Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 33–37. Pergamon Press. Printed in Great Britain

PLASMA CORTISOL, TESTOSTERONE, ANDROSTENEDIONE AND LUTEINIZING HORMONE (LH) IN A NON-COMPETITIVE MARATHON RUN

A. DESSYPRIS, K. KUOPPASALMI and H. ADLERCREUTZ

Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, 00290 Helsinki 29, and Endocrine Research Unit, University of Helsinki, Minerva Foundation Institute for Medical Research, P.O.B. 819, 00101 Helsinki 10, Finland

(*Received* 21 *April* 1975)

SUMMARY

Plasma cortisol, testosterone, androstenedione and LH were determined in 14 men (27–58 years old) taking part in a non-competitive marathon (42·2 km). After the run the mean values showed a rise in cortisol and androstenedione and a fall in testosterone; these changes were statistically highly significant. After the marathon a significant correlation was found between the values for testosterone and androstenedione. Comparisons of the changes in cortisol, testosterone and androstenedione in relation to the control level show that significant correlations existed between the percentage increases in cortisol and androstenedione, and between the percentage decrease in testosterone and percentage increase in androstenedione. Plasma LH response varied, but the mean value after the run did not differ from the mean control value at the same time of day. One very fit subject, who ran the marathon in only 182 min, did not show any decrease in testosterone and his LH increased by more than 100%. One subject, who collapsed after running 15 km, had very low testosterone and LH values, although his cortisol value was unchanged as compared with his control value. The results suggest that during prolonged strenuous exercise LH and androgens, in addition to cortisol, play some role in promoting endurance in men.

INTRODUCTION

Physical exercise alters the blood levels of many hormones, including insulin [1–8], glucagon [2, 8], growth hormone [1, 2, 5–7, 9, 16], cortisol and other 17-hydroxycorticosteroids [2, 5–7, 10–17, 40], androgens [16, 17], aldosterone [14] and renin activity [14, 18]. Plasma luteinizing hormone (LH) seems to remain unaltered [2, 16, 17]. Hormonal metabolism may also be altered, as has been demonstrated for cortisol [15]. However, the degree and direction of these hormonal changes may be influenced by genetic factors, physical fitness, training schedule and diet, and the nature, severity and duration of the exercise.

A marathon run constitutes an extremely strenuous endurance test and to our knowledge the hormonal changes associated with it are not yet fully documented. The studies reported here may throw more light on the role of endogenous hormones during severe physical exercise. A preliminary report has been published on some of the findings [19].

EXPERIMENTAL

Material. Blood specimens were obtained from 13 Finnish amateur sportsmen aged 27 to 40. They were all in training, having practiced running regularly to increase their stamina. They had been medically examined some weeks before the event and were physically fit.

Subject No. 12 was highly trained and fit. He ran the marathon in only 182 min and his condition afterwards was exceptionally good. Subject No. 13 had influenza at the time of the race, but felt able to participate. He collapsed after 66 min, having run about 15 km. Subject No. 14 was a 58-year-old Greek, a former marathon prize winner; his physical condition was good as a result of rural life, active work and running.

Experimental conditions. The second non-competitive marathon took place in Athens (20 October 1973) over the classical course. The temperature was high for the season, about 30°C in the shade. Water, orange juice, Isotonic® drink (containing sugar, fructose, citric acid, sodium chloride, sodium carbonate, monosodium phosphate, monopotassium phosphate, potassium chloride and riboflavin) were available *ad libitum* every 5 km and at the finish (42·2 km) before blood sampling. The samples were withdrawn into heparinized tubes within 30 min of completion of the run. They were centrifuged within 60 min of withdrawal and the plasma was kept frozen (−20°C) until analysed. Control samples were taken in Finland at 12.00–13.00 h and 16.00–17.00 h (no time difference between Greece and Finland), the approximate times of the start (12.30 h) and finish of the marathon, some

days before (Nos. 3, 5, 7, 13, 14) or after the run. This was necessary because of the obvious difficulties of blood withdrawal at the start of the race and the rise of cortisol in anticipation of muscular exercise [5, 7, 13, 40].

Methods. All assays were carried out in duplicate. In some instances the analyses were repeated, especially if the values in the first analysis were very high or very low. Adequate quality controls were included in all series of determinations.

Cortisol was determined in 0·1 ml of plasma by a competitive protein-binding assay [20] based on Murphy's technique, with plasma of late twin pregnancy as the source of transcortin. Normal values at 8.00 h are 0·22–0·70 μmol/l. Afternoon values are 30% lower [21].

Testosterone was determined by a modification of the method of Ismail *et al.* [22]. After extraction of the steroids 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 and 0·00001% Tween 20. The sample is incubated overnight at 4°C with antiserum (raised in rabbits against testosterone-3-BSA Searle Diagnostics, High Wycombe, Bucks., England) and [1,2,6,7-^{3}H]-testosterone (The Radiochemical Centre, Amersham). Bound and unbound testosterone are separated with Dextran-coated charcoal in borate buffer (2·5 g Norit-A and 2·5 g Dextran T 70 in 1 litre of the borate buffer).

The normal values for men at 8.00 h are 14 to 38 nmol/l.

Androstenedione was determined by a direct method, based principally on the technical bulletin of Endocrine Sciences Inc. (Tarzana, California) (February 1972) and using their antiserum No. AN 6–22 (raised in rabbits against a 6-linked bovine serum albumin conjugate). The cross-reaction with testosterone is only 2%. The hormone is extracted from 0·5 ml of plasma with 2 × 2·5 ml of petroleum ether (b.p. 30–60°C) 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 the borate buffer used for testosterone assay. Suitable amounts (100–250 μl) of the buffer are used 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 done as for testosterone.

Plasma luteinizing hormone (LH) was determined by a double antibody solid phase technique (DASP) (Seuderling, Karonen and Adlercreutz, to be published) mainly according to Den Hollander and Schuurs [23]. The antiserum and purified human LH were from the National Institute of Arthritis and Metabolic Diseases (National Institutes of Health, Bethesda, Maryland). The LH was labelled with ^{125}I by coupling the lactoperoxidase to a cross-linked copolymer of maleic anhydride and butanediol divinyl ether (E. Merck) and using the coupled enzyme for iodination [24]. Human Pituitary Luteinizing Hormone (68/40) (Medical Research Council, London) was used for the standard curves. Antigen (unknown or standard), labelled antigen and first antibody were incubated for 48 h in a volume of 550 μl. Insolubilized second antibody (sheep antiserum to rabbit-gamma-globulin), immunosorbent 5·5 ml diluted in 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), and 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 during 18 months was 14·2%. 67 analyses of a human LH reference preparation LER-907 during a 2-year period gave a coefficient of variation of 9·6%. The reference values for normal men are 4 to 20 U/l. Statistical treatment of the results was carried out by the *t*-test according to de Jonge [25].

RESULTS

Hormone values in control samples and after the marathon, with the running times, of the participants, are given in Table 1.

In all participants the control values of cortisol were within normal limits, the mean value for the group Nos. 1–11 being 0·42 μmol/l at 12.30 h and 0·26 μmol/l at 16.30 h. The increase in the mean value after the marathon, compared with the 16.30 h value, was statistically highly significant (to 1·15 μmol/l; $P < 0·001$). Participant No. 12 had one of the highest values (1·45 μmol/l), whereas No. 13 had a very low value (0·26 μmol/l), and No. 14 had a value similar to the mean of the group.

The control values of testosterone were within normal limits for all participants. For the group the mean control values were 21·6 μmol/l at 12.30 h and 23·4 μmol/l at 16.30 h and, compared with the latter value, the mean value after the marathon had fallen highly significantly (to 14·1 μmol/l; $P < 0·001$). In participant No. 12, in contrast to all the other runners, the testosterone value rose slightly (from 33·2 to 36·0 μmol/l). A remarkable decrease of plasma testosterone, down to 3·0 μmol/l, was found in participant No. 13, who collapsed. The testosterone value of subject No. 14 fell in the same way as in the group.

The mean control androstenedione values for the group were 7·4 μmol/l at 12.30 h and 5·5 μmol/l at 16.30 h; compared with the latter value the mean value after the marathon had risen highly significantly to 12·4 μmol/l ($P < 0·005$). The result for participants Nos. 12 and 14 resembled the mean value for the group.

The mean control LH values for the group were 15·6 U/l at 12.30 h and 17·3 U/l at 16.30 h, and the mean value after the marathon was 18·8 U/l, which

Table 1. Hormone values in control samples and after the marathon. The first value is the 12.30 h control, the second value the 16.30 h control and the third value the postmarathon one

	Subject No.	Cortisol μmol/l			Testosterone nmol/l			Androstenedione nmol/l			LH U/l			Running time, min
The group	1	0·22	0·26	1·42	8·5	10·7	5·3	n.d.	n.d.	n.d.	13·9	14·9	19·1	210
	2	0·74	0·34	0·97	11·9	20·7	7·9	4·6	6·5	7·3	20·9	23·6	26·7	210
	3	0·65	0·44	1·50	30·7	27·9	23·0	10·0	4·4	12·1	13·2	9·9	19·9	215
	4	0·20	0·16	0·96	11·5	15·1	7·5	9·3	5·4	9·8	11·9	15·5	18·6	225
	5	0·45	0·23	0·83	34·8	20·7	10·3	10·3	6·1	12·3	9·5	12·3	25·2	246
	6	0·35	0·16	0·99	38·1	39·2	29·1	7·5	6·4	23·3	15·4	13·4	14·7	246
	7	0·35	0·22	1·86	21·0	13·0	8·9	6·6	4·6	17·0	14·2	19·0	26·6	250
	8	n.d.	0·18	1·20	n.d.	41·6	25·3	n.d.	5·3	16·6	n.d.	15·2	10·2	260
	9	0·32	0·19	1·14	15·5	19·2	6·5	4·6	4·4	7·1	12·2	19·0	14·3	260
	10	0·63	0·40	0·55	10·7	10·2	6·2	8·4	5·0	8·1	29·4	22·0	15·7	267
	11	0·36	0·31	1·28	32·9	39·4	25·5	5·4	6·7	10·7	14·6	25·9	15·5	283
Mean value		0·42	0·26	1·15	21·6	23·4	14·1	7·4	5·5	12·4	15·6	17·3	18·8	
S.D.		0·18	0·10	0·36	11·5	11·8	9·4	2·2	0·9	5·2	5·7	5·0	5·5	
	12	0·54	0·44	1·45	27·4	33·2	36·0	6·0	5·4	11·6	23·4	23·3	48·7	182
	13	0·40	0·24	0·26	13·9	11·0	3·0	n.d.	4·8	n.d.	17·5	17·2	2·0	66 (15 km)
	14	n.d.	0·45	1·24	n.d.	19·9	11·4	n.d.	4·6	7·7	n.d.	22·2	11·5	277

n.d. = not determined.

does not differ significantly from the mean control value at 16.30 h. After the marathon a rise of more than 100% (from 23·3 to 48·7 U/l) was found in subject No. 12, whereas in participant No. 13 the value was very low (2·0 U/l).

In participant No. 14 the LH values were comparable to those of the group.

There was no correlation between the plasma levels of cortisol and testosterone, cortisol and androstenedione, or testosterone and androstenedione in the control samples (12.30 h and 16.30 h). However, a significant correlation ($r = 0{\cdot}66$; $P < 0{\cdot}05$) was found between the plasma levels of testosterone and androstenedione in the post-marathon samples.

Comparisons of the changes in cortisol, testosterone and androstenedione after the marathon in relation to their respective control level at 16.30 h show that significant correlation existed ($r = 0{\cdot}68$; $P < 0{\cdot}05$) between the percentage increases in cortisol and androstenedione. In addition, a significant ($r = 0{\cdot}69$; $P < 0{\cdot}05$) correlation between the percentage decrease in testosterone and percentage increase in androstenedione was observed.

DISCUSSION

The physiology of marathon runners has been studied extensively (for a review see 26). Of the hormones investigated in the present study, however, only cortisol has been measured before in a marathon and found to be greatly elevated (mean 2·54 μmol/l). Plasma cortisol may increase even before exercise, depending upon the fitness and training [5, 7, 13, 40]. During strenuous exercise it increases and the longer the run the greater the increase [5, 6, 12, 40]. All our runners showed an increase in plasma cortisol, the mean level being over 400% higher than the control values, which agrees with previous findings in a marathon [2, 5, 40] and in a 70-km cross-country ski race [14]. The adrenal response to exercise is not uniform but depends upon the fitness [2] and training of the individual, which increases its adrenal capacity to respond [11].

The old Greek's cortisol response corresponds with the findings in a 67-year old marathon runner [40], and strengthens the view that the adrenal response to exercise in trained subjects is not impaired by age. Participant's No. 13 cortisol value after collapse indicates a poor adrenal response. In a race of 15·5 km six competitors collapsed and cortisol was elevated in all but one. The explanation was unknown, as subsequent investigation revealed normal adrenal stimulation in response to insulin hypoglycaemia and to Synacthen® test [5]. Afterwards, a Synacthen® test on our subject who collapsed showed that his adrenal function was normal. No heat stroke or dehydration was diagnosed.

Plasma testosterone (or androgens) increases with a change from rest to activity [27, 28] or during exercise [16, 17]. In one study [30], exercise did not cause any change in plasma testosterone levels. The mechanism of the 60% decrease of testosterone during the marathon is not immediately obvious. Our observations cannot be compared with opposite results of Sutton *et al.* [16, 17], because the latter measured total androgens during less exhausting exercise. The decrease we observed is comparable to that found after surgery [29–31], after ACTH administration [32, 42], and among army cadets subjected to extreme physical activity [33]. The changes in the other hormones studied were also similar to those observed previously [29–32].

Finally, the heat (30°C)—especially for Finns—may have influenced their testosterone, as suggested by *in vivo* and *in vitro* experiments in animals [34, 35]. In the very fit subject (No. 12) the slight increase in testosterone is consistent with the very high LH. In the

other participants the mean LH value after the race did not differ from the control value, which confirms that exercise does not affect mean plasma LH [2, 16, 17]. The reason for the very low values for cortisol, LH and testosterone in the man who collapsed is not clear, but a central nervous system collapse may be postulated, perhaps caused by the slight influenza.

The significant increase in androstenedione for all participants after the marathon is probably a result of increased adrenal activity.

Afternoon control values for cortisol were lower than the values at noon, as expected. With testosterone no such decrease was observed. The 4 h interval may have been too short for a detectable decrease, but it is possible that the diurnal variation [36, 37] is modified in physically active individuals. Lipsett *et al.* [27] found a smaller drop in testosterone in active subjects than at rest. A decrease in androstenedione was observed in afternoon control samples. This agrees with the finding that androstenedione decreases during the day more than twice as much as testosterone [38]. No significant change in LH was observed during these four afternoon hours.

In the male, androstenedione is secreted mainly by the adrenal; its concentration in adrenal venous blood increases after ACTH stimulation [32, 42] and it is precursor of testosterone. Increased stimulation of adrenal androgen biosynthesis by ACTH during the marathon would therefore account for the significant correlation between the post-marathon testosterone and androstenedione levels. It would also explain the significant correlation between the percentage increases of cortisol and androstenedione.

In three individuals (Nos. 3, 6, 7) in whom testosterone decreased only moderately there was a great increase of androstenedione and it is possible that their testosterone decrease was partially compensated for by increased adrenal androstenedione (and testosterone?) production. It is likely that the stress caused a decrease in the production of testosterone by the testes, as has been observed in dogs [41].

It is concluded that marathon running causes marked alterations in plasma cortisol, testosterone and androstenedione, to some degree connected with the physical fitness of the athletes. Further research is needed to clarify the role of hormones in the endurance of runners. Not only will such studies contribute to the solution of medical problems associated with mass participation in sport [5] but it will also be of great value if we could distinguish in advance between those for whom the marathon is to be recommended and those for whom it may be dangerous [43]. We must learn more about the collapses during marathon as described by us and by Sutton [5] and which caused the death of Philippides, the dispatch-runner, on his arrival at Athens after the Battle of Marathon on 22 September 490 B.C. [44].

Acknowledgements—We wish to thank Miss Inga Wiik and Mrs Pirjo Keskinen for skilful technical assistance. We also extend our thanks to the National Pituitary Agency (University of Maryland, School of Medicine), the National Institute of Arthritis, Metabolic and Digestive Diseases, Bethesda, U.S.A., for supplying the human LH and anti-human LH serum and the National Institute for Medical Research, London, England, for supplying the human ICSH used as a standard for LH. We are grateful for grants from the Ford Foundation, New York, The Finnish Ministry of Education and from the Sigrid Jusélius Foundation, Helsinki.

We wish to thank the Hellenic Amateur Athletic Association (S.E.G.A.S.) and the Hellenic Association of Sports Medicine for their help. Invaluable technical assistance was provided by the scientific and laboratory personnel of the 2d. Regional Blood Centre of Athens (the King Paul Hospital, Goudi, Head: Dr. T. Mandalaki-Yannitsioti), for centrifugation and storage of the samples; we extend our thanks to them as well as to the Greek and Finnish physicians who supervised the different steps. Finally, the willingness of the participants to give blood samples, especially after the race was the basis on which the whole investigation rested. We thank them very sincerely.

REFERENCES

1. Schalch D. S.: *J. Lab. clin. Med.* **69** (1967) 256–269.
2. Sutton J. R., Young J. D., Lazarus L., Hickie J. B. and Maksvytis J.: *Austral. Ann. Med.* **18** (1969) 84–90.
3. Hermansen L., Pruett E. D. R., Osnes J. B. and Giere F. A.: *J. appl. Physiol.* **29** (1970) 13–16.
4. Pruett E. D. R.: *J. appl. Physiol.* **28** (1970) 199–208.
5. Sutton J., Coleman M. J., Millar A. P., Lazarus L. and Russo P.: *Med. J. Austral.* **2** (1972) 127–133.
6. Hartley L. H., Mason J. W., Hogan R. P., Jones L. G., Kotchen T. A., Mougey E. H., Wherry F. E., Pennington L. L. and Ricketts P. T.: *J. appl. Physiol.* **33** (1972) 602–606.
7. Hartley L. H., Mason J. W., Hogan R. P., Jones L. G., Kotchen T. A., Mougey E. H., Wherry F. E., Pennington L. L. and Ricketts P. T.: *J. appl. Physiol.* **33** (1972) 607–610.
8. Böttger I., Schlein E. M., Faloona G. R., Knochel J. P. and Unger R. H.: *J. clin. Endocr. Metab.* **35** (1972) 117–125.
9. Hunter W. M., Fonseka C. C., Passmore R.: *Science* **150** (1965) 1051–1053.
10. Staehelin D., Labhart A., Froesch R. and Kägi H. R.: *Acta endocr., Copenh.* **18** (1955) 521–529.
11. Cornil A., De Coster A., Copinschi G. and Franckson J. R. M.: *Acta endocr., Copenh.* **48** (1965) 163–168.
12. Davies C. T. M. and Few J. D.: *J. appl. Physiol.* **35** (1973) 887–891.
13. Mason J. W., Hartley L. H., Kotchen T. A., Mougey E. H., Ricketts P. T., Jones L. G.: *Psychosom. Med.* **35** (1973) 406–414.
14. Sundsfjord J. A., Strømme S. B., Refsum H. E. and Aakvaag A.: *Acta endocr., Copenh* Suppl. 177 (1973) 184.
15. Few J. D.: *J. Endocr.* **62** (1974) 341–353.
16. Sutton J. R., Coleman M. J., Casey J., Lazarus L.: *Brit. Med. J.* **1** (1973) 520–522.
17. Sutton J. R., Coleman M. J. and Casey J. H.: *Med. Sci. Sports* **6** (1974) 72.
18. Kotchen T. A., Hartley L. H., Rice T. W., Mougey E. H., Jones L. G. and Mason J. W.: *J. appl. Physiol.* **31** (1971) 178–184.
19. Adlercreutz H. and Dessypris A.: *Brit. Med. J.* **2** (1974) 726.
20. Leclerq R. U., Copinschi G., Bruno O. D. and Franckson J. R. M.: Fonds de la recherche Scientifique Medicale, Belgique, Groupes de Contact. (1969) 7–20.
21. Dessypris A.: *Ärztl. Lab.* **19** (1973) 231–233.

22. Ismail A. A. A., Niswender G. D. and Midgley R. Jr.: *J. clin. endocr. Metab.* **34** (1972) 177–184.
23. Den Hollander F. C. and Schuurs A. H. W. M.: In Radioimmunoassay Methods (Edited by K. E. Kirkham and W. M. Hunter) (1971) pp. 419–422, Livingston, Edinburgh.
24. Karonen S-L., Mörsky P., Siren M. and Seuderling U.: *Analyt. Biochem.* **67** (1975) 1–10.
25. De Jonge H.: Inleiding tot de Medische Statistiek. Deel II. Verhandeling van het Nederlands Institut voor Praeventive Geneeskunde, Leiden, 2nd edn. (1964) pp. 486–487.
26. Costill D. L.: *J. Am. med. Assoc.* **221** (1972) 1024–1029.
27. Lipsett M. B., Wilson H., Kirschner M. A., Korenman S. G., Fishman L. M., Sarfaty G. A. and Bardin C. W.: In *Recent Progress in Hormone Research* (Edited by G. Pincus) Academic Press, New York, Vol. 22 (1966) p. 245, p. 252.
28. Briggs M. H., Garcia-Webb P., Cheung T.: *Brit. med. J.* **3** (1973) 49–50.
29. Carstensen H., Terner N., Thorén L. and Widc L.: *Acta Chir. scand.* **138** (1972) 1–5.
30. Carstensen H., Amér B., Amér I. and Wide L.: *J. steroid Biochem.* **4** (1973) 45–55.
31. Nakashima A., Koshiyama K., Uozumi T., Monden Y., Hamanaka Y., Kurachi K., Aono T., Mizutani S. and Matsumoto K.: *Acta endocr., Copenh.* **78** (1975) 258–269.
32. Beitins I. Z., Bayard F., Kowarski A. and Migeon C. J.: *Steroids* **21** (1973) 553–563.
33. Kreuz L. E., Rose R. M. and Jennings J. R.: *Archs Gen. Psychiat.* **26** (1972) 479–482.
34. Bedrak E., Samoiloff V., Sod-Moriah U. A. and Goldberg S.: *J. Endocr.* **51** (1971) 489–498.
35. Gomes W. R., Butler W. R. and Johnson A. D.: *J. anim. Sci.* **33** (1971) 804–807.
36. Rose R. M., Kreuz L. E., Holaday J. W., Sulak K. J. and Johnson C. E.: *J. Endocr.* **54** (1972) 177–178.
37. Piro C., Fraioli F., Sciarra F. and Conti C.: *J. steroid Biochem.* **4** (1973) 321–329.
38. Crafts R., Llerena L. A., Guevara A., Lobotsky J. and Lloyd C. W.: *Steroids* **12** (1968) 151–163.
39. Wieland R. G., de Courcy C., Levy R. P., Zala A. P. and Hirschmann J.: *J. clin. Invest.* **44** (1965) 159–168.
40. Sutton J. R. and Casey J. H.: *J. clin. Endocr. Metab.* **40** (1975) 135–138.
41. Tcholakian R. K. and Eik-Nes K. B.: *Am. J. Physiol.* **221** (1971) 1824–1826.
42. Doerr P. and Pirke K. M.: *Acta endocr. Copenh.* **78** (1975) 531–538.
43. Kavanagh T., Shephard R. H. and Pandit V.: *J. Am. med. Assoc.* **229** (1974) 1602–1605.
44. Loukianos: *Τπέρ τοῦ 'εν τῇ προσαγορεύσει πταίσματος.* (The works of Lucian of Samosata: Vol. 2, p. 36: A slip of the tongue in salutation. Translated by H. W. Fowler and F. G. Fowler, Oxford University Press. 1949).