

ORIGINAL ARTICLE

Adaptation of the hypothalamic-pituitary hormones during intensive endurance training

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Summary

Objective Physical activity leads to changes in the hypothalamic-pituitary hormonal system. However, acute and long-term adaptations have not yet been precisely characterized. In this study, the changes of the hormonal system as a result of marathon training and running a marathon were examined. In particular, we focused on adaptations of the hypothalamic-pituitary-adrenocortical (HPA) axis, regarding the activation or inactivation of cortisol to cortisone by the 11 β -hydroxysteroid-dehydrogenase system (11 β -HSD).

Design Patient measurements: 8 healthy women and 11 healthy men volunteered for this study. Blood samples, 24-h urine and a dexamethasone suppression test were analysed for metabolic and hormonal parameters at five different dates 12 weeks around a marathon.

Results Cortisol and ACTH values decreased significantly 2 days after the marathon, whereas the activity of the whole body 11 β -HSD-1 was up-regulated. An increased suppression of cortisol levels was observed in the dexamethasone suppression test after 6 weeks of reduced training levels. Ghrelin was elevated 2 days after the marathon. Only minor changes in the other hypothalamic-pituitary-hormonal axes could be observed. However, the free androgen index increased significantly after 6 weeks of reduced training.

Conclusions The HPA system appeared to become chronically activated by continuous physical training and therefore less sensitive to the dexamethasone suppression test. The acute stress of the marathon led to a central exhaustion of the HPA system with a paracrine counteraction by the activation of the 11 β -HSD system. Changes in the other hypothalamic-pituitary hormonal axes were the result of long-term differences in training levels and were not altered by the marathon.

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Introduction

Physical activity is known to induce various endocrine changes^{1,2} such as an activation of the hypothalamic-pituitary-adrenocortical (HPA) system^{3–5} but varies between acute and long-term adaptations.⁶ In particular, relatively short-term intensive physical activity leads to increased plasma levels of glucocorticoids like cortisol. This is apparently required to cope with the higher demand of energy as a result of the acute stress, e.g. running a marathon.⁷ Data concerning long-term physical activity or endurance training are not conclusive. In some studies it was suggested that chronic stress or physical training leads to a pseudo-Cushing's syndrome with elevated cortisol levels or insufficient suppression in the dexamethasone suppression test.^{8,9} On the other hand there are also studies that showed a decrease in cortisol levels after long-term endurance training or competition.^{10,11} Furthermore, in some studies no changes in cortisol metabolism were found.¹² In addition to the mechanism of the cortisol synthesis or production in response to exercise, there is no information about the activation or inactivation of cortisol to its inactive metabolite, cortisone, by the 11 β -hydroxysteroid-dehydrogenase system (11 β -HSD).^{13,14} The NADP⁺/H-dependent 11 β -HSD type 1 (11 β -HSD-1) enzyme functions *in vitro* as a bidirectional oxidoreductase and is expressed ubiquitously. *In vivo*, 11 β -HSD-1 acts mainly as a reductase and activates inactive cortisone to cortisol. Thereby it is believed that 11 β -HSD-1 modulates intracellular concentrations of active glucocorticoids and occupancy of the glucocorticoid-receptor. The main function of 11 β -HSD-2 is the protection of the unselective mineralocorticoid receptor, which has similar affinity to cortisol and aldosterone. This isoenzyme converts large amounts of cortisol to inactive cortisone, thus allowing the less concentrated aldosterone to bind to the mineralocorticoid receptor.

Besides the changes in glucocorticoid secretion and metabolism following physical activity, the response of other hypothalamic-pituitary

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hormones to endurance training are still not clear. The changes in the growth hormone/insulin-like growth factor-I (GH/IGF-I) axis as a result of endurance training seem to depend on many factors. Short-term activity leads to an increase of GH, IGF-1 and IGF-BP3,^{15,16} whereas there is little information about chronic physical activity. In elderly men, no differences in IGF-I, GH and IGF-BP3 levels were found between endurance-trained and normal men. However, IGF-BP1/2 levels were slightly different in both groups.¹⁷

A recently discovered hormone, with strong GH-releasing properties, is ghrelin.¹⁸ Ghrelin, a 28-amino acid peptide, is produced by the enterocrine cells of the gastric mucosa. It is the natural ligand of GH secretagogue receptor and stimulates GH secretion more potently than GH-releasing hormone (GHRH).¹⁹ In addition, ghrelin also stimulates the release of corticotropin releasing hormone (CRH), vasopressin, ACTH, cortisol, prolactin and aldosterone,^{19–21} whereas its own secretion seems to be inhibited by somatostatin.^{22,23} Short-term exercise did not lead to changes in ghrelin levels.²⁴ The effects of prolonged exercise are unknown.

The aim of this study was to assess the adaptations of the hypothalamic-pituitary hormonal system to endurance training and specifically to running a marathon.

Materials and methods

Subjects

Eight healthy women between 26 and 55 years old and 11 healthy men between 30 and 66 years old volunteered for this study. The BMI was $21.6 \pm 1.1 \text{ kg/m}^2$ and $24.0 \pm 1.6 \text{ kg/m}^2$ for the female and for the male athletes, respectively. The VO_2max was $42.9 \pm 2.1 \text{ ml/kg} \cdot \text{min}$ for the female athletes and $48.3 \pm 1.8 \text{ ml/kg} \cdot \text{min}$ for the male athletes. A training log over the whole study period of 12 weeks was carried out by each runner. All volunteers trained the whole time in Berlin at an altitude of about 80 m above sea level and all participants were requested not to change nutritive behaviour.

All volunteers were screened for serious health problems or use of any drugs by interview, and an examination was performed by an experienced sports physician. The experimental protocol was approved by the institutional review board, and all subjects gave written informed consent.

Experimental design

Metabolic parameters were analysed at five different dates around the 30th real-Berlin marathon:

Date 1: 6 weeks before marathon

Date 2: 10 days before marathon

Date 3: 2 days after real-Berlin marathon

Date 4: 10 days after marathon

Date 5: 6 weeks after marathon

The participants came at 2 days between 8:00 and 9:00 h for blood withdrawal. Before the first day they also collected urine for 24 h. At date 1, blood samples were taken for basal values of cortisol, ACTH, total testosterone, sex hormone binding globulin (SHBG), LH, FSH, insulin-like growth factor-1 (IGF-1), insulin-like growth factor-binding protein 3 (IGF-BP3), TSH, free thyroxine (fT4), ghrelin and

C-reactive protein (CRP). The volunteers received 1 mg dexamethasone, which they were asked to take at 12:00 h on the same day. At 8:00 h of the second day, blood samples were taken for cortisol and ACTH after 1 mg dexamethasone.

Methods

After sampling in ethylenediaminetetraacetic acid or serum tubes, blood was immediately chilled on ice and centrifuged; aliquots were frozen at -20°C until assayed. Blood samples were analysed for cortisol, ACTH, testosterone, SHBG, LH, FSH, IGF-1, IGF-BP3, TSH, fT4 by the full automatic chemiluminescence-immunoassay system IMMULITE from DPC Biermann (Bad Nauheim, Germany). Free androgen index was calculated by $\text{FAI} = \text{testosterone} \times 100 / \text{SHBG}$. CRP was measured with COBAS MIRA from Roche (Lörrach, Germany). Free cortisone, free cortisol, tetrahydrocortisol (THF), αTHF and tetrahydrocortisone (THE) concentrations in 24-h urine samples were analysed by RIA.²⁵ $11\beta\text{-HSD-1}$ activity was calculated by the ratio $(\text{THF} + \alpha\text{THF}) / \text{THE}$, and $11\beta\text{-HSD-2}$ activity by the ratio free cortisol/free cortisone.

Plasma ghrelin was analysed in all samples from individual subjects in duplicate in the same assay. Immunoreactive total human plasma ghrelin was measured by RIA (Phoenix Pharmaceuticals, Mountain View, CA, USA). Intra- and interassay CV was 5.3% and 13.6%, respectively.²⁶

Statistics

Statistical calculations were performed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

All values are given as mean value and standard error. Paired analysis was performed by Wilcoxon test. An alpha-error below 5% was considered statistically significant.

Results

In the time before the marathon, there was a significantly higher training level (date 2: $57.5 \pm 4.3 \text{ km/week}$, date 4: $20.5 \pm 4.0 \text{ km/week}$, $P < 0.05$), which is shown by the number of kilometres per week (Fig. 1). The 42.195 kilometres of the marathon are not included in training amounts at date 3 ($28.44 \pm 5.1 \text{ km/week}$).

The data for ACTH and cortisol are shown in Fig. 2. There was a significant decrease of ACTH (date 1: $21.5 \pm 2.1 \text{ ng/l}$, date 3: $16.7 \pm 1.4 \text{ ng/l}$, date 5: $23.3 \pm 3.2 \text{ ng/ml}$, $P < 0.05$) and cortisol (date 1: $483.5 \pm 37.0 \text{ nmol/l}$, date 3: $370.7 \pm 25.8 \text{ nmol/l}$, date 5: $446.6 \pm 43.3 \text{ nmol/l}$, $P < 0.05$) 2 days after the marathon, but no differences were observed in relation to high and low training levels. All ACTH and cortisol values were in the physiological normal range.

The results of the dexamethasone suppression test showed significantly lower levels of cortisol 6 weeks after the marathon with lower training levels (Fig. 3). Significant differences were seen only in date 5 (date 1: $40.2 \pm 4.5 \text{ nmol/l}$, $P = 0.008$, date 5: $31.6 \pm 3.7 \text{ nmol/l}$, $P = 0.037$). No differences were found in relation to the marathon on date 3.

Values for $11\beta\text{-HSD-1}$ activity and $11\beta\text{-HSD-2}$ activity are shown in Fig. 4. Whereas the activity of the $11\beta\text{-HSD-2}$ appeared to

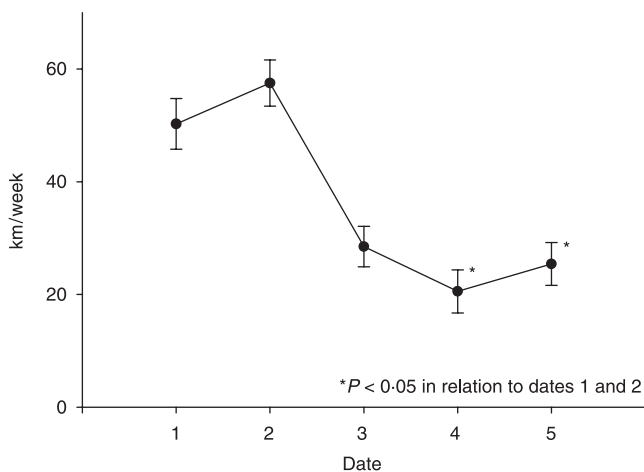


Fig. 1 Kilometres per week between the five examinations (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real-Berlin marathon (training amounts did not include the 42.195 kilometres of the marathon), date 4: 10 days after marathon, date 5: 6 weeks after marathon). After the marathon there was a significant decrease in running intensity ($P < 0.05$).

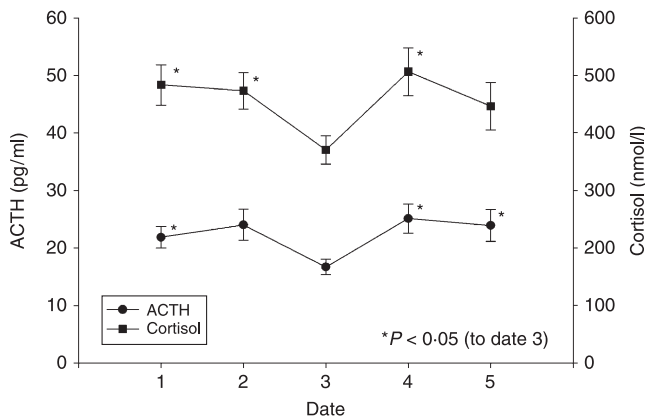


Fig. 2 Changes of ACTH and cortisol levels in relation to the marathon. Significant changes ($P < 0.05$) in relation to date 3 (2 days after marathon) were marked with * (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real-Berlin marathon, date 4: 10 days after marathon, date 5: 6 weeks after marathon).

be unaffected, the activity of the 11β -HSD-1 was significantly higher 2 days after the marathon (date 2: 1.18 ± 0.09 , $P = 0.013$, date 4: 1.06 ± 0.06 , $P = 0.006$).

Thyroid-stimulating hormone was significantly lower 6 weeks after the marathon (date 1: 1.13 ± 0.2 mU/l, date 5: 1.03 ± 0.16 mU/l, $P = 0.024$), but free T4 showed no significant changes (date 1: 22.5 ± 0.8 pmol/l, date 5: 23.3 ± 0.7 pmol/l, $P = 0.205$). TSH and fT4 values were in the physiological normal range, but TSH was in a lower normal range, whereas fT4 was in the high normal range at all time points.

IGF-1 and IGF-BP3 were also unaltered in the observed 12 weeks (IGF-1: date 1: 132.1 ± 8.7 μ g/l, date 3: 141.2 ± 8.8 μ g/l, date 5: 147.9 ± 11.5 μ g/l, $P > 0.05$; IGF-BP3: date 1: 4.1 ± 0.2 mg/l, date 3: 4.0 ± 0.1 mg/l, date 5: 4.3 ± 0.2 mg/l, $P > 0.05$). Only in men did we find a significant increase of IGF-1 (date 1: 126.7 ± 9.0 μ g/l, date

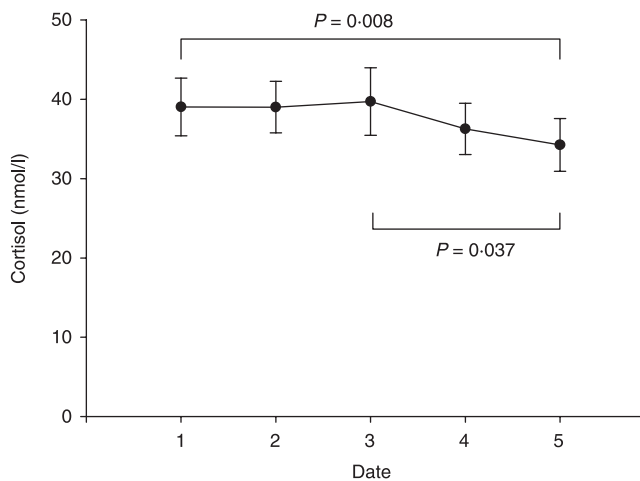


Fig. 3 Cortisol after 1 mg dexamethasone suppression test. Significant changes were marked with brackets. (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real-Berlin marathon, date 4: 10 days after marathon, date 5: 6 weeks after marathon).

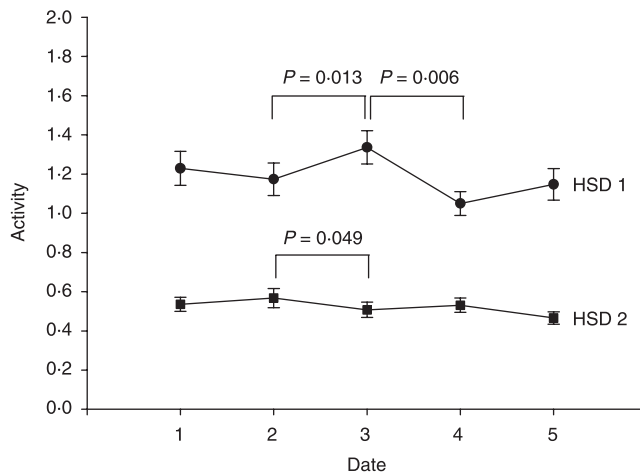


Fig. 4 Activity of HSD 1 (circles) and HSD 2 (squares) in 24-h urine. Significant changes in relation to date 3 (2 days after marathon) were marked with brackets. (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real-Berlin marathon, date 4: 10 days after marathon, date 5: 6 weeks after marathon).

3: 141.0 ± 8.8 μ g/l, $P = 0.036$) and IGF-BP3 (date 3: 3.7 ± 0.2 mg/l, date 5: 4.2 ± 0.2 mg/l, $P = 0.045$).

Ghrelin levels were basically not different during the study (Fig. 5). Only in relation to 6 weeks after the marathon was there a slight difference (date 3: 588.4 ± 51.8 ng/l, date 5: 512.17 ± 41.8 ng/l, $P = 0.031$).

Changes in sex hormones were studied only for men, because of different cycle times in female volunteers. In male volunteers, no changes were observed for LH and FSH (data not shown). However, there was a significant increase from all dates to date 5 of the free androgen index (FAI) (date 1: 38.3 ± 3.4 , date 2: 41.7 ± 3.3 , date 3: 46.0 ± 6.4 , date 4: 51.1 ± 3.4 , date 5: 58.9 ± 9.5 , $P < 0.05$), which was calculated by testosterone (date 1: 15.4 ± 1.5 nmol/l, date 2: 16.2 ± 1.3 nmol/l, date 3: 17.3 ± 1.6 nmol/l, date 4: 19.4 ± 1.8 nmol/l, date 5: 21.8 ± 2.9 nmol/l) and SHBG values (date 1: 40.0 ± 4.5 nmol/l,

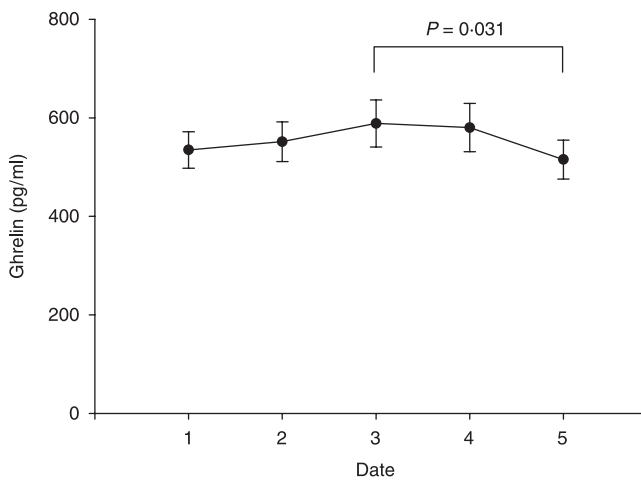


Fig. 5 Plasma samples were taken at all five dates in the morning and were analysed for ghrelin. Significant changes were marked with brackets. (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real,- Berlin marathon, date 4: 10 days after marathon, date 5: 6 weeks after marathon).

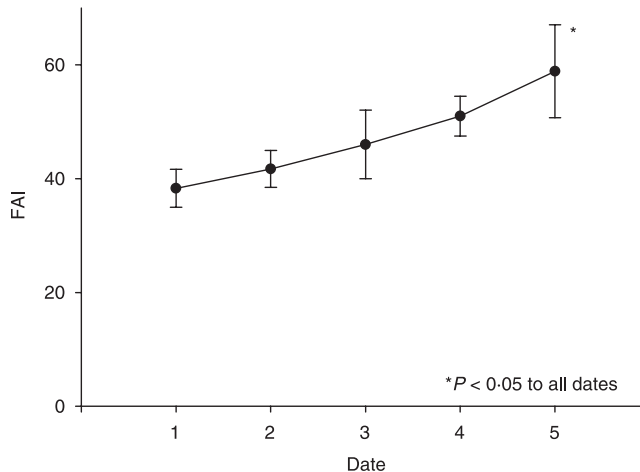


Fig. 6 For all male volunteers, free androgen index was calculated by testosterone \times 100/SHBG. FAI at date 5 (6 weeks after marathon) was significantly higher compared with all other dates. (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real,- Berlin marathon, date 4: 10 days after marathon, date 5: 6 weeks after marathon).

date 2: 39.1 ± 4.5 nmol/l, date 3: 37.4 ± 4.3 nmol/l, date 4: 37.9 ± 4.4 nmol/l, date 5: 37.4 ± 4.3 nmol/l) (Fig. 6).

We also aimed to detect general inflammation or possible infections. Therefore, CRP was measured at all time points (Fig. 7). There was a significant increase of CRP in the physiological normal range 2 days after the marathon (date 3: 5.4 ± 0.6 mg/l, $P < 0.005$).

Discussion

In this study, the effects of intensive endurance training on the hypothalamic-pituitary axis were examined.

Previous reports suggested that endurance training is associated with subclinical hypercortisolism,^{3,8} whereas others reported even

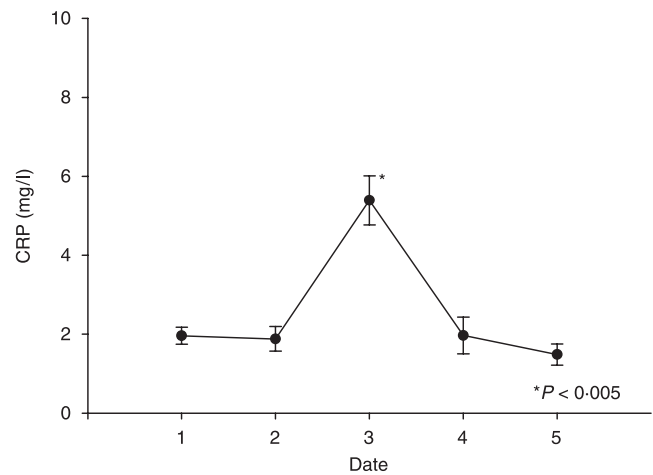


Fig. 7 CRP levels at all dates. CRP at date 3 (2 days after the marathon) was significantly higher compared with all other dates. (date 1: 6 weeks before marathon, date 2: 10 days before marathon, date 3: 2 days after real,- Berlin marathon, date 4: 10 days after marathon, date 5: 6 weeks after marathon).

lower basal cortisol levels after endurance training.^{10,11} In this study no changes in basal cortisol or ACTH basal levels between a high training status (at date 1 or 2) and a low training status (at date 4 or 5) were found. However, there was a trend to an up-regulation of the HPA system, because the dexamethasone suppression test, which is an indicator for the sensitivity of the HPA system, showed significant lower levels of cortisol after 6 weeks of lower training levels in relation to high training levels. In combination with the results of unaltered basal cortisol levels, it seems that the sensitivity of the HPA axis is changed. At a training status with an elevated training amount, the HPA axis is more resistant to dexamethasone than in a moderate training status with only a few kilometres per week. Similar results were found in a cross-sectional study performed in endurance-trained and untrained men, although a dexamethasone-CRH test was applied in this study.⁹ It is also known that the tissue sensitivity to glucocorticoids in endurance-trained men is decreased.²⁷ Changes in sensitivity to glucocorticoids may explain the discrepancy between repeated and prolonged exercise-induced HPA axis activation and the lack of metabolic consequences of such increased cortisol secretion.

We found reduced plasma levels of cortisol and ACTH 2 days after the marathon. This was probably an acute effect of the marathon and not related to training changes. The results suggest that the extreme physical stress of the marathon⁷ causes a short-term exhaustion of the ACTH-cortisol axis.

As a result of the exhaustion of the ACTH-cortisol axis after the marathon, the tissues expressing 11 β -HSD-1 may regulate cortisol metabolism against the low-circulating cortisol levels. Indeed, the activity of the 11 β -HSD-1 was increased 2 days after the marathon, whereas the activity of the 11 β -HSD-2 was slightly reduced. Thus, the impaired sensitivity of the systemic HPA axis appears to be counterbalanced by increased availability of cortisol by increased local activation from cortisone to the more active cortisol. Similar results are found in the circadian rhythm of the 11 β -HSD activity. Low

cortisol values at midnight are maybe compensated by a higher activity of 11β -HSD-1.²⁸

Generally, CRP levels were relatively low in the investigated individuals, which was probably to the result of the ongoing endurance training.^{29,30} The extreme physical stress during the marathon caused a substantial increase of CRP values as detected 2 days after the race. Although elevation of CRP usually indicates an ongoing systemic inflammatory reaction³¹ we found no elevated cortisol levels like that found in ill persons.³² Interestingly, there were no differences between high and low training levels. However, the time periods with the respective training levels may have been too short to see any changes.

Although there was a trend towards higher ghrelin levels directly after the marathon, no dramatic differences were seen. This result is in line with a recent publication showing that acute exercise does not change circulating ghrelin levels in humans.²⁴ The slightly increased ghrelin levels 2 days after the marathon could be still a sign of a negative energy balance after the marathon, which is known to induce higher ghrelin levels.^{33,34}

The changes of IGF-1 and IGF-BP3 were not significant, but there was a trend towards increasing levels of IGF-1 and IGF-BP3 during lower training levels. However, these findings are only significant in men, but not in women. One year of endurance training above the lactate threshold has been shown to cause an increase in basal 24-h pulsatile GH release.³⁵ Interestingly, subjects training below the lactate threshold did not show any change in the GH release, indicating that training intensity may be important in regulating the GH axis. Therefore, the study group was perhaps too inhomogeneous to see any differences in the hypothalamic-pituitary-growth hormone axis. We found no acute effect 2 days after the marathon, whereas other studies have shown that IGF-1 and IGF-BP3 increase after acute exercise.^{36,37} However, the duration of the postexercise elevation of IGF-1 and IGF-BP3 might be short and we thus might have missed a peak directly after the marathon. Additionally in our study, all investigated individuals had a relatively high training status and the IGF-1/IGF-BP3 levels might have reached a relative plateau, explaining that there was no additional increase after the acute exercise.

The thyroidal axis was also altered by marathon training. TSH, during the time of lower training exercise, was significantly lower than during intensive training, whereas fT_4 was also higher after a phase of lower training exercise, but these changes were not significant. Similar results with increased levels of TSH and slightly reduced levels of T_3 after 3 months were found in obese women.³⁸ Acute exercise does not induce any changes in the thyroidal axis.³⁹ In accordance with these findings, there was no acute effect 2 days after the marathon. The data concerning thyroidal hormones and exercise are very controversial. Regarding the thyroid hormone response to endurance exercise in humans, there exist studies with increases and decreases in TSH levels.⁴⁰ A possible cause of these differences between studies and individuals might be variations in plasma volume after exercise, i.e. haemoconcentration or haemodilution. Therefore, the effects of endurance training on thyroid function still remain to be fully elucidated.

In male participants, the hypothalamic-pituitary-gonadal axis was altered in this study. LH and FSH did not show any significant variances during this study, whereas LH slightly increased and FSH decreased during the period of lower training intensity. The free

androgen index (FAI) was significantly higher after reduction of endurance exercise. Changes in testosterone values through physical activity depend of the kind of training. Lower testosterone levels have been shown in endurance-trained men compared with sedentary controls,^{41,42} whereas resistance-trained persons seem to have higher basal testosterone levels.^{43,44} Thereby endurance- and resistant-trained persons had lower testosterone levels than sedentary control subjects.⁴⁵ It is also known that high cortisol levels, e.g. in patients with Cushing's syndrome, lead to a suppression of the gonadal axis with lowered values for testosterone.⁴⁶ The present study supports the previously found results. Thereby this lowered testosterone level does not seem to induce pathological reactions like decreased bone density⁴⁷ or decreased physical fitness.⁴⁸ Furthermore, testosterone concentrations have been shown to increase after an acute bout of resistance or endurance exercise.^{49,50} In response to prolonged endurance exercise (e.g. running a marathon), testosterone levels will typically decline.^{51,52} In this study, the time after the marathon (2 days) was probably too long to see any acute changes resulting from the marathon.

In summary, this study has shown acute and prolonged changes in pituitary hormone axes as a result of marathon training and running a marathon. In particular, the adaptations in the HPA system showed decreased levels of cortisol after the marathon and decreased sensitivity of glucocorticoid-regulation during a stage of high training intensity. Furthermore, cortisol metabolism by the 11β -HSD system is altered after the marathon. Concerning the acute down-regulation of ACTH and cortisol after the marathon, further studies with dynamic testing (CRH test, metyrapone test) would be interesting.

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