin plasma level during the exercise compared with the control condition, as well as a reduction in subjective hunger during suggesting that acylated ghrelin responds differently from total ghrelin to acute exercise [15]. It is possible that a strong adrenergic stimulation could, at least in part, be responsible for a suppressive effect on ghrelin during high-intensity exercise thereby overriding the as yet unknown stimulatory mechanisms activated by low-intensity exercise. Recent study has shown that exercise-induced ghrelin suppression may be mediated by activated adrenergic system [54]. Because a role a physiological modulator of ghrelin concentration was postulated for insulin, this hormone could be, at least in recovery from exercise time, responsible for decrease in ghrelin plasma level.

These findings indicate that during exercise other regulatory factors than ghrelin are of greater relevance and the biological impact of ghrelin’s rise during low-intensity exercise remains to be elucidated. A lack of an association between changes of plasma ghrelin levels and corresponding hunger/satiety scores has been reported previously during several types of test meals [55, 56]. It is worth to note that physical highly intensive exerstion, results in a health related quality of life [57] thanks to the diet modification.

**CONCLUSIONS**

It was found that acute exercise, particularly high intensity, have markedly different effects on appetite and energy intake. HIE intervention temporarily suppressed hunger and motivation to eat sensations but food intake was augmented in these conditions. This exercise-induced anorexia was observed only during, and for a short time after exercise. A redistribution of blood flow from the splanchnic circulation, towards the skeletal muscles observed during exercise, could be implicated in this phenomenon. The increased food intake in response to acute exercise may be the result of cognitive factors including attitudes and beliefs associated with exercise, such as that exercise increases hunger or the common behavior of using food as reward for exercising. A possible candidate to explain changes of food intake is ghrelin. The present data suggest that low rather than high-intensity exercise stimulates ghrelin levels. The impact of exercise on appetite and subsequent energy balance could not be fully explained by changes in postprandial levels of ghrelin. More research is needed to explain the mechanisms behind the post exercise adjustments in short-term appetite control. Future investigations may also seek to characterize responses in other populations, particularly the overweight/obese because it is with these individuals where the findings may hold most clinical relevance.

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