Plasma β-Endorphin, Corticotrophin and Growth Hormone Responses to Exercise in Pubertal and Prepubertal Children

O. Bouix [1], *J. F. Brun* [1], *C. Fédou* [1], *E. Raynaud* [1], *B. Kerdélhué* [2], *V. Lenoir and A. Orsetti* [1]

[1] Department of Physiology, Faculty of Medicine, Montpellier, France

[2] Reproductive Neurobiology Unit, CNRS-INRA, Jouy-en-Josas, France

Summary

An increase in plasma β-endorphin concentrations during exercise has been reported in adult men and women by several investigators. However, very little is known about this physiological hormonal response to exercise in children. In this study, we investigated plasma β-endorphin, ACTH and GH responses to exercise in 40 prepubertal and pubertal children. Subjects were recruited as part of a population of children and adolescents presenting growth retardation and were selected on the basis of the absence of any clinical or biological signs of endocrine or metabolic disease. There were 16 girls and 24 boys with 24 prepubertal and 16 pubertal individuals. A standardised 15 min workload on cycloergometer was used to progressively increase the heart rate of the children up to 90% of the theoretical maximal value. Exercise resulted in a significant increase ($p < 0.01$) in plasma β-endorphin (mean ± SEM) (4.26 ± 0.47 vs 5.74 ± 0.56 fmol/ml), ACTH (3.71 ± 0.41 vs 6.2 ± 0.62 fmol/ml) and GH (147 ± 29 vs 364 ± 67 fmol/ml). The percentage of children with significant hormonal response to exercise was about 75% for each of the 3 hormones but only 3 of the 40 children studied did not show any hormonal response to exercise. Exercise-induced increases in plasma β-endorphin and ACTH were significantly correlated ($p < 0.01$). By contrast, there was no significant relationship between GH and β-endorphin or ACTH values. Furthermore, whereas exercise-induced plasma GH increase was significantly higher in pubertal than in prepubertal children ($p < 0.001$), corresponding β-endorphin and ACTH levels were quite similar in the two groups. Our findings give evidence for comparable exercise-induced increase in plasma β-endorphin levels in either pubertal or prepubertal normal short children. The similarity of the β-endorphin responses to exercise between prepubertal and pubertal children does not support the controversial hypothesis that plasma β-endorphin modulates GH response to exercise.

Key words

Children – Exercise – β-Endorphin – Growth Hormone

Introduction

Exercise-induced changes in plasma β-endorphin concentrations are now well documented in adult men or women (*Berk, Tan, Anderson* and *Reiss* 1981; *Colt, Wardlaw* and *Frantz* 1981; *Farrell, Gates, Maksud* and *Morgan* 1982; *Gambert, Garthwaite, Pontzer, Cook, Tristani, Duthie, Martinson, Hagen* and *McCarty* 1981). Recent studies clearly show that changes in plasma β-endorphin are related to the intensity and the duration of exercise (*Donevan* and *Andrew* 1978; *Farrell* 1985; *Goldfarb, Hatfield, Armstrong* and *Potts* 1990; *Kraemer, Dziados, Marchitelli, Gordon, Harman, Mello, Fleck, Frykman* and *Triplett* 1993; *Rahkila, Hakala, Alén, Salminen* and *Laatikainen* 1988). The β-endorphin response to exercise has been recently demonstrated in junior athletes (*Kraemer, Fray, Warren, Stone, Fleck, Kearney, Conroy, Maresh, Weseman, Triplett* and *Gordon* 1992) as well as in non trained adolescent girls and boys (*Gerra, Terzi, Delsignore, Caccavari, Gaggiotti, Maestri, Ugolotti, Chiodera* and *Coiro* 1991) but to our knowledge, the exercise-induced plasma β-endorphin release has not yet been studied in children in relation to pubertal development.

Exercise-induced increase in plasma β-endorphin concentration is associated with a parallel rise in plasma ACTH (*Rahkila* et al. 1988), suggesting a common anterior pituitary origin for both peptides (*Fraioli, Moretti, Paolucci, Alicicco, Crescenzi* and *Fortunio* 1980; *Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale* and *Gloom* 1977). However, the basic physiological function of circulating endogenous opiates response to exercise remains unclear. It has been demonstrated that changes in plasma endogenous opiates may be responsible for certain of the hormonal alterations associated with exercise (*Bouix, Najimi* and *Orsetti* 1993; *Grossman, Bouloux, Price, Drury, Lam, Turner, Thomas, Besser* and *Sutton* 1984; *Grossman* and *Sutton* 1985; *Morley* 1981). In particular, the opiate antagonist naloxone has been shown to inhibit exercise-induced GH release in highly trained athletes (*Moretti, Fabri, Gnessi, Cappa, Calzolari, Fraioli, Grossman* and *Besser* 1980) suggesting a stimulatory effect of plasma β-endorphin on GH responsiveness. However, other studies have failed to demonstrate any relationship between β-endorphin and GH secretion, either in resting or exercising normal man (*Bramnert* and *Hokfelt* 1987; *Mayer, Wessel* and *Kobberling* 1980; *Spiler* and *Molitch* 1980). Since puberty is associated with profound changes in the hypothalamus-anterior pituitary axis responsiveness and regulation, we have addressed the questions of 1) the influence of puberty on exercise-induced β-endorphin release and 2) the

Horm. metab. Res. 26 (1994) 195–199

Received: 15 Oct. 1993 Accepted: 10 Dec. 1993

relationship between β-endorphin and GH response to exercise in a population of normal prepubertal and pubertal short children.

Materials and Methods

Subjects

Forty healthy children (24 boys, mean age 12.85 ± 0.054 years and 16 girls, mean age 10.87 ± 0.47 years) were studied during a medical check-up for idiopathic growth retardation in order to measure their exercise-induced plasma GH response. After completion of the clinical and biological check-up, all the children in whom any endocrine or metabolic abnormality was diagnosed were excluded from the study. Height standard deviation scores ranged from −0.5 to −3.8. The stage of pubertal development was assessed using the descriptive standards proposed by *Tanner* (1962). The number of subjects within each pubertal group was as follows: stage 1, 14 boys and 10 girls; stage 2, 4 boys and 4 girls; stage 3, 5 boys and 2 girls; stage 4, 1 boy. All children were following regular school sport programs but were not involved in any particular physical training. None were receiving any medication at the time of the study. Informed consent was obtained from all subjects and their parents after the nature of the study was fully explained.

Exercise test

Children (or their parents) were instructed to maintain normal diet and physical activity for 3 days before the test. Each subject was tested 2 hours after consumption of his usual breakfast at home. An indwelling catheter was inserted into a forearm vein and was kept patent with physiological heparinized saline for 30 minutes, before the first blood sample (t_{-15}). Then the subject was seated on a cycle ergometer (Bodyguard) and another basal blood sample was obtained just before the beginning of exercise (t_0). Heart rate was continuously monitored using bipolar chest electrodes. A 15 minute graded workload on cycloergometer was used to progressively increase the heart rate of the children up to 90% of the theoretical maximal value which was maintained for the last 5 minutes of cycling. Exercise intensity was therefore proportional to working capacity of each subject. Additional blood samples were obtained at the end (t_{15}) and after 10 min recovery (t_{25}). Physical working capacity was determined by using the W170 index which is the power corresponding to a heart rate of 170/min divided by the weight of the subject (*Wahlund* 1948). We previously demonstrated the advantages of this simple and physiological test in the diagnosis of GH deficiency in short children (*Fedou, Jesuran, Brun, Mirouze, Jaffiol* and *Orsetti* 1987).

Hormone measurements

Blood samples were drawn on ice in tubes containing 50 µl EDTA and 100 µl aprotinin (Antagosan, Hoechst, Paris, France) for β-endorphin and ACTH measurements of 50 U heparin/ml whole blood for GH determination. Blood was immediately centrifuged 10 min at 3000 rpm at 4 °C and plasma was kept frozen at −20 °C until analysed. All samples from a given individual were measured in the same assay. A specific radioimmunoassay developed in our laboratory (*Kerdélhué, Bethea, Ling, Chretien* and *Weiner* 1982) was used for measuring plasma β-endorphin concentrations. Plasma β-endorphin was concentrated and purified by previous extraction on SEP-Pack C18 columns (Waters Inc., Milford, MA, USA). Then 0.1 ml of a 1/500 dilution of the β-endorphin antiserum, various quantities of unlabelled β-endorphin (0.9 fmol to 2.9 pmol) and related peptides and various volumes of serum were incubated in a total volume of 0.5 ml of buffer (0.1 M sodium phosphate buffer, pH 7.5, containing 0.15 M NaCl, 0.1% gelatine and 0.05% Triton X-100) at 4 °C for 24 hours. Then 0.1 ml of approximately 15,000 cpm of human ^{125}I-labelled β-endorphin was added and the assay mixture was incubated at 4 °C for 72 hours. Bound and free hormones were separated by addition of 0.2 ml of a 1/20 dilution of an anti-rabbit gamma-globulin serum in the above buffer which also contains 1% of normal rabbit serum and 10^{-2} M of EDTA. After 24 h of incubation at 4 °C, 1.5 ml of buffer was added, the tubes were centrifuged for 40 min (1500 g, 4 °C) and the supernatant was poured off. Radioactivity in the precipitate was counted with a gamma counter. The sensitivity of this RIA was 5–10 pg per tube. β-lipotropin, which contains the complete amino acid sequence of β-endorphin, had less than 2% cross reactivity with β-endorphin. Plasma ACTH and GH concentrations were determined using a solid phase two-sites immunoradiometric assay (ELSA-ACTH and ELSA-HGH, CIS bio international, Gif-sur-Yvette, France). In both assays, one monoclonal antibody was coated on the solid phase and a second ^{125}I radiolabelled antibody was used as tracer. The radioactivity bound to the solid phase was related to the amount of ACTH or GH in the sample. The detection limit was 0.5 fmol/ml for ACTH and 2 fmol/ml for GH. Intra-assay coefficients of variation were 6.2% and 2.4% for ACTH and GH respectively.

Statistical analysis

Values are given as mean ± SEM. Statistical comparisons between groups were performed using Wilcoxon test, Mann-Whitney U-test or non-paired and paired t tests where appropriate. Linear regression analysis was performed by the least-squares method. Statistical significance was accepted at a $p < 0.05$.

Table 1 Clinical and hormonal characteristics of prepubertal and pubertal boys and girls.

	Boys		Girls	
	Prepubertal	Pubertal	Prepubertal	Pubertal
n	14	10	10	6
Age (years)	11.54 ± 0.52	14.89 ± 0.70	10.01 ± 0.54	12.33 ± 0.49
Height (m)	1.34 ± 0.03	1.47 ± 0.02	1.25 ± 0.03	1.42 ± 0.02
Weight (kg)	29.91 ± 1.88	37.73 ± 1.44	25.34 ± 1.48	35.34 ± 2.59
Growth retardation (standard deviation)	−2.26 ± 0.18	−2.16 ± 0.35	−2.03 ± 0.29	−2.10 ± 0.27
W170 (w/kg)	2.32 ± 0.21	2.26 ± 0.19	1.98 ± 0.20	2.12 ± 0.20
Hormonal responses to exercise (fmol/ml):*				
GH	+87 ±53	+444 ±142	+65 ±63	+395 ±117
ACTH	+2.69 ± 1.56	+2.54 ± 0.74	+1.96 ± 0.98	+3.09 ± 0.67
B-Endorphin	+1.64 ± 0.64	+1.15 ± 0.48	+1.71 ± 0.72	+2.22 ± 1.22

Data are presented as mean ± SEM
*Plasma GH, ACTH and β-endorphin changes from t_0 to t_{15}.

Results

Table **1** presents the characteristics of the 40 children according to sex and pubertal status. Each exercising child reached the desired theoretical power value (power range 50 to 130 watts). Fifteen minutes of exercise resulted in a significant increase in plasma β-endorphin levels (t_0 4.26±0.47 vs t_{15} 5.74±0.56 fmol/ml, $p<0.01$). Mean peak value was found immediately after the exercise test but plasma β-endorphin concentration remained significantly elevated after 10 min recovery (t_{25} 5.21±0.56 fmol/ml, $p<0.05$). However, the exercise-induced increase in plasma β-endorphin was observed in only 30 (75%) of the 40 children, ranging from +0.57 to +7.52 fmol/ml. Children in whom no β-endorphin response was detected were not different from the others regarding age, sex, pubertal status or growth retardation. Plasma levels of ACTH and GH also significantly increased ($p<0.01$) after exercise (ACTH, t_0 3.71±0.41 vs t_{15} 6.20±0.62 fmol/ml and GH t_0 147±29 vs t_{15} 364±67 fmol/ml). The respective percentage of children responding to exercise was 78% for ACTH and 75% for GH. Overall, only 3 of the 40 children did not show any increase in either plasma ACTH, GH or β-endorphin levels during exercise. They were 2 boys and 1 girl, aged 15, 13 and 10 years with pubertal stages 1, 2, and 1 and a degree of growth retardation of −3.5, −2 and −1 standard deviations respectively. Exercise-induced increases in plasma β-endorphin and ACTH levels were significantly correlated ($n=40$, $r=0.681$, $p<0.01$) (Fig. **1**). By contrast, exercise-induced increase in plasma GH concentrations showed no significant relationship with β-endorphin or ACTH values. There were no significant differences in β-endorphin, ACTH or GH responses to exercise between boys and girls. By contrast, when comparing prepubertal and pubertal children groups in both sexes (Table **1** and Fig. **2**), the exercise-induced plasma GH release appeared to be significantly lower in prepubertal children ($p<0.001$), whereas the corresponding plasma β-endorphin and ACTH responses to exercise were not different between prepubertal and pubertal children. Plasma β-endorphin, ACTH and GH values, either before or after exercise, showed no correlation with individual physical working capacity, age, pubertal status or level of growth retardation.

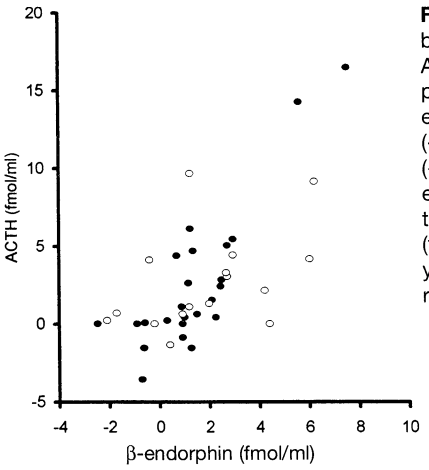

Fig. 1 Relationship between plasma ACTH and β-endorphin changes during exercise in boys (--●--) and girls (--○--). Results are expressed as delta $t_{15}-t_0$ values (fmol/ml). n = 40, y = 0.70 + 1.13x, r = 0.681, p < 0.01.

Discussion

This study shows that a 15 min submaximal standardised exercise induces a significant increase in β-endorphin, as well as ACTH and GH plasma levels in normal prepubertal and pubertal short children. In contrast to the higher GH response that we observed in pubertal compared to prepubertal children, β-endorphin and ACTH values were not significantly different between the two groups. The group of children studied here is part of a population attending a medical check-up for short stature. After completion of the clinical examination and biological analysis, all children in whom endocrine or metabolic abnormalities were detected were excluded from the study. Therefore, the 40 children included in our study can be considered as normal short children, free of any somatotropic, gonadotropic, thyrotropic or corticotropic dysfunction. Although caution is needed for extrapolating our results to a general children population, it seems reasonable to hypothesize that the increase in β-endorphin which is found in both normal adults and normal short children does also occur in children with normal height.

The plasma β-endorphin response to exercise in man is now well documented. This response is mainly dependent on intensity and duration of exercise (*Donevan* and *Andrew* 1987; *Farrell* 1985; *Rahkila* et al. 1988). Indeed, a critical threshold intensity of at least 70% $\dot{V}O_2$max for a minimum of 15 minutes seems to be necessary to obtain a significant increase in β-endorphin levels (*Goldfarb* et al. 1990). However, almost all these studies have been conducted in adult or adolescents and, to our knowledge, little information is available on β-endorphin response to exercise in children before and during puberty. Our results show that like in adults, a 15 min cycling at 90% $\dot{V}O_2$max results in a significant increase in mean plasma β-endorphin concentration in either pubertal or prepubertal children. However, about 25% of the children do not show significant changes in β-endorphin, ACTH or GH and 3 of them do not present any hormonal change. The lack of β-endorphin response to exercise as observed in some children is not related to age, pubertal status or growth retardation. The use of a submaximal exercise intensity and the relatively short time of cycling could explain, at least in part, the lack of hormonal changes in some children. The correlation between ACTH and β-endorphin response to exercise is in favour with a parallel release of these two hormones by corticotrophs in this population. A simultaneous release of ACTH and β-endorphin has been demonstrated in trained or untrained adults in response to exercise (*Gambert* et al. 1981; *Rahkila* et al. 1988) as well as in experimental animals and in vitro on cultured pituitary cells (*Guillemin* et al. 1977; *Vale, Rivier* and *Yang* 1978).

Several studies report significant responses of growth hormone to exercise. However, studies on the effect of endogenous opiates on exercise-induced plasma GH response are both scarce and controversial. In normal untrained man, the GH response to high intensity exercise is either unchanged (*Mayer, Wessel* and *Kobberling* 1980) or enhanced (*Grossman* et al. (1984) by previous naloxone injection. In contrast, this response has been found to be significantly blunted by naloxone in a population of highly trained athletes (*Moretti* et al. 1980). Thus, the role of opiates in modulating GH secretion at exercise remains unclear. In resting human, β-endorphin itself administered intravenously does not seem to have any effect on basal plasma GH levels while inducing a significant increase in the levels of prolactin and decline in the concentrations of luteinizing hormone concentrations (*Reid, Hoff, Yen* and *Li* 1981). Additionally, baseline GH values are not modified by naloxone

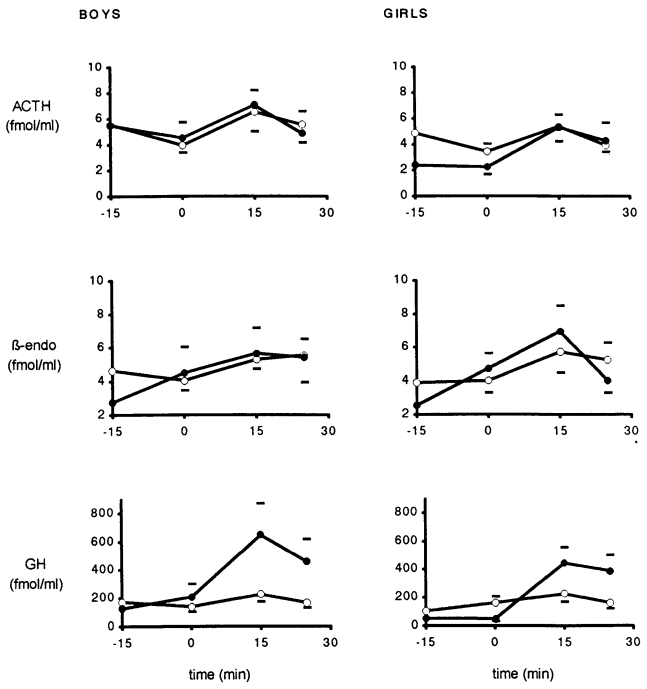

Fig. 2 ACTH, β-endorphin (β-endo) and GH responses to exercise in prepubertal (-○-) and pubertal (-●-) boys and girls.

injection (*Delitala, Grossman* and *Besser* 1983). In contrast, the enkephalin, or at least the enkephalin analogue DAMME has proven to be able to stimulate GH secretion in resting human (*Stubbs, Delitala, Jones, Jeffcoate, Edward, Ratter, Besser, Bloom* and *Alberti* 1978). This effect is only slightly counteracted by naloxone suggesting that δ-opiate receptor subtype is preferentially involved in this stimulatory pathway. The present study shows that plasma GH and β-endorphin variations during exercise are quite discordant. Moreover, whereas plasma GH response to exercise is significantly higher in pubertal than in prepubertal children, β-endorphin as well as ACTH increases do not differ between the two groups. Thus, our results seem to indicate that circulating β-endorphin is not involved in the modulation of the exercise-induced GH release. It must be noted, however, that endogenous opiates might stimulate GH release by acting synaptically as neurotransmitters in the hypothalamus. It has been shown that the opiate blocker naloxone enhances the increase in plasma GH induced by the α-2 adrenergic agonists clonidine and guanfacine (*Bramnert* and *Hokfelt* 1987). Therefore the action of opiates on GH could involve modulation of noradrenergic stimulatory effects by enkephalinergic neurones, rather than hormonal action of circulating β-endorphin.

Short periods of muscular activity reaching 90% of maximal heart rate seem to be usual events in the life of children and do not represent an extreme situation on the edge of physiological conditions. Our results indicate that strong physical activity, as performed in everyday child life, induces a β-endorphin rise in short, and presumably also in normal girls and boys before and after onset of puberty. Unlike plasma GH response which increases with puberty, β-endorphin response does not seem to be affected by pubertal status.

References

Berk, L. S., S. A. Tan, C. L. Anderson, G. Reiss: β-endorphin response to exercise in athletes and non-athletes. Med. Sci. Sports Exerc. 13: 134–137 (1981)

Bouix, O., A. Najimi, A. Orsetti: Effect of naloxone on the insulin response after exercise in rats. In vivo and in vitro study. Diabetologia 36 (suppl. 1): A21 (1993)

Bramnert, M., B. Hokfelt: The influence of naloxone on exercise-induced increase in plasma pituitary hormones and the subjectively experienced level of exhaustion in healthy males. Acta Endocrinol. (Copenhagen) 115: 125–130 (1987)

Colt, E. W. D., S. L. Wardlaw, A. G. Frantz: The effect of running on plasma β-endorphin. Life Science 28: 1637–1640 (1981)

Delitala, G., A. Grossman, M. Besser: Differential effects of opiates peptides and alkaloids on anterior pituitary hormone secretion. Neuroendocrinol. 37: 275–279 (1983)

Donevan, R. H., G. M. Andrew: Plasma β-endorphin immunoreactivity during graded cycle ergometry. Med. Sci. Sports Exerc. 19: 229–233 (1987)

Farrell, P. A., W. K. Gates, M. G. Maksud, W. P. Morgan: Increases in plasma β-endorphin/β-lipotropin immunoreactivity after treadmill running in humans. J. Appl. Physiol. 52 (5): 1245–1249 (1982)

Farrell, P. A.: Exercise and endorphins. Male responses. Med. Sci. Sports Exerc. 17: 89–93 (1985)

Fedou, C., M. Jesuran, J. F. Brun, J. Mirouze, C. Jaffiol, A. Orsetti: Exercise-test and neurogenic regulation of growth hormone secretion. J. Endocrinol. Invest. 10 (Suppl. 2): 79 (1987)

Fraioli, F., C. Moretti, D. Paolucci, E. Alicicco, F. Crescenzi, G. Fortunio: Physical exercise stimulates marked concomitant release of beta-endorphin and adrenocorticotropic hormone (ACTH) in peripheral blood in man. Experientia 36: 987–989 (1980)

Gambert, S. R., T. L. Garthwaite, C. H. Pontzer, E. E. Cook, F. E. Tristani, E. H. Duthie, D. R. Martinson, T. C. Hagen, D. J. Mc Carty: Running elevates plasma β-endorphin immunoreactivity and ACTH in untrained human subjects. Proc. Soc. Exp. Biol. Med. 168: 1–4 (1981)

Gerra, G., C. Terzi, R. Delsignore, R. Caccavari, M. T. Gaggiotti, D. Maestri, G. Ugolotti, P. Chiodera, V. Coiro: Neuroendocrine responses to physical exercise in adolescents with low self esteem and frustration. Neuroendocrinol. Lett. 13: 367–375 (1991)

Goldfarb, A. H., B. D. Hatfield, D. Armstrong, J. Potts: Plasma β-endorphin concentration: response to intensity and duration of exercise. Med. Sci. Sports Exerc. 22: 241–244 (1990)

Grossman, A., P. Bouloux, P. Price, P. L. Drury, K. S. L. Lam, T. Turner, J. Thomas, G. M. Besser, J. Sutton: The role of opioid peptides in the hormonal responses to acute exercise in man. Clin. Sci. 67: 483–491 (1984)

Grossman, A., J. R. Sutton: Endorphins: what are they? How are they measured? What is their role in exercise? Med. Sci. Sports Exerc. 17: 74–81 (1985)

Guillemin, R., T. Vargo, J. Rossier, S. Minick, N. Ling, C. Rivier, W. Vale, F. Gloom: Beta endorphin and adrenocorticotrophin are secreted concomitantly by the pituitary gland. Science 197: 1367–1369 (1977)

Kerdélhué, B., C. L. Bethea, N. Ling, M. Chretien, R. I. Weiner: β-endorphins concentrations in serum, hypothalamus and central gray of hypophysectomized and mediobasal hypothalamus lesioned rats. Brain Res. 231: 85–91 (1982)

Kraemer, W. J., A. C. Fray, B. J. Warren, M. H. Stone, S. J. Fleck, J. T. Kearney, B. P. Conroy, C. M. Maresh, C. A. Weseman, N. T. Triplett, S. E. Gordon: Acute hormonal responses in elite junior weightlifters. Intern. J. Sports Med. 13: 103–109 (1992)

Kraemer, W. J., J. E. Dziados, L. J. Marchitelli, S. E. Gordon, E. A. Harman, R. Mello, S. J. Fleck, P. N. Frykman, N. T. Triplett: Effects of different heavy-resistance exercise protocols on plasma β-endorphin concentrations. J. Appl. Physiol. 74: 450–459 (1993)

Mayer, G., J. Wessel, J. Kobberling: Failure of naloxone to alter exercise-induced growth hormone and prolactin release in normal men. Clin. Endocrinol. 13: 413–416 (1980)

Moretti, C., A. Fabri, L. Gnessi, M. Cappa, A. Calzolari, F. Fraioli, A. Grossman, G. M. Besser: Naloxone inhibits exercise-induced release of PRL and GH in athletes. Clin. Endocrinol. 13: 413–416 (1980)

Morley, J. E.: The endocrinology of the opiates and opioid peptides. Metabolism 30: 195–205 (1981)

Rahkila, P., E. Hakala, M. Alén, K. Salminen, T. Laatikainen: β-endorphin and corticotropin release is dependent on a threshold intensity of running exercise in male endurance athletes. Life Science 43: 551–558 (1988)

Reid, R. L., J. D. Hoff, S. S. C. Yen, C. H. Li: Effects of exogenous β-endorphin on pituitary hormone secretion and its disappearance rate in normal human subjects. J. Clin. Endocrinol. Metab. 52: 1179–1184 (1981)

Spiler, I. J., M. E. Molitch: Lack of modulation of pituitary hormone stress response by neural pathways involving opiates receptors. J. Clin. Endocrinol. Metab. 50: 516–520 (1980)

Stubbs, N. A., A. Delitala, G. Jones, W. J. Jeffcoate, C. R. W. Edward, S. J. Ratter, G. M. Besser, S. R. Bloom, K. G. M. M. Alberti: Hormonal and metabolic responses to an enkephalin analogue in normal man. Lancet 2: 1255–1259 (1978)

Tanner, J. M.: Growth and adolescence. Second edition. Blackwell, Oxford (1962)

Vale, W., C. Rivier, L. Yang: Effects of purified hypothalamic corticotrophin-releasing factor and other substances on the secretion of adrenocorticotrophin and β-endorphin-like immunoreactivities in vitro. Endocrinology 103: 1910–1915 (1978)

Wahlund, H.: Determination of physical working capacity. Acta Med. Scand. 215: 1–78 (1948)

Requests for reprints should be addressed to:

Dr. O. Bouix

Service d'Exploration Physiologique
des Hormones et des Métabolismes
Hopital Lapeyronie
371 avenue du Doyen G. Giraud
F-34295 Montpellier Cédex 5
France