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EFFECT OF STRENUOUS ANAEROBIC RUNNING EXERCISE ON PLASMA GROWTH HORMONE, CORTISOL, LUTEINIZING HORMONE, TESTOSTERONE, ANDROSTENEDIONE, ESTRONE AND ESTRADIOL

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SUMMARY

Plasma growth hormone (GH), cortisol, luteinizing hormone (lutropin, LH), testosterone, androstenedione, estrone and estradiol levels were investigated before and after strenuous anaerobic running exercise of short duration in five male runners. After the exercise there were statistically significant increases in the mean plasma concentrations of GH (233%), LH (49%), testosterone (13%) and androstenedione (34%). Plasma cortisol increased only slightly. The testosterone and androstenedione concentrations in the samples taken 6 h after exercise were below the control levels (51 and 40%, respectively). This effect was more pronounced than during the day of normal activity when in the same subjects the testosterone concentration decreased only 12%, and the androstenedione concentration did not change at all. Exercise affected plasma estradiol levels in the same way as it did testosterone and estrone levels in the same way as cortisol. Control values were not reattained for some of the hormones until more than 24 h after the run. In this study the most fit runner showed greater LH, testosterone, androstenedione and cortisol response than the least fit runner, who had a more elevated GH level after the run.

INTRODUCTION

Exercise is known to elicit marked changes in blood hormone levels, especially, in those hormones which are involved in the regulation of energy balance. Previous studies dealing with exercise and growth hormone (GH) have been reviewed by Shephard *et al* [1], cortisol by Shephard *et al.* [1] and by Tharp [2], insulin by Métivier [3] and catecholamines by von Euler [4]. Many investigations have been undertaken with the purpose of clarifying the role of hormones in prolonged aerobic exercise [5–10], but our knowledge still remains very fragmentary. Fewer investigations have been carried out on the effect of strenuous anaerobic exercise on hormonal systems [11].

The aim of this investigation was to study the endocrine response to intermittent strenuous anaerobic running. Changes in blood and muscle biochemistry were investigated simultaneously. The latter findings are presented in detail in another communication [12]. The results of this study demonstrate that the blood levels of all hormones investigated showed considerable alteration during and after strenuous anaerobic exercise.

EXPERIMENTAL

Material

Five male runners, 21–23 years old, participated in the study. The experiment was carried out between 11 and 12 a.m. in the Olympic Stadium, Helsinki, in rather cold weather (5–10°C). Before the test exercise all runners had a warm up, which lasted about 15 min. Then, they each ran 300 m at their maximal speed, three times, resting for five min between the first and second, and three min between second and third run. The entire test exercise lasted about 10 min. At the end of the third run all five athletes were completely exhausted. Their blood pH immediately after the run was 6.85–7.00. This kind of exercise schedule is used by 400 m and 800 m runners when they are preparing for competitive exercise [12].

The anaerobic fitness of the runners was determined, using as a criterion the times taken to run 200 and 400 m. The fastest runner was the most fit and the slowest runner the least fit.

Venous blood samples were taken from the athletes before the warm up (at 11 a.m.), immediately (less than 30 s) after the test exercise and 30 min, 1, 3, and 6 h, and 1 and 3 days afterwards. All blood samples were taken with the subject in a recumbent position. The samples for GH, cortisol, testosterone, androstenedione, estrone, estradiol and LH were collected into heparinized glass tubes. All blood samples were immediately placed in an ice-bath and centrifuged in the cold (+4°C). The plasma was removed as soon as possible (within minutes) and stored at −20°C until analysed. The diurnal variation in plasma cortisol,

testosterone and androstenedione were studied in the same subjects throughout a day of normal activity.

Methods

All assays were carried out in duplicate. Adequate quality controls were included in all series of determinations.

Cortisol was determined by an enzymatic fluorometric method [13].

Testosterone was determined by a modification of the method of Ismail *et al.* [14]. After extraction of the steroid from the ammonium sulphate precipitate and evaporation of the organic solvent, the residue is taken up in 0.133 mol/l borate buffer, pH 8.0, containing 0.02% gelatin. The sample is incubated overnight at +4°C with antiserum (raised in rabbits against testosterone-3-b.s.a., Searle Diagnostic, High Wycombe, Bucks, England) and [1,2,3,4-^{3}H]-testosterone (The Radiochemical Centre, Amersham). Bound and unbound testosterone were separated using Dextran-coated charcoal in borate buffer (2.5 g Norit-A and 2.5 g Dextran T 70 in one l of the borate buffer).

Androstenedione was determined by a direct method based on the technical bulletin issued by Endocrine Sciences Inc. (Tarzana, California) (February 1972) and using their antiserum No. AN 6-22 (raised in rabbits against a 6-linked b.s.a. conjugate). The cross-reaction with testosterone is only 2%. The hormone is extracted with 2 × 2.5 ml of petroleum ether (b.p. 30–60°C) from 0.5 ml of plasma, to which 0.002 μCi of [1,2-^{3}H]-androstenedione is added as internal standard. After evaporation of the solvent, the residue is dissolved in 1 ml of borate buffer (as used in the testosterone assay) and suitable aliquots (100–250 μl) are taken for radioimmunoassay. Antiserum and 0.0125 μCi [1,2-^{3}H]-androstenedione are added, and the mixture is incubated overnight at +4°C. Separation of bound and unbound androstenedione is carried out, as for testosterone.

Plasma luteinizing hormone (*lutropin, LH*) was determined using a double antibody solid phase technique (DASP) [15], for the most part as described by Den Hollander and Schuurs [16]. The antiserum and purified human LH were obtained from the National pituitary agency (NIAMDD, National Institutes of Health, Bethesda, Maryland). The LH was labelled with ^{125}I by coupling lactoperoxidase to a cross-linked copolymer of maleic anhydride and butanediol divinyl ether (E. Merck) and using the coupled enzyme for iodination [17]. MRC reference material of Human Pituitary Lutropin (68/40) (World Health Organization, prepared by the International Laboratory for Biological Standards, Mill Hill, London, N.W.7) was used for the standard curves (assuming a value of 77 U/ampoule).* Antigen (unknown or standard), labelled antigen and first antibody were incubated at +4°C for 48 h in a vol. of 550 μl. Insolubilized second antibody (sheep antiserum to rabbit-gamma-globulin, Organon, Oss, Holland) (immunosorbent, 5.5 ml, diluted with 50 ml of 0.02 mol/l sodium phosphate buffer, pH 7, containing 0.02 mol/l NaCl, 0.005 mol/l Na-EDTA and 0.1% w/v merthiolate) was added (500 μl). The tubes were rotated at room temperature for 6 h and then centrifuged. The supernatant was discarded and the solid phase washed and counted. The inter-assay coefficient of variation for a pooled sample analysed 17 times over a period of 18 months was 14.2%. The coefficient of variation obtained for 67 analyses of human LH/FSH reference preparation (LER-907) over a 2-year period was 9.6%.

Growth hormone (*GH*) was measured using the human growth hormone radioimmunoassay kit obtained from CEA-IRE-SORIN, Paris, France with the following modifications: 0.1 ml of plasma and standard antigen are first incubated for 6 h at +37°C with 0.1 ml of the primary antibody, and after this 0.1 ml of labelled antigen is added to the tubes. The mixture is then incubated for 18 h at +37°C, after which 500 μl of insolubilized second antibody (immunosorbent-linked) (sheep antiserum to guinea-pig gamma-globulin, Organon, Oss, Holland) is added. The tubes are rotated at room temperature for 6 h and then centrifuged. The supernatant is discarded and solid phase washed and counted.

Plasma estradiol-17β and estrone were determined essentially as described previously [18–24], with the following modifications: The antiserum for estrone was obtained from Endocrine Sciences Inc. (Tarzana, California) (August 1972) (Antiserum No E17-94). The antiserum for estradiol was from Searle Diagnostic (High Wycombe, Bucks, England). The hormones are extracted from 2 ml of plasma (male) with 2 × 5 ml of an ethyl acetate–petroleum ether mixture (1:4 v/v), to which 0.0025 μCi of [2,4,6,7-^{3}H]-estrone and 0.0025 μCi of [2,4,6,7-^{3}H]-estradiol are added as internal standards. The micro columns of Sephadex LH-20 for hormone purification are prepared in pasteur pipettes (0.5 cm. × 5 cm.). The Sephadex LH-20 is first allowed to swell in a flask containing column eluting solvent, benzene–methanol (91:9 v/v), overnight. After this the Sephadex is washed five times with benzene–methanol (1:1 v/v) and four times with the column eluting solvent mixture. After washing, the column material is packed into pasteur pipettes and the samples, after evaporation of the extraction solvent, are transferred to the columns with 2 × 0.1 ml of the eluting solvent. The estrone and estradiol are eluted in the following way: the first 1.1 ml of the solvent is discarded, after this a 1.2 ml portion containing the estrone is collected, the following 0.4 ml of eluent is discarded, and the next 1.8 ml of solvent, containing the estradiol, is collected. After evaporation, the estrone and estradiol containing residues are dissolved in 1.0 ml of borate buffer, pH 8.0 (0.133 mol/l); 0.5 ml of the buffer solution is used for

* In our previous studies the results have been calculated assuming a value of 40 U/ampoule as originally suggested.

radioimmunoassay, and 0.4 ml for recovery measurement. 0.1 ml of the appropriate antiserum and 0.0125 μCi [2,4,6,7-^{3}H]-estrone or 0.0125 μCi [2,4,6,7-^{3}H]-estradiol are added and the mixture is incubated overnight at +4°C (or for 3 h at room temperature). The standard curve is prepared by measuring the dose-response of known amounts (5 to 200 pg) of estrone and estradiol, in duplicate. The separation of unbound and antibody-bound steroid is performed in the usual way, at +4°C, with 0.2 ml of Dextran-coated charcoal in borate buffer (6.25 g Norit A and 6.25 g Dextran T 70 in 1 l of borate buffer).

Statistics. The matched pair *t*-test was used for the statistical treatment of the results.

RESULTS

The control levels of plasma cortisol before running, in all five participants (mean 0.25 μmol/l), were within normal limits. Immediately after the test exercise the increase seen in cortisol concentration was not significant (mean 0.30 μmol/l). In the samples taken 6 h and 24 h after the run the mean cortisol concentration was below the mean control value and the mean values obtained 3 days after the run, but it did not differ significantly from the control level (Fig. 1). During the control day of normal activity an expected decrease in plasma cortisol concentration was observed, but the pattern of change was clearly different from that seen on the test exercise day (Fig. 2.)

The control values for plasma ,testosterone (mean 20.6 nmol/l at 11:00 a.m.) were within normal limits for all participants. Immediately after the test exercise the mean testosterone concentration (mean 23.3 nmol/l) was higher (13%) than the mean value before the run ($P < 0.05$). Thirty min (mean 16.8 nmol/l) and 60 min (mean 14.0 nmol/l) afterwards plasma testosterone levels were about 25% lower than the control values ($P < 0.05$), 6 (mean 10.0 nmol/l, $P < 0{,}001$) and 24 h (15.8 nmol/l, $P < 0.005$), after the run they were still significantly lower than resting values; after 3 days, the values were the same as

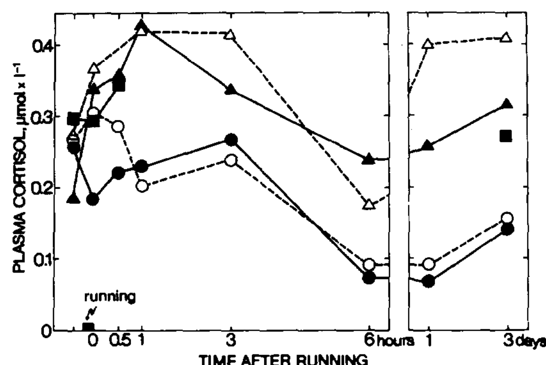

Fig. 1. Changes in plasma cortisol levels during and after strenuous short-term running exercise. The various symbols represent the values for each of the five test subjects.

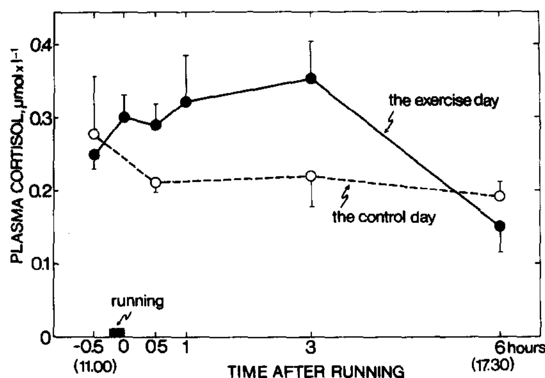

Fig. 2. Changes in plasma cortisol levels in the five test subjects during the exercise day (closed circles) and during the control day of normal activity (open circles). The values are expressed as mean ± S.E.M. (n = 5).

before the experiment (Fig. 3). During the day of normal activity there was a decrease (12%) in mean testosterone concentration over the same time period, but the quantitative change was very slight as compared to that observed during the exercise day (Fig. 4).

Under basal conditions the mean plasma androstenedione concentration was 8.3 nmol/l. Immediately

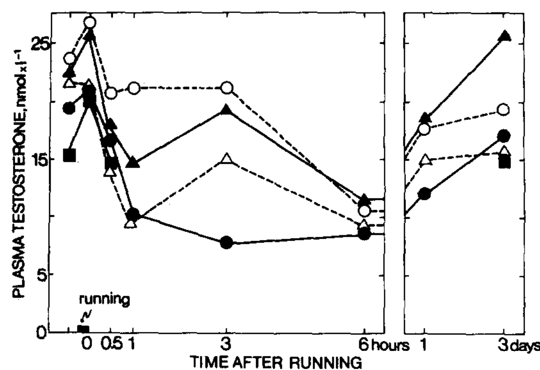

Fig. 3. Changes in plasma testosterone levels during and after strenuous short-term running exercise. The various symbols represent the values for each of the five test subjects.

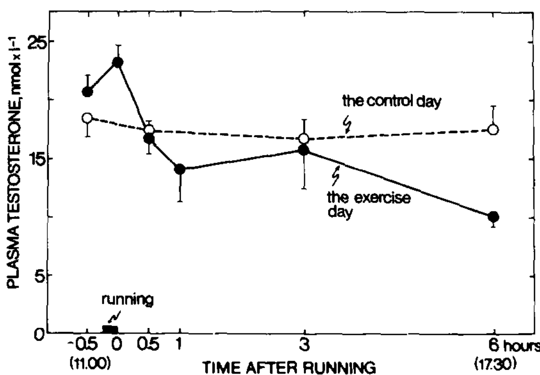

Fig. 4. Changes in plasma testosterone levels in the five test subjects during the exercise day (closed circles) and during the control day of normal activity (open circles). The values are expressed as mean ± S.E.M. (n = 5).

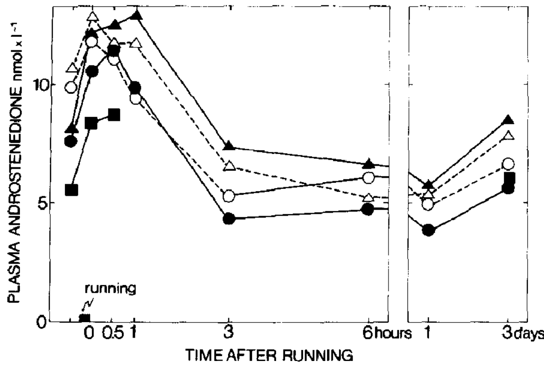

Fig. 5. Changes in plasma androstenedione levels during and after strenuous short-term running exercise. The various symbols represent the values for each of the five test subjects.

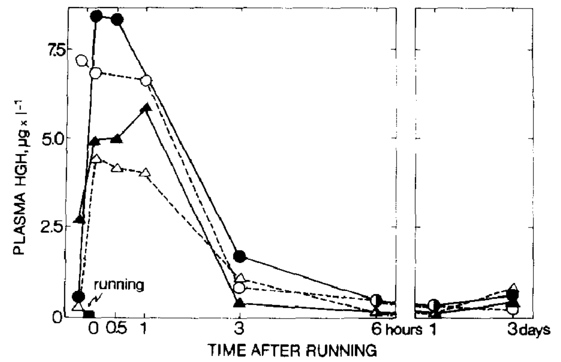

Fig. 7. Changes in plasma GH levels during and after strenuous short-term running exercise. The various symbols represent each of four of the test subjects.

after the exercise the androstenedione concentrations (mean 11.1 nmol/l) were elevated about 34% ($P < 0.005$), elevated values were also seen 30 min after the run (mean 11.0 nmol/l, $P < 0.02$). Three (mean 5.8 nmol/l), 6 (mean 5.7 nmol/l) and 24 h (mean 5.0 nmol/l) afterwards the values were markedly lower than the control values ($P < 0.05$–0.01). The pre-exercise level had been reattained three days after the run (Fig. 5). During the day of normal activity plasma androstenedione concentrations did not change at all.

Plasma LH levels were significantly elevated (49%) above resting values (mean 21.2 IU/l) immediately after the test exercise (mean 31.6 IU/l, $P < 0.01$). Thirty min after the run (mean 28.7 IU/l) plasma LH was still significantly elevated ($P < 0.02$). The pre-exercise level was reattained within three h (Fig. 6).

Plasma GH increased from a mean resting value of 2.7 μg/l to 6.2 μg/l after exercise, an increase of 233%. Because the control value in one of the test subjects was very high, 7.3 μg/l, the increase immediately after the run was not significant. The reason for this high control value is not known, but one explanation might be the psychological stress in this kind of situation, which is known to elicit an increase in GH secretion [25]. However, none of the runners

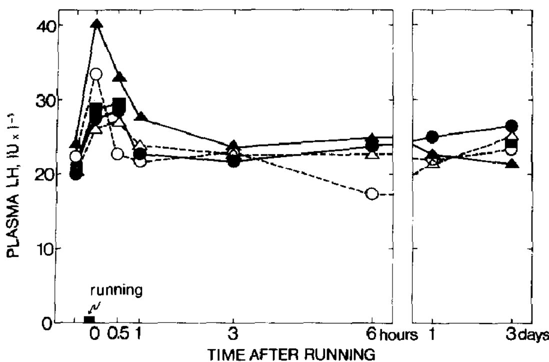

Fig. 6. Changes in plasma LH levels during and after strenuous short-term running exercise. The various symbols represent the values for each of the five test subjects.

showed any anticipatory responses in plasma cortisol levels before the run. When the values immediately after the run are compared with those three days after (mean 0.46 μg/l) the increase is highly significant ($P < 0.01$); the GH values 30 min after the exercise (mean 6.0 μg/l) were still > 1 000% higher than the resting values recorded three days later. If the peak values 0–30 min after the run are compared to the 3 h values (mean 1.1 μg/l) a decrease of more than 80% in GH concentration ($P < 0.01$) is evident. The reason for this pronounced decrease is not known. Six and 24 h after the exercise the values were normal (Fig. 7). Before the exercise the participants mean plasma estrone concentration was 133 pmol/l. The decrease in the mean estrone concentration after the run was not statistically significant. Resting values were reattained by the third day after the run (Table 1). Mean plasma estradiol concentrations increased from 53 pmol/l to 113 pmol/l after the run. This represents an increase of 156%, but because of large individual variation in estradiol concentration the changes were not statistically significant. Resting values were reached on the 3rd day (mean 53 pmol/l) after the run (Table 1).

A slight hemoconcentration, as judged from plasma protein, blood hemoglobin and hematocrit determinations, was observed during the exercise, but this was not sufficient to cause the increased hormone levels observed immediately after the exercise [12].

DISCUSSION

The effects of short-term anaerobic exercise has been previously studied very little [11]. The results of the present investigation show that changes in blood hormone levels during and after anaerobic exercise are different in many respects from those which are seen during prolonged aerobic exercise [5–10].

It has been demonstrated that anticipation of muscular exercise may significantly affect plasma cortisol levels in human subjects prior to exercising [26]. Furthermore, there may be a fall in plasma cortisol dur-

Table 1. Changes in plasma estrone and estradiol levels during and after strenuous short-term running exercise. The values are expressed as mean ± S.E.M. (n = 4)

	Pre-exercise	Time after running						
		0 min	30 min	60 min	3 h	6 h	24 h	72 h
Estrone pmol/l	113 ± 33	118 ± 12	121 ± 16	122 ± 15	112 ± 19	77 ± 12	72 ± 6	134 ± 17
Estradiol pmol/l	53 ± 18	113 ± 46	136 ± 43	80 ± 13	74 ± 22	108 ± 38	90 ± 14	53 ± 3

ing the warm up before the actual contest if the work load does not exceed a critical stimulation level [27].

The changes in plasma cortisol levels after the test exercise were not very great. In addition, none of the runners showed any anticipatory responses in cortisol levels before the run. It is surprising that we did not find any clear increase in mean cortisol levels after the run, but when these are compared to the mean cortisol level at the same time on the day of normal activity a slight increase after exercise is apparent. However, during and after long distance running much more pronounced increases in plasma cortisol have been reported [5, 8]. In exercise of short duration the running time may not be sufficiently long to cause massive adrenocortical response. Davies and Few [28] have reported that during heavy aerobic exercise a significant increase in plasma cortisol is first demonstrable 20 min after the beginning of exercise; our short anaerobic exercise lasted only 10 min.

In previous studies, exercise has not been found to elicit changes in plasma LH levels [8, 29, 30]: Only in one study was an increase in plasma LH (about two-fold) seen in a very fit subject after long distance running [5]. After short-term anaerobic exercise, LH levels increased significantly. The reason for this rise is not known, but it would be of interest to establish whether or not training and psychological "self motivation" have any effect on the LH response to exercise.

Plasma testosterone has been found to increase, decrease, or remain unchanged after aerobic exercise [5, 29, 31]. These diverging observations are probably due to the different levels of fitness of the test subjects and/or different types and duration of the test exercises. After short-term exercise there was a significant increase in plasma testosterone. Although LH also increased the testosterone rise cannot be the result of LH stimulation because the increases occurred simultaneously. The rise in testosterone may be due to increased release from the testis. However, other mechanisms may be involved as discussed previously by Sutton *et al.* [29]. Decreased metabolism of testosterone due to a drop in hepatic blood flow during exercise may also contribute to the increase in plasma testosterone [32]. In three of the runners we found relatively high values in the samples taken 3 h after the exercise. At that time there was a small peak or delay in the decrease in testosterone which continued up to 6 h. Alford *et al.*, have reported that a positive correlation exists between plasma testosterone values and the LH level in samples taken 2–3 h previously [33]. This suggests that the delay in the testosterone decrease 3 h after the run may be caused by the rise in LH values immediately after the test exercise. Six hours after the run the testosterone values were low, less than 50% of the pre-exercise values ($P < 0.001$). This decrease was much more pronounced than the normal diurnal variation (between 11:00 a.m. and 17:00 p.m.) in plasma testosterone levels (12%) in the test subjects.

During long distance running plasma androstenedione levels have been found to rise [5]. This is probably due to increased adrenal production. However, other factors may also have contributed to the increase in plasma androstenedione after short-term anaerobic exercise since during such exercise there is only a slight increase in adrenal activity. The rise in adrostenedione levels was more prolonged than that in testosterone. Thus an inhibition of the conversion of androstenedione to testosterone would explain the prolonged rise in androstenedione and the drop in testosterone observed between 30 min and 60 min after the run. Six hours and 24 h after the test exercise the androstenedione levels, like the testosterone levels, were very low. These changes are not due to diurnal variation, because during the day of normal activity no significant variations in plasma androstenedione concentrations were apparent. Further research is needed to clarify the reasons of these changes in plasma androstenedione.

As estrone and its precursors are mainly of adrenal origin, it was not surprising that cortisol and estrone levels reached their lowest values at the same time. It can be observed that the changes in mean estradiol and estrone levels are reciprocal, but at the present stage of this study no explanation for this phenomenon can be offered. It was also evident that the rises in mean estradiol concentrations occurred after a corresponding increase of mean plasma testosterone levels. This may be of some relevance as testosterone is the immediate precursor of estradiol.

Several authors have found that aerobic exercise increases plasma GH concentration [7, 8, 9, 34], but this increase depends on many factors. The rise is higher during competitive than noncompetitive exercise [7]; Hansen has reported that the increase in GH was larger during 20 min of heavy work than during 20 min of moderate exercise [35]; it has also been found that the rate of GH return to basal levels was

much slower in unfit than in fit subjects [36]. During the very strenuous anaerobic exercise of our experiment the GH increased, but to a lesser extent than has been reported for aerobic exercise of long duration. The rise also occurs earlier than during aerobic exercise [34].

The mechanism for the increase in blood GH that is observed after exercise is still unclear, but it seems likely that the control of GH release in exercise is mediated by adrenergic functions found locally in the hypothalamus [37]. It is also possible that humoral factors effect GH release. Lassarre *et al.* have suggested that the level of circulating hormone and anaerobic glycolysis may be connected [34]. However, it seems unlikely that changes in blood FFA could have a marked effect on GH release during exercise [38]. In addition, the stimulation of GH release during exercise may be mediated through a common afferent pathway in the spinal cord which Newsome and Rose have concluded to be active during surgical stress [39].

The changes in GH after exercise are partly related to alterations in energy metabolism. Adamson *et al.*, have reported that there was a significant increase in plasma GH during sleep after daytime exercise as compared to sleep following rest days. They suggested that higher levels of GH reflect reparative processes taking place during sleep [40].

In the present study it was found that the most fit and the least fit of the participants had different patterns of changes in their plasma hormone levels. The most fit subject had comparatively high LH, testosterone and androstenedione levels after the run while the least fit runner had comparatively low levels. In the most fit subject the increase in cortisol was most pronounced, while in the least fit runner there was a slight decrease after the run. The increase in GH was much more pronounced in the least fit runner than in the most fit runner. Other studies have also demonstrated that fitness may affect the hormonal response during and after exercise [5, 9, 36].

It can be concluded that short-term anaerobic exercise causes marked changes in plasma hormone concentrations. The pattern of these hormonal changes differs in many respects from that observed during and after exercise of long duration. Individual differences were great, and it would seem that the reaction of very fit and less fit persons to both aerobic and anaerobic exercise may be quite different, at least in terms of hormonal response.

At this stage of the study we cannot speculate about possible beneficial or harmful effects of these pronounced alterations in hormone metabolism in individuals who train regularly. Longitudinal studies are needed before this question can be answered.

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