Growth Hormone Response Induced by a Respiratory Muscle Endurance Training in Healthy Subjects

Abstract

To date, the large majority of studies evaluating growth hormone (GH) response to acute physical exercise has been performed involving gross muscle groups. To the best of our knowledge, none has evaluated the effects of a respiratory muscle endurance training (RMET) on hormonal secretions, particularly on GH release, though some respiratory devices have been widely used in athletes to train respiratory muscles and to improve cardiopulmonary function and physical performance. 8 healthy men underwent an incremental progressive RMET protocol of 11 daily sessions, obtained through the use of a specifically designed respiratory device (Spiro Tiger®). The 12th session of RMET (15 min duration: 1 min at a respiration rate of 28 acts/min, 5 min at 32 acts/min, 5 min at 34 acts/min, 4 min at 36 acts/min) was associated with blood samplings for determination of GH, cortisol, ghrelin, glucose, and lactate (LA) levels. GH and cortisol responses significantly increased after a 15-minute RMET session, which, in contrast, inhibited ghrelin secretion. There was a minimal, though significant, increase in LA levels with a significant elevation in glycemia. A 15-minute RMET session, administered after a 11-days incremental progressive RMET protocol, was capable of stimulating GH and cortisol release and suppressing ghrelin secretion. Optimization of incremental progressive RMET protocols would be important to maximize the positive chronic effects of this intervention on somatotropic function and muscle performance.

Introduction

Physical exercise is the most potent physiologic stimulus for growth hormone (GH) release [1]. GH levels start to increase 10–20 min after the onset of exercise, peak either at the end or shortly after exercise and remain elevated for up to 2 h following the end of exercise [2]. The magnitude of the GH response to exercise is influenced by age [3–5], gender, body composition, physical fitness, intensity, type, and duration of exercise. The physiologic mechanisms through which GH secretion increases during exercise are not completely known, but changes in body temperature, blood lactate (LA) levels, and pH have all been hypothesized [1].

In addition to stimulating GH secretion, physical exercise also acts the hypothalamic-pituitary-adrenal axis (HPA) with an increase in circulating levels of cortisol [6].

To date, the large majority of studies have been performed evaluating hormonal responses to acute physical exercise involving gross muscle groups, mainly of lower limbs [7]. To the best of our knowledge, to date no studies have been devised on the effects of a respiratory muscle endurance training (RMET) on hormonal secretions, particularly on GH and cortisol releases, though respiratory devices have been widely used in athletes to train the endurance of respiratory muscles and improve cardiopulmonary function and, indirectly, physical performance [8–11].

Several mechanisms have been hypothesized to account for the positive effects of RMET on physical performance, such as the attenuation of diaphragm fatigue via an RMET-induced increase in the strength and endurance of the respiratory muscles, which become more mechanically efficient in sustaining the alveolar gas exchanges during whole-body exercise. Furthermore, by executing regular sessions of RMET, the respiratory muscles would undergo a process of adaptation, thereby preventing any reflex vasoconstriction effects on the locomotor muscle vasculature and...
so resulting in a long-lasting physical performance [12]. Anyway, the potential (acute) metabolic and (chronic) anabolic effects of an enhanced GH function by RMET cannot be ruled out [13]. Similarly, the benefits of the physiotherapeutical application of RMET in cystic fibrosis [14] or asthma [15] might be related not only to an increase in the size of type II fibers of the external intercostal muscles [16] or an improvement of alveolar gas exchanges [17], but also to changes in circulating levels of anti-inflammatory hormones, such as glucocorticoids (cortisol), which have a pharmacotherapeutical relevance in these conditions.

Therefore, the aim of the present study was to evaluate, in healthy subjects, the effects of a single acute session of RMET with a specifically designed respiratory device on circulating levels of GH and cortisol. As ghrelin, a 28-amino acid peptide produced by the stomach, an organ anatomically close to diaphragm, is a potent GH releaser and also promotes ACTH and cortisol release [18], RMET-induced changes of ghrelin levels were also evaluated.

Materials and Methods

Study design
The study was approved by the Ethics Committee of the Italian Institute for Auxology and was conducted in accordance with the principles expressed in the Declaration of Helsinki. To compare the effects of a single acute session of RMET (see below for details) on GH, cortisol, and ghrelin, a group of healthy volunteer subjects, recruited amongst friends and colleagues, was admitted to the study.

Subjects
Written informed consent was obtained from 8 healthy men (age: 26.3 ± 4.2 years, height: 178 ± 0.1 cm, body mass: 70.6 ± 1.8 kg; fat free mass, FFM: 89.2 ± 2.4%) following detailed explanation of experimental procedures and associated risks. All subjects were habitually active and involved in training on a regular comparable basis, and none of them had any signs of musculoskeletal disorder. The subjects were asked not to perform any exercise for at least 48 h before and during the experimental period, and not to take any medication or nutritional supplements. There were no significant differences in the alimentary habits of the individual subjects of the study group.

Testing
All the subjects admitted to the study performed a specific respiratory training incremental protocol, described in Table 1, by using a specific device (Spiro Tiger®, Idiag, Fehraltorf, Switzerland). The Spiro Tiger® training device consists of a hand-held unit with a respiratory pouch and a base station (Fig. 1). The specific properties of the device allow for personalized respiratory training through maximal inspirations and expirations without hypocapnia, and without the limitation resulting from involvement of lower limb muscles. To avoid hypocapnia in the presence of hyperpnoea, the device features a 2 way piston valve to a rebreathing bag. As the patient breathes out through the mouth-piece, the rebreathing bag stores part of the expired air, which contains increased concentrations of carbon dioxide. Once the rebreathing bag is filled to its capacity, a valve opens and allows

| Table 1 Respiratory training protocol performed by each subject before undertaking the single acute session of RMET on the experimental day (12th) to evaluate hormonal and metabolic responses. |
|-----------------|---------------------------------|
| 0 day | Theory, explanations, demonstration, and 10 min training. Breath co-ordination and choice of the rebreathing bag |
| 1st day | 6 min duration (2 min at a respiration rate of 28 acts/min, 2 min at 30 acts/min, 2 min at 32 acts/min) |
| 2nd day | 8 min duration (2 min at a respiration rate of 28 acts/min, 2 min at 30 acts/min, 2 min at 32 acts/min, 2 min at 34 acts/min) |
| 3rd day | 10 min duration (2 min at a respiration rate of 28 acts/min, 2 min at 30 acts/min, 2 min at 32 acts/min, 2 min at 34 acts/min, 2 min at 36 acts/min) |
| 4th day | 15 min duration (1 min at a respiration rate of 28 acts/min, 4 min at 30 acts/min, 4 min at 32 acts/min, 3 min at 34 acts/min, 3 min at 36 acts/min) |
| 5th, 6th day | 15 min duration (1 min at a respiration rate of 28 acts/min, 4 min at 30 acts/min, 4 min at 32 acts/min, 2 min at 34 acts/min, 2 min at 36 acts/min) |
| 7th, 8th, 9th, 10th, 11th day | 15 min duration (1 min at a respiration rate of 28 acts/min, 4 min at 30 acts/min, 4 min at 32 acts/min, 4 min at 34 acts/min, 2 min at 36 acts/min) |

Fig. 1 Spiro Tiger®, which consists in a held unit endowed with specific valve and respiratory pouch (1). Personal training target values are entered into the basic unit and serve to monitor the breathing frequency and depth during training (2). The display provides instruction such as “breathe faster” and a bar shows the respiration depth (3). The breathing frequency is paced by a moving light and brief sounds (4). In case of substantial deviation from the ideal training frequency, the Spiro Tiger® issues optical and acoustic warnings (5). The data collected during a training session are passed by cable to the basic station store signals (6).
the rest of the expired air to be released into the environment. The valve shuts when expiration finishes and inspiration starts. Inspiration empties the rebreathing bag first (filled with the exhaled air containing an increased concentrations of carbon dioxide), then the valve opens and some fresh outside air is inspired at the end of each inspiration.

Personal training target values are entered into the base unit and are used to monitor the breathing frequency and depth during training. The base station in the hand-held computer monitors the breathing frequency, sets threshold limits for breathing patterns, and displays visual and acoustic feedback so as to allow the subject to breathe within the threshold values for normocapnia. The base station also stores time and frequency of each exercise session, thus allowing the patient and his/her health care provider to retrieve and review the data.

The choice of the respiratory air pocket was based on approximately 50–60% of the ventilatory capacity evaluated by spirometry (CPD, Medical Graphics corp., MN, USA).

The 12th session of training (1 min at a respiration rate of 28 acts/min, 5 min at 32 acts/min, 5 min at 34 acts/min, 4 min at 36 acts/min), performed after an overnight fast, was associated with blood sampling for GH determinations before (basal) and 0′ (T0), 10′ (T10), 30′ (T30), 60′ (T60), and 120′ (T120) after the end of the training session. Serum concentrations of ghrelin and cortisol were determined before, at T0, T30 and T60; blood LA and serum glucose levels were measured before and immediately after the end of the session (T0).

FFM was assessed by bioelectric impedance analysis (Human-IM scan, DS Medigroup, Milan, Italy), performed in early morning after an overnight fast. Oxygen saturation was monitored by a pulse oximeter (Palmsat Handheld Oximeter, Nonin Medical Inc., Plymouth, MN, USA) during the entire period of the test.

Blood sampling and measurements

While the baseline blood sample was obtained by syringe venipuncture, the remaining blood samples were drawn through an indwelling cannula inserted into an antecubital vein kept patent with a continuous infusion of isotonic saline. All blood samples were allowed to clot, centrifuged for 5 min to obtain serum, and immediately stored at −20 °C for the next analysis.

GH concentrations were determined by a commercially available immunometric kit (Immulite 2000, DPC, Los Angeles, CA, USA). Intra- and inter-assay coefficients of variation for this assay were 2.5% and 6%, respectively. The sensitivity of the method was 0.01 ng/ml.

Cortisol levels were determined using a commercial ELISA kit (IBL-Hamburg GmbH, Hamburg, Germany). Intra- and inter-assay coefficients of variation for this assay were <8.0% and <15%, respectively. The sensitivity of the method was 1.5 ng/ml.

Total immunoreactive ghrelin concentration was determined with a commercial radioimmunological assay (RIA) kit (Millipore, Research Park Drive, St. Charles, Missouri, USA). The lower and upper detection limits were 93 pg/ml and 6000 pg/ml, respectively, whereas intra- and inter-assay coefficients of variation (at 1500 pg/ml) were 3.3% and 17.8%, respectively.

Serum glucose levels were measured by commercial colorimetric methods (Sigma Diagnostics, St. Louis, Missouri, USA). The sensitivity of the method was 2.16 mg/dl.

All samples were run in the same assay to minimize inter-assay variability.

Before starting the standardized warm-up, a small blood sample (5μl) was obtained from the earlobe for the determination of basal LA concentration. The blood sample at T0 was obtained from the earlobe of the other side. Blood LA was measured by a portable analyzer (Lactate Pro, Akray, Japan); for this method intra- and inter-assay coefficients of variation were 3% and 7%, respectively. The sensitivity of the method was 0.8 mmol/l.

Statistical analysis

All data were presented as means (±SEM). Peaks of GH and cortisol referred to the means of the highest measured values for each individual, while nadir of ghrelin the mean of the lowest measured values.

Circulating levels of GH, cortisol, and ghrelin were analyzed using one-way analysis of variance (ANOVA). Specific differences were identified using a paired t-test with Bonferroni correction for multiple comparisons. Student’s t-test for paired data was used to compare values of LA, glycemia, and oxygen saturation at baseline and T0. Correlations between couples of parameters (peak values of GH and cortisol, levels of LA at T0, and nadir value of ghrelin) were calculated by the least squares regression approach. Statistical significance was accepted at p < 0.05.

Results

GH responses significantly increased (p < 0.01) at the end of the 15-minute session of RMET, which was performed after a 11-days long-lasting respiratory training incremental protocol (from a mean basal value of 0.9 ± 0.4 ng/ml up to a peak value of 15.7 ± 4.0 ng/ml). Although GH responses were actually present in all subjects (peaks ranging between 4 and 40 ng/ml), the post-exercise peaks occurred at different times (at 0’ in 5/8 and in 3/8 at 30’ post-exercise). GH levels remained significantly higher at 60’ than those recorded at baseline (6.4 ± 1.8 ng/ml, p < 0.05), complete return to normal being present only after 120’ post-exercise (1.7 ± 0.5 ng/ml, p = NS) (Fig. 2).

The 15-minute RMET session resulted also in a significant increase in cortisol levels, from a basal mean value of 142.9 ± 9.4 ng/ml up to 149.2 ± 11.4 ng/ml at T0 (p < 0.01) and 184.1 ± 12.2 ng/ml at T120.

![Fig. 2](Fig. 2) GH responses to a 15-minute RMET session (from basal to T0). Evaluation was performed at resting before the test (basal) and 15 min after at the times indicated. Values are expressed as mean ± SEM. p < 0.05 vs. basal.
T30 (vs. basal, p<0.01). After 60’ from the end of the test, cortisol levels were still significantly higher than those found at baseline (168.4±6.8 ng/ml vs. basal; p<0.05) (Fig. 3).

After the 15-minute RMET session, ghrelin concentrations progressively declined, reaching the nadir at T30 (561.9±45.2 pg/ml vs. basal=711.9±73.1 pg/ml, p<0.05), returning to the baseline values at 60’ (645.2±45.7 pg/ml, p=NS) (Fig. 3).

Oxygen saturation never dropped below 96% in any subject throughout the protocol. There was no statistical difference in oxygen saturation before and after RMET (basal vs. T0: 98.3±0.3% and 96.8±0.3%, respectively, p=NS). LA concentrations significantly increased after exercise (from a mean baseline value of 1.2±0.1 mmol/l up to 2.3±0.2 mmol/l at T0; p<0.05). The 15-minute RMET session induced a significant increase in glycemia (90.1±1.6 mg/dl at baseline vs. 104.5±6.2 mg/dl at T0, p<0.05). No significant correlations were found between GH and cortisol peaks, ghrelin nadir, and the level of LA at T0.

**Discussion**

Understanding the effects of RMET on physical performance (e.g., large muscle mass dynamic exercise, such as running, cycling, and rowing) is an important issue in sport physiology because such supplemental training has the potential to improve physical performance even in endurance athletes [12]. Although controversy exists regarding the effect of RMET on physical performance [19], several recent studies in healthy subjects have shown that execution of specific protocols of RMET is associated with enhanced endurance exercise performance, at least when the exercise tests require the subjects to work at about 70–80% of their maximal capacity or less [8–11].

The mechanisms underlying the apparent enhancement in exercise performance following RMET are unclear. Plausible hypotheses include among others a delay of respiratory muscle fatigue, a redistribution of blood flow from respiratory to locomotor muscles, a decrease in the perception of respiratory, and limb discomfort [12].

Hormonal changes induced by RMET, such as those of GH, have not been so far investigated. As GH is a well-recognized ergogenic agent, often abused in sport for doping purposes [13], the potential metabolic and anabolic effects of an (endogenously) enhanced GH function by RMET should not be ruled out.

The results of the present study show that respiratory training through a specific commercial device is associated in healthy subjects with a significant increase of GH secretion, markedly greater than that previously recorded with whole body vibration (WBV) [20] or neuromuscular electrical stimulation (NMES) [21] and comparable to that evoked by high-intensity muscle voluntary contraction (MVC) [7].

A wide range of studies in several mammalian species, including humans, have shown acute increases in the plasma concentrations of glucocorticoids in response to submaximal, maximal, and supramaximal exercise [6]. In the present study, a 15-minute RMET session stimulated the secretion of cortisol, which reached the peak 20 min after that of GH.

Ghrelin, which is known to stimulate GH and ACTH/cortisol releases [18], does not seem to participate in the acute GH and cortisol responses to RMET, which, in the present study, were followed by a progressive decrease in circulating levels of ghrelin. Although the effect of exercise on circulating levels of (total) ghrelin is controversial, in a recent study [22], a negative correlation was found between ghrelin and catecholamines, which generally increase in plasma after acute exercise [23]. These results suggest that exercise-induced ghrelin suppression might be, in part, mediated by the activation of adrenergic system. The latter hypothesis is contradicted, however, by the finding of a stimulation of ghrelin secretion by β1-adrenergic agonists [24].

As a blockade of central β-adrenergoreceptors by propranolol has been reported to exert a stimulatory effect on GH and ACTH secretion during acute exercise [25], a non-catecholamine-mediated mechanism would need to be invoked to explain the RMET-induced release of GH and cortisol.

Suppression of ghrelin secretion after a RMET session might suggest that GH and cortisol releases are the consequence of an exercise-induced hypoglycemia [26], which in humans reduces circulating levels of ghrelin [27]. However, in the present work, the occurrence of a RMET-induced increase in glycemia rules out this possibility. Although both hyperglycemia and hypoglycemia may occur during exercise, serum glucose concentration usually remains relatively constant. These different effects of exercise on the glucose metabolism are due to changes in both the hormonal milieu and in the availability of hepatic glycogen and gluconeogenic precursors.
sors, which depend on many factors, including the type, intensity and duration of exercise and training and diet of the athlete [28]. It is notable that hepatic glucose output during exercise may be stimulated not only by glucagon and catecholamines, but also by GH and cortisol [29]. Hypoxia, acidosis and hyperlactemia, which occur during high-intensity anaerobic exercise, markedly stimulate GH and cortisol secretion [30]. However, in the present study, production of LA was minimal, although significant, and oxygen saturation did not change after the test, suggesting a negligible role of acid-base imbalance on RMET-stimulated GH and cortisol secretion. Accordingly, there was no correlation between GH peaks and LA levels after RMET.

Subjects, recruited into this study, were not (psychologically) stressed, being well-trained in undergoing acute sessions of RMET. Therefore, a potential RMET-induced (psychological) stress should not be invoked to explain the ensuing GH and cortisol secretion [31]. Although many mechanisms may be hypothesized, one (intriguing) possibility is to postulate the existence of (mono- or polysynaptic?) nervous efferents from the diaphragm to the hypothalamus, which are capable of regulating the activity of neurons located in specific hypothalamic nuclei, such as those of GH releasing hormone (GHRH) in the arcuate nucleus and corticotrophin (CRH) neurons in the paraventricular nucleus. With this hypothesis RMET would stimulate GH and cortisol release by contracting diaphragm muscle and activate GHRH- and CRH neurons via afferent nervous pathways.

Granted that ghrelin is not involved in the RMET-induced GH secretion, this does not exclude the possibility that GH may exert an effect on ghrelin secretion. In fact, as a negative correlation has been reported between circulating levels of ghrelin and GH after an acute exercise [32], the RMET-stimulated GH release might have inhibited ghrelin secretion via a negative feedback system. This view is in accordance with the results of Dall et al. [33], who found a decrease in ghrelin levels during GH replacement therapy in GH-deficient patients.

RMET-stimulated GH secretion could be incorporated as a provocative test in the diagnosis of GH-deficiency. The main advantage would be the not-invasive nature of this procedure, which would not require administration of any pharmacological stimulus [34]. However, a simplification of the RMET protocol would be advisable in a pediatric context. Further studies are mandatory to compare the diagnostic performance of RMET with other classical provocative tests in endocrinological practice.

Before concluding, a few clinical considerations should be mentioned. RMET is often used as one component of the rehabilitative plan in patients with asthma or COPD, who report an improvement in exercise tolerance, breathlessness, and quality of life [15,35]. These benefits might be related to an improvement of cardiopulmonary function such as that found in healthy subjects. However, the results of the present study suggest the causal involvement of RMET-induced changes in circulating levels of hormones endowed with anti-inflammatory properties, such as glucocorticoids (cortisol), which have a pharmacological relevance in these conditions. Further studies are mandatory to confirm this hypothesis.

In conclusion, the present study shows that a 15-minute RMET session is capable of stimulating, in healthy subjects, GH and cortisol release, and of suppressing ghrelin secretion. Notably, the RMET-induced GH-releasing effect is comparable in its potency to that observed after more stressful and prolonged protocols of aerobic exercise. Optimization of protocols based on RMET, a noninvasive and easily reproducible approach, might be beneficial not only in sport to improve the physical performance of athletes, but also in clinical practice when there is the need to stimulate GH/IGF-I function and muscle performance in patients with an impairment of motor capacity required to perform normal daily activities (i.e., severely obese or elderly people).

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