Hormonal Responses to a Resistance Exercise Performed Under the Influence of Delayed Onset Muscle Soreness

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ABSTRACT
Hormonal responses to an unaccustomed knee-extension exercise (E1; 5 times 10 repetitions with 40% load of 1RM [1 repetition maximum] followed by 2 sets until exhaustion) were compared in 6 men with the corresponding responses to an identical exercise performed 2 days later under the influence of delayed onset muscle soreness (DOMS) (E2). Both exercises were performed with a variable-resistance machine causing exhaustion with significantly fewer repetitions than a normal constant-resistance knee-extension device does. The E1 induced DOMS as expected, but the 1RM, the total work done, and the repetition number and frequency were not different in the 2 exercises. In the 2 sets to failure, the mean repetition number varied between 17 and 25. The exercise-induced norepinephrine, epinephrine, testosterone, cortisol (COR), and growth hormone (GH) increases were similar in the 2 exercises, although the overall level of COR and GH, including the preexercise concentrations, tended to decline in the second exercise. The results may thus suggest that the hormonal response to resistance exercise is not significantly altered when performed soon after an unaccustomed exercise bout leading to DOMS.

Key Words: hormones, variable-resistance exercise, muscle damage


Introduction
We have recently found a significant attenuation in plasma epinephrine (E) response to an exhausting knee-extension exercise 3 days after an unaccustomed exercise session leading to the development of delayed onset muscle soreness (DOMS), although the exercise intensity remained unchanged (22). This decrease in E response could not be explained by declined emotional stress caused by the muscular discomfort still present in the second exercise. It is well known that an acute exercise bout, especially if it contains eccentric muscle contractions (8), results in the disruption of contractile tissue and initiates an immune-mediated inflammatory response, which is in turn associated with DOMS (16, 17, 23). The local acute response to tissue injury involves the production of cytokines, which are released at the site of inflammation, facilitating an influx of lymphocytes, neutrophils, monocytes, and other cells that participate in the healing of the tissue. In addition, the classical stress-related hormones, norepinephrine (NE), E, cortisol (COR), growth hormone (GH), and possibly also testosterone (TES), have important immunomodulatory roles (21). However, the interaction between the immune system and hormonal release is bidirectional. The release of cytokines may increase sympathoadrenal activity as well as the secretion of COR from the adrenal cortex and GH from the anterior pituitary gland (18). In line with this it has been shown that the GH concentration response may be higher in resistance exercise with muscle damage than without it (13). It has also been shown earlier, by using the eccentric exercise model, that 1 previous training session may provide a protective effect for further muscle damage, even if the second exercise is performed before full recovery and restoration of muscle function (7). Therefore, a possible explanation for the decreased E response in our earlier study (22) could be a lower stimulus from the exercising muscles because of the protective effect of the first exercise.

The present experiments were designed as an extension of our earlier study (22) to examine more precisely the possible decrease in the plasma E response...
in the second exercise after the unaccustomed first exercise bout. In addition, the serum COR, GH, and TES responses were measured to see if they also decline in the second exercise.

**Methods**

**Experimental Approach to the Problem**

The resistance exercises in the present study were performed with a variable-resistance knee-extension machine (David 200, David International Ltd., Vantaa, Finland). In this machine a cam in the force transmission system is used to vary the resistance with knee angle to follow the specific force–angle relationship of the muscle (11). It has been shown earlier that with this machine exhaustion may be reached with about half the repetitions at a given load level as compared with a normal constant-resistance knee-extension device (10). We have also observed that exhausting, unaccustomed exercise with this machine clearly leads to DOMS, peaking approximately 2 days later when, however, no force production decline or only a slight one still exists (22). Therefore, assuming that the exercise loading would not have to be significantly reduced in the second exercise, and thus being able to keep the exercise stimulus (total work performed and exercise duration) relatively unchanged, this exercise model was used in the present study to examine the hormonal responses to a resistance exercise performed soon after an unaccustomed exercise bout. The second exercise was timed to the second recovery day, when the DOMS was expected to peak. If decreases in the stress hormone response were found, this might further reinforce the role of the muscle damage protective effect of the first exercise in explaining the decline, as it has been suggested earlier that exercise stress may even increase when performed with sore muscles (9).

**Subjects**

Six healthy men (27 ± 3 years, 179 ± 6 cm, and 77.2 ± 8.4 kg) volunteered to participate in the present study. All the subjects were physically active and exercised regularly 3–6 times per week, of which approximately 1–3 times per week were devoted to resistance training. However, none of them had used in the training the variable-resistance knee-extension machine used in the present study. The subjects were also advised not to perform any other type of knee-extension exercises during the 2 weeks preceding the study. The subjects were thoroughly familiarized with all testing procedures before the study, and their written informed consent was obtained. The Ethics Committee approved the study.

**Protocol**

The subjects adhered to a list of pretest instructions, including no exercise or ingestion of alcohol for 24 hours before the tests. The first test day started with blood sampling in the morning after 12 hours of fasting (from 7 to 8 AM) and continued with a light standardized breakfast that was similar for both testing days. After that, the subjects were allowed to ingest some food until 4 hours before the exercise, but none of them made use of this opportunity. No caffeine or other stimulants were allowed before the exercise, but the subjects were allowed to drink water freely. None of the subjects used nicotine. The first resistance exercise session (E1) started between 1 and 3 PM. The exercise protocol consisted of a total of 7 sets of bilateral knee extensions at 40% load of the 1RM (1 repetition maximum) with the variable-resistance exercise machine. The subjects first performed 5 sets of 10 repetitions (Esubmax), and then 2 sets of repetitions were continued until exhaustion (Emax1 and Emax2). The same exercise machine was also used for measurements of isometric maximal voluntary contraction (MVC) before and after the exercise to evaluate the exercise-induced fatigue. A summary of the study protocol is presented in Figure 1. All the subjects performed the entire protocol once again after 48 hours of rest (E2). DOMS was assessed with the use of a questionnaire (scale: 0 = no soreness, 5 = intolerable soreness) during the 5 days following E1.

**Blood Sampling and Analyses**

Arterialized venous blood was used in the present study to determine more accurately the arterial plasma catecholamine (CA) concentrations (12). In conventional blood samples from the antecubital vein, the plasma NE concentration is known to be disproportionately influenced by forearm sympathetic nerve activity, and the E secretion, on the other hand, may be underestimated because of forearm tissue extraction of E (12). To obtain arterialized venous blood, the fingers and knuckles of the right hand were placed in 40°C water 5 minutes before the preexercise sample, while the subjects were seated on the exercise machine, and were kept in water during the whole exercise session. A winged infusion set (either G22.0.60.20 mm or G22.0.70.20 mm, Danlab, Helsinki, Finland) was inserted into a vein on the back of the hand and advanced as far as possible in the distal direction. The morning resting blood samples were taken from the antecubital vein in a lying position.

The preparation of the CA eluates has been presented in detail previously (22). The CA in the eluates (50 µl) was measured by high-pressure liquid chromatography with a multichannel electrochemical detector (ESA CoulArray, model 5600). For the analysis of NE and E, an Inertsil ODS-3 column (4.0 × 150 mm, 3 µm; GL Sciences Inc., Tokyo, Japan) and a citric acid–monochloroacetic acid–acetonitrile buffer (pH 3.4), as mobile phase, were used. Flow rate was 0.6 ml·min⁻¹. For calibration purposes, known CA standards were treated in the same way as were the samples, and the
peak height ratios (relative to the peak height of the internal standard) of unknown CA were compared with those of the synthetic standards (CoulArray Software 1.003). The detection limit of CA standards in the method described was 0.2 nmol·L⁻¹ and the interassay coefficient of variation, 5±6%.

Serum samples for the other hormonal analyses were kept frozen at −25°C until assayed. The concentrations of serum total TES (TESₜₒₜ) were measured by the Chiron Diagnostics ACS:180 automated chemiluminescence system, with a sensitivity of 0.4 nmol·L⁻¹ and coefficient of intra-assay variation of 6.7%. The concentrations of serum free TES (TES₉ₒ) were measured by radioimmunoassay kits from Diagnostic Products Corp. (Los Angeles, CA) (sensitivity 0.52 pmol·L⁻¹ and coefficient of intra-assay variation 3.8%).

Serum GH concentrations were analyzed by 2-site fluoroimmunometric methods, using the AutoDELFIA analyzer (Wallac Oy, Turku, Finland) (0.01 µg·L⁻¹ and 2.7%) and serum COR by radioimmunassay kits from Orion Diagnostica (Espoo, Finland) (0.05 µmol·L⁻¹ and 4.0%). Fingertip blood samples were analyzed for hematocrit and hemoglobin, and plasma volume shifts were calculated (6). The fingertip blood samples (50 µl) for the blood lactate ([La⁻]ₒ) were analyzed enzymatically (Boehringer Mannheim, Germany), and the blood glucose (GLU) levels were determined photometrically (HemoCue, Ängelholm, Sweden). The morning samples were analyzed for serum creatine kinase (CK) activity (Boehringer Mannheim).

**Comparison of Exercise Loading**

The bilateral concentric 1RM was measured before both exercises, as described earlier (22), to determine the exercise load. During the exercise sets the range of movement in the knee joint (from 90° to full extension 180°) was controlled with a light signal at the desired extension angle. In addition, the actual displacement of the weight stack during each repetition was calculated using the lever arm angle recordings for the exact determination of the external work done (Weight calculated as the product of the force and the vertical displacement of the weights). In each movement cycle both the duration of the concentric extension phase (1 second) and that of the eccentric lowering of the weights (1 second) was indicated to the subject by an auditory feedback.

Electromyographic activity from the vastus lateralis, vastus medialis, and rectus femoris muscles of the right thigh was recorded during all measurements. The mean value of the average electromyographic (EMG) signal amplitudes of the 3 muscles in the MVC measurement was taken before the exercise for further analysis to represent the maximal voluntary activation level of the knee extensor muscles (aEMG_max). A corresponding aEMG parameter was also calculated for every set of knee extensions and normalized for the preexercise aEMG_max to represent the level of central command during the exercise contractions (aEMG_exc). To evaluate the exercise-induced fatigue the MVC was also measured after the exercise. The methods of the EMG and MVC measurements and analysis have been presented in detail previously (22).

Heart rate (fₜ) was recorded telemetrically during the exercise session, using a Polar Vantage NV heart rate monitor (Polar Electro, Kempele, Finland). The highest fₜ recorded during each part of the exercise was taken for further analysis.

**Statistical Analyses**

The results were analyzed statistically using 2-way analysis of variance for repeated measures and Student’s t-test for paired data. A power analysis for the statistically significant differences in the hormone concentrations between the 2 exercises was also performed (1). Before these analyses the normality of the data dis-
Table 1. The 1RM, exercise load, number of repetitions, external work done (W_{ext}), level of central command (aEMG_{max}), and peak heart rates (f_{p}) in the submaximal part of the exercise (E_{submax}) and in the 2 maximal sets (E_{max1} and E_{max2}).

<table>
<thead>
<tr>
<th>E1 (n = 6)</th>
<th>E2 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1RM (kg)</td>
<td>154 ± 29</td>
</tr>
<tr>
<td>Exercise load (kg)</td>
<td>63 ± 13</td>
</tr>
<tr>
<td>Repetitions</td>
<td>E_{submax}</td>
</tr>
<tr>
<td>E_{max1}</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>E_{max2}</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>W_{ext} (kJ)</td>
<td>E_{submax}</td>
</tr>
<tr>
<td>E_{max1}</td>
<td>9.2 ± 2.4</td>
</tr>
<tr>
<td>E_{max2}</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td>aEMG_{max} (% aEMG_{max})</td>
<td>E_{submax}</td>
</tr>
<tr>
<td>E_{max1}</td>
<td>63.7 ± 7.7</td>
</tr>
<tr>
<td>E_{max2}</td>
<td>71.6 ± 10.8</td>
</tr>
<tr>
<td>f_{p} (b·min⁻¹)</td>
<td>E_{submax}</td>
</tr>
<tr>
<td>E_{max1}</td>
<td>164 ± 11</td>
</tr>
<tr>
<td>E_{max2}</td>
<td>161 ± 10</td>
</tr>
</tbody>
</table>

† Mean ± SD; 1RM = 1 repetition maximum; EMG = electromyogram.
* Significantly different from E1, p < 0.05.
** Significantly different from E1, p < 0.01.

Results

The DOMS peaked just before E2 (2.3 ± 1.6) and declined thereafter, but the CK activity increase did not reach the level of statistical significance (E1: 240 ± 203 U·L⁻¹ vs. E2: 333 ± 163 U·L⁻¹, NS). No differences between the 2 exercises were observed in 1RM, the number of repetitions performed, W_{ext} or f_{p}, but the aEMG_{max} was lower in E2 than in E1 (Table 1). The mean repetition frequency varied between 0.48 and 0.52 Hz during both exercise sessions (NS). The MVC decreased similarly after the 2 exercises (E1: 1,916 ± 363 N vs. 1,411 ± 184 N, p < 0.01, and E2: 1,862 ± 257 N vs. 1,477 ± 246 N, p < 0.001), whereas no changes in the aEMG_{max} were observed in either exercise (E1: 356 ± 87 μV vs. 383 ± 106 μV, NS, and E2: 363 ± 112 μV vs. 360 ± 121 μV, NS). The [La⁻]₅ levels were similar in the 2 exercise sessions, but the GLU level tended to be lower after E2 than after E1 (Figure 2).

No differences between the exercise sessions were observed in the plasma NE responses, whereas the E concentration after E_{max2} was lower in E2 than in E1 (Figure 3, statistical power 0.85). However, in both exercise sessions the highest E concentration was observed in most of the subjects after E_{max1}, and no differences between the individual peak plasma E levels were found (E1 3.5 ± 0.8 nmol·L⁻¹ vs. E2 3.4 ± 0.6 nmol·L⁻¹, NS). In E2 the COR and GH concentrations tended to be lower than in E1 (Figure 4, statistical power 0.75–0.95), but no differences in the serum TES_{tot} and TES_{free} levels were observed between the 2 exercise sessions.

Plasma volume decreases after E_{submax} (E1: 11.9 ± 3.8% vs. E2: 7.3 ± 4.8%, NS) and after E_{max2} (E1: 14.6 ± 5.2% vs. E2: 10.6 ± 3.5%, NS) were similar in E1 and E2, and they could totally explain the increases in serum TES_{tot} and GH concentrations in both exercises.

Discussion

The present exercise protocol with the variable-resistance exercise machine led to rapid exhaustion despite the seemingly low exercise load (40% of 1RM). The mean repetition number in the last 2 sets (17±25 repetitions) clearly indicates that the load of 40% 1RM does not correspond to that of a constant-load exercise machine. However, it has been shown earlier that in this machine, exhaustion may be reached with only about half the repetitions (16 ± 3.5) as compared with a normal constant-resistance knee-extension device (32.3 ± 9.0) using a 60% load of 1RM (10). Therefore, it is clear that the exercise loading used in the present study corresponds to at least a load of 60% of 1RM in a normal constant-load exercise machine. Actually, the recently reported maximal number of repetitions in the bilateral leg press with an 80% load of 1RM in untrained men (20.3 ± 5.7) and in power lifters (21.0 ± 1.8), leading to a postexercise [La⁻]₅ level of about 7 mmol·L⁻¹ in both groups, was very similar to that in the present study (14). Taking the relatively small
active muscle mass (knee-extensor muscles, bilaterally) into account, the extremely exhaustive nature of the present exercise protocol is also evident from the postexercise [La\(^-\)]\(_b\) level (over 10 mmol·L\(^{-1}\) in both exercise sessions).

The first resistance exercise session in the present study induced DOMS in every subject as expected. In this respect, in addition to the unfamiliar exercise machine itself, the eccentric lowering phase of the weights in each movement cycle was probably especially important (4, 8). However, the subjective peak soreness ratings showed large variation between 1 (very slight soreness) and 5 (intolerable soreness). In addition, despite the fact that the first exercise also induced a large acute decline in MVC (26%), which could not be explained by a decline in central command (aEMG\(_{\text{max}}\)), only slight decreases in MVC (3%) and 1RM (4%) were found in E2 as compared with E1 (NS). Furthermore, although the true peak CK values may have been missed in the present study because blood samples were taken only 2 days after E1 (5), the serum CK activity increase also did not reach the level of statistical significance. Therefore, it is obvious that the possible muscle damage was rather moderate in the present study. This, however, made it possible for the exercise loading not to have to be decreased significantly in the second exercise. Indeed, exercise load (both relative and absolute), exercise duration, number of repetitions, repetition frequency, \(W_{\text{ext}}\), postexercise [La\(^-\)]\(_b\), and \(f_c\) responses were similar in the 2 exercises. The only difference between the 2 exercises was the slightly but significantly lower aEMG\(_{\text{exc}}\) normalized for the aEMG\(_{\text{max}}\) in E2 than for that in E1, suggesting a lower relative level of central command during the second exercise than during the first one. However, because EMG was only measured unilaterally, the activation of the knee-extensor muscles in the other thigh is not known.

Despite the fact that the exercise stimulus was almost identical in E1 and E2, some differences in the hormone concentrations between the 2 exercise sessions could be observed. Regardless of the unaltered NE response, the postexercise E concentration was sig-
Serum total testosterone (TES<sub>tot</sub>), free testosterone (TES<sub>free</sub>), cortisol (COR), and growth hormone (GH) concentrations. Significantly different from pre-exercise a <i>p</i> < 0.05, b <i>p</i> < 0.01, and c <i>p</i> < 0.001. Significantly different from the previous value d <i>p</i> < 0.05 and e <i>p</i> < 0.01. Significant difference between the exercises * <i>p</i> < 0.05.

In the present study the exercise-induced increases in GH and TES<sub>tot</sub> concentrations from pre-exercise were similar in E1 and E2 and could be explained completely by the plasma volume shift. The TES<sub>free</sub> concentration increase, which should not be influenced by the plasma volume change, also suggested a similar slight increase in both exercise sessions. No increase in COR concentration was observed in either exercise. Therefore, it is obvious that the stimulus for the increase of these hormone concentrations in the second exercise was not decreased by a possible muscle damage protective effect of the first exercise.

The trend for the declined GH and COR concentrations in the pre-exercise samples in the second exercise is difficult to explain. However, because the resting levels of these hormones in the morning were similar before the 2 exercises and the pre-exercise samples were drawn 12 minutes after the cessation of the measurements of 1RM and MVC, it is possible that the influence of these measurements was decreased, although the possible mechanism is unclear. It has been suggested earlier that the COR concentration at rest may be even increased when the leg muscles are sore because of the stress associated with normal ambulatory movements (9). Furthermore, it is also difficult to say whether the observed decline had any physiological influence. In the case of GH it must be kept in mind that multiple variants of GH exist in the circulation (2) and that different variants may respond differently to exercise stimulus (20). In the present experiments only the immunoassayable form of GH could be measured. The reason for the slight but significant GLU concentration decrease in E2 is unclear. It is, however, of interest that both COR and GH are involved in GLU regulation during exercise (19). No decrease in the GLU concentration during resistance exercise was observed earlier (e.g., 15, 24).

**Practical Applications**

The present results did not suggest any changes in resistance exercise-induced increases of the circulating NE, E, COR, GH, TES<sub>tot</sub>, and TES<sub>free</sub> concentrations significantly lower in E2 than in E1, supporting our earlier observations (22). However, because the individual peak E concentrations, already observed in most of the subjects after E<sub>max1</sub> were identical in E1 and E2, the results may not suggest a decrease in the responsiveness of the adrenal medulla to sympathetic input in the second exercise, as we have suggested earlier (22). Nevertheless, the results are in line with an earlier study showing that the plasma E concentration may peak during a repetitive high-intensity exercise rather than after it (3) and suggest that in this type of exercise it is also important to measure the plasma E concentration during the exercise and not just before and after it.
when the exercise was performed after an unaccustomed exercise bout leading to DOMS. Therefore, although the overall level of the serum COR and GH, including the preexercise concentrations, tended to decline in the second exercise, it may be suggested that the hormonal response to a resistance exercise is not significantly altered when performed with sore muscles. However, this does not mean that a proper recovery after damaging resistance exercise would not be feasible in practical strength training programs.

References


Acknowledgments

This study was supported in part by a grant from the Ministry of Education, Finland.

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