0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 7
Printed in U.S.A.

Serum Leptin Levels in Male Marathon Athletes before and after the Marathon Run*

ALFONSO LEAL-CERRO, PEDRO P. GARCIA-LUNA, RICARDO ASTORGA, JUANA PAREJO, ROBERTO PEINO, CARLOS DIEGUEZ, AND FELIPE F. CASANUEVA

Division of Endocrinology, Hospital Virgen del Rocio (A.L.-C., P.P.G.L., R.A., J.P.), Sevilla; and Endocrine Section and the Departments of Medicine (F.F.C., R.P.) and Physiology (C.D.), University of Santiago de Compostela, Santiago de Compostela, Spain

ABSTRACT

Leptin is a hormone produced by the adipocytes to regulate food intake and energy expenditure at the hypothalamic level. It is commonly accepted that the main determinants of leptin secretion are the net amount of body fat and the mean size of adipocytes. On the contrary, important vectors of energy flux in the organism, such as food intake and energy expended on exercise, are not thought to be regulators of that secretion. To understand whether leptin is regulated by an acute energy expenditure such as strenuous exercise, 29 male athletes who had trained for marathon running were studied before and after a marathon run and compared with 22 nonobese, age-, sex-, and body mass index (BMI)-matched sedentary controls.

Controls and marathon athletes showed no differences in BMI or fat-free mass. Marathon runners showed a strong reduction in total fat mass (6.2 ± 0.4 kg; 9.1 ± 0.5% of body fat) compared with controls (12.3 ± 0.5 kg; 16.1 ± 0.5% of body fat; $P < 0.05$). This difference in body composition was paralleled by a mean serum leptin level that in marathonians (2.9 ± 0.2 μg/L) was significantly ($P < 0.05$) reduced compared with that in controls (5.1 ± 0.6 μg/L). It is remarkable that the ratio of leptin per kg body fat, showed a very good agreement between the two groups, 0.40 ± 0.04 μg/L·kg for controls and 0.46 ± 0.03 μg/L·kg for marathonians. In the two groups, leptin was correlated with both body weight, BMI, and fat mass ($P < 0.001$).

The marathon trajectory was the standard 42.195 km accomplished in an average time of 3 h, 17 min, 7 s, with a calculated energy expenditure of over 2800 Cal. After the marathon run, a water imbalance occurred, with a significant decrease in body weight and an increase in serum albumin. A significant ($P < 0.05$) reduction in leptin values was observed after the run (2.6 ± 0.2 μg/L) compared with before (2.9 ± 0.2 μg/L), which was more relevant considering the relative hemoconcentration.

In conclusion, 1) compared with sedentary subjects, leptin levels are reduced in male marathon runners in parallel with the relevant reduction in total body fat; 2) expressed as a ratio of leptin per kg body fat, no differences were observed between marathonians and controls; and 3) after an energy expenditure of 2800 Cal in the marathon run, a reduction in leptin levels occurred. Strong changes in energy expenditure may regulate serum leptin levels in man. (*J Clin Endocrinol Metab* **83:** 2376–2379, 1998)

THE ADIPOCYTE hormone leptin is thought to serve as a signal to inform the central nervous system about the state of fat stores (1–4). Compared with those in normal weight controls, serum leptin levels are high in obese humans (5–7) and are severely reduced in underweight subjects (8–10). Besides its role in obesity and metabolic disorders, the participation of leptin in new and previously unexpected hormonal functions has been described, for example, in the regulation of GH secretion (11), gonadal function and gestation (12–14), and placental function (15).

It is commonly admitted that both the net amount of fat and the mean size of individual adipocytes are the main positive regulators of circulating leptin (7, 16). Moreover, experimental interventions such as short term fasting or overfeeding led in a few days to a decrease or a rise, respectively, in circulating leptin levels (17). However, other undetermined factors should operate to explain facts such as the gender differences in leptin concentrations or the pulsatility of leptin in serum (13, 18, 19). In fact, insulin, glucocorticoids, and thyroid and gonadal hormones participate in the regulation of leptin secretion by the adipocytes (20). Interestingly, although apparently unrelated hormonal signals regulate it, factors directly linked to energy regulation, such as meal consumption, dietary energy source, or exercise-mediated energy expenditure, do not seem to acutely contribute to the serum levels of leptin (5, 21), and they are operative only after inducing evident changes in body composition. Although low leptin levels have been seen in women athletes (20), in aged women after an exercise program (16), and in long distance runners (22), such levels merely reflect the reduction in adipose tissue mass of the studied individuals. The main problem in evaluating the role of energy expenditure *per se* in leptin regulation is to find an adequate experimental design with strenuous exercise capable of inducing high energy expenditure in such a short time that does not alter the fat mass and can be performed in nonfasting subjects to prevent the action of insulin over the adipocyte.

In the present work, serum leptin concentrations have been assessed in a group of male marathon athletes both before and after a marathon run and in matched controls. The two aims of the study were 1) to determine the levels of leptin in a group of highly trained male athletes compared with

Received August 14, 1997. Revision received November 17, 1997. Rerevision received March 30, 1998. Accepted April 6, 1998.

Address all correspondence and requests for reprints to: F. F. Casanueva, M.D., Ph.D., P.O. Box 563, E-15780 Santiago de Compostela, Spain. E-mail: meffcasa@uscmail.usc.es.

* This work was supported by grants from Fondo de Investigacion Sanitaria, Spanish Ministry of Health, Xunta de Galicia, and Fundacion Salud 2000.

those in matched controls, and 2) to observe the effect on leptin concentrations of the most intense model of exercise-mediated energy expenditure, *i.e.* a run of 42.195 km performed in less than 3.5 h (2800 Cal).

Subjects and Methods

The subjects of this study were 29 nonprofessional male athletes trained for marathon running who at the moment of the study were training at a high level. Their mean age was 37.1 ± 1.7 yr (range, 22.0–51.3 yr), weight was 67.7 ± 1.0 kg, height was 168 ± 3 cm, and body mass index (BMI) was 23.9 ± 0.3. They were studied both the day before and after finishing a marathon run. As controls, 22 nonobese males with little exercise activity in the normal range for the Spanish population were studied. Their mean age was 34.6 ± 1.5 yr (range, 23.0–48.2 yr), weight was 75.6 ± 1.7 kg, height was 176 ± 01 cm, and BMI was 24.1 ± 0.4. They were selected on the basis of being sex, age, and BMI matched with the marathonians. None of the subjects presented actual or past history of endocrinological or metabolic disease. The study was approved by the hospital ethical committee, and informed consent was previously obtained from all participants.

Standing height was measured using a portable direct reading Harpenden stadiometer. Weight was determined by means of a calibrated electronic scale. The mean BMI, defined as weight in kilograms divided by the square of height in meters, was calculated. Fat mass was determined by tetrapolar bioelectrical impedance (Human-IM Scan, Ditosystem, Barcelona, Spain), measured at 50 kHz. Total body water was estimated using sex-specific equations (23, 24). Fat-free mass was assumed to have a hydration constant of 0.73 and was calculated using the formula: fat-free mass = total body water/0.73.

To prevent circadian variations, blood samples were obtained from the controls and the marathonians before and after running at the same time. Blood samples were always obtained after a light breakfast in the morning (1000–1200 h) using a standard venipuncture technique, and after clotting at 4 C, the serum was separated by centrifugation and was stored at −20 C until assay. Marathon runners were assessed both the day before and the day of the marathon after finishing the run. Controls were assessed once.

Serum leptin levels were measured in duplicate by RIA for leptin using commercial kits (Human Leptin RIA, Linco Research, St. Charles, MO). The limit of sensitivity was 0.5 μg/L; the intraassay coefficient of variation was 8.3%, and the interassay coefficient of variation was 6.2%. Plasma GH was measured by an immunoradiometric assay (Bio Merieux, Madrid, Spain), with intraassay coefficients of variation of 5% and 5.6%, respectively, and interassay coefficients of variation of 6% (1.6 μg/L) and 4.4% (19.0 μg/L), respectively. Albumin was determined using an automatic analyzer (Cobas, F. Hoffman-La Roche Ltd., Basel, Switzerland). Samples from each patient were assayed at the same time.

Results are presented as the mean ± SEM of absolute values, and the data were compared using a nonparametric test (Wilcoxon) and paired Student's *t* tests when appropriate. The effects of age, weight, height, BMI, percent body fat, and lean body mass on leptin values and their relationships were assessed by simple linear correlation (Pearson's test). $P < 0.05$ was considered significant.

Results

Compared with nonobese controls, marathon athletes presented no differences in BMI or fat-free mass. Furthermore, marathonians showed a strong reduction in the total fat mass (6.2 ± 0.4 kg; 9.1 ± 0.5% of body fat) compared with controls (12.3 ± 0.5 kg; 16.1 ± 0.5% of body fat; Fig. 1). This important difference in body composition was paralleled by the serum level of leptin found in marathonians (2.9 ± 0.2 μg/L), which was significantly ($P < 0.05$) reduced compared with that in controls (5.1 ± 0.6 μg/L). Interestingly, when leptin concentrations were calculated by each kilogram of body fat, a very good agreement was observed in the two groups (0.40 ± 0.04 μg/kg for controls and 0.46 ± 0.03 μg/kg for marathonians).

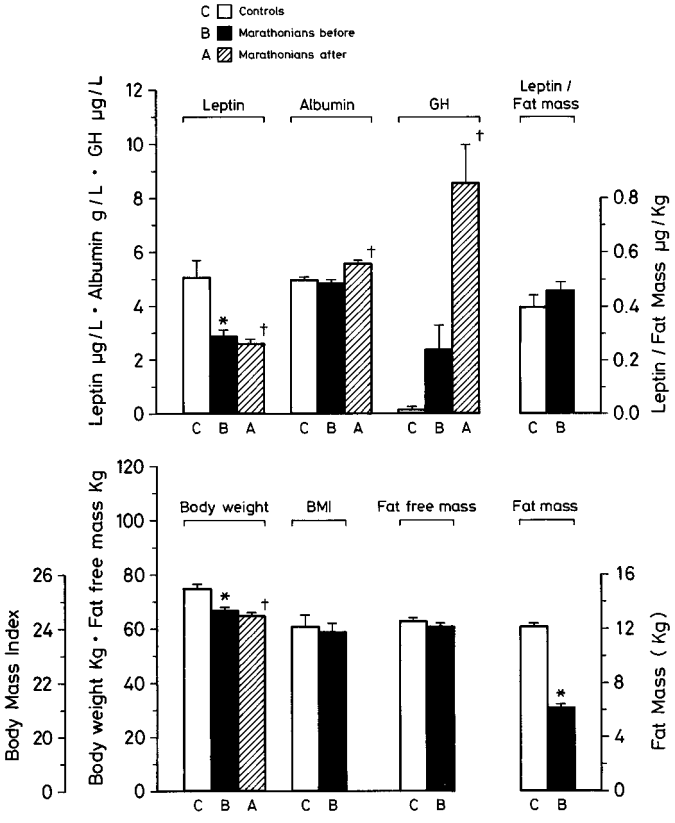

FIG. 1. Mean ± SEM serum leptin levels and several auxological parameters in control sedentary subjects and in marathon athletes before and after running the marathon (42.1 km in 3 h, 17 min). *, $P < 0.05$ *vs.* control subjects; +, $P < 0.05$ *vs.* marathonians before the run.

The marathon distance was the standard length of 42.195 km performed in an average time of 3 h, 17 min, 7 s (range, 2 h, 28 min, 00 s to 3 h, 53 min, 52 s). The calculated energy expenditure in the marathon run was over 2800 Cal (25). When marathonians were analyzed after the marathon run, it was evident that a water imbalance had occurred despite their free access to water at regular intervals. In fact, a significant ($P < 0.05$) decrement in body weight was observed together with an increase in serum albumin concentration (4.9 ± 0.06 *vs.* 5.6 ± 0.09 mg/dL; $P < 0.05$; Fig. 1). Similarly, an increase in GH values was observed, an expected change after intense exercise (26), without changes in insulin-like growth factor I (data not shown). After the marathon run, a significant decrease ($P < 0.05$) was observed in serum leptin (2.6 ± 0.2 μg/L) compared with that before the run (2.9 ± 0.2 μg/L), a value more relevant considering the relative hemoconcentration that occurs.

When individual leptin values were analyzed (Fig. 2), a good correlation was observed in each of the 2 groups between leptin and BMI, body weight, and total fat mass (all $P < 0.001$). Interestingly, a very good correlation was observed between leptin and total fat mass in the marathonians. On the contrary, in the control subjects, a considerable dispersion in leptin levels was observed for any fat mass value. When individual leptin values of marathon athletes obtained both before and after the run were plotted against total fat mass calculated before the run, a change in the correlation

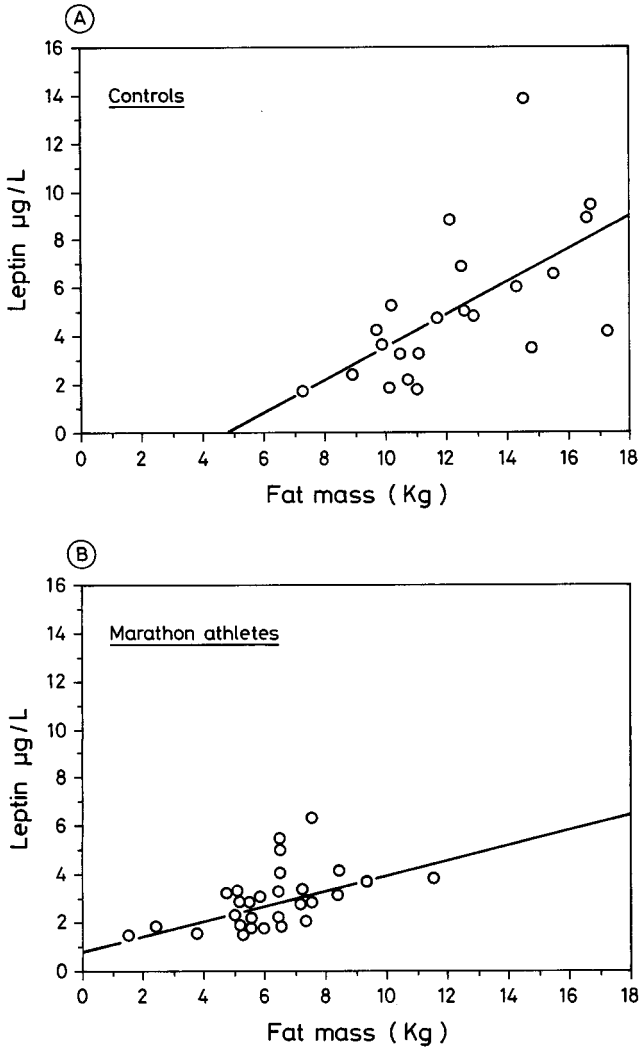

FIG. 2. Scattergram of individual serum leptin levels plotted against total fat mass in control sedentary subjects (A; $y = 0.678 \times 3.234$; r = 0.61; $P < 0.001$) and marathon athletes before running the marathon (B; $y = 0.324 \times + 0.956$; r = 0.50; $P < 0.001$).

line was evident (Fig. 3), and in 17 subjects a reduction in leptin levels was observed.

Discussion

The extraordinary conservation of the leptin molecule through species suggests that it played a key role in metabolism throughout the evolution. Leptin should be viewed as a hormone that in man regulated the neuroendocrine adaptations to fasting and the fluxes of energy in a world characterized by food shortage and enhanced energy expenditure (27, 28). Although both short term fasting and physical exercise led after some days to a reduction in serum leptin levels, no acute changes in leptin have been reported after either food intake or physical exercise. To understand the role of acute energy expenditure in nonfasting subjects, in the present work a group of trained marathon runners was studied before and after the marathon run, with blood samples obtained at similar times of the day to avoid changes due to circadian variations (29, 30).

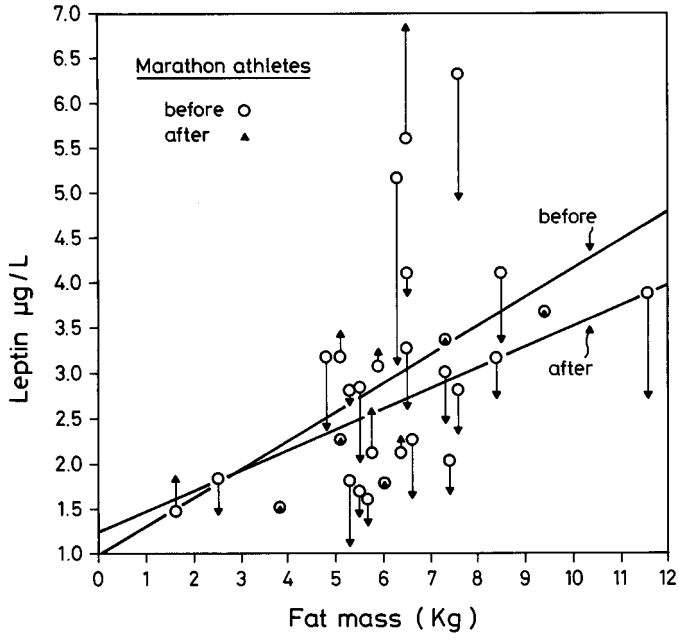

FIG. 3. Scattergram of individual serum leptin levels plotted against total fat mass (kilograms) in the marathon athletes before (*white symbol*) and after (*arrowhead*) running the marathon.

Athletes trained for marathon running underwent a very complex and systematic training over several years that led to profound changes in body composition, with the net result of a total fat mass near half that in normal controls. As observed in this work, a strong reduction in leptin levels paralleled the reduction in body fat, as previously observed in other athletes (20, 22). A good correlation in each group was observed for leptin with BMI, body weight, and total fat mass, again confirming in this selected population that the main determinant of circulating leptin concentrations is the amount of adipose tissue in the individual even when the amount of this tissue is severely reduced. Interestingly, the correlation of leptin with total fat mass or BMI was very good in marathonians, whereas a high dispersion was found in the sedentary controls. It appears that with higher amounts of fat deposits, other factors regulating leptin secretion are operative, and the expected stoichiometry between adipocyte mass and leptin values becomes less strict. Interestingly, if leptin values are expressed as the ratio per kg body fat, no difference between sedentary controls and runners was observed, suggesting that this index might be of utility in further studies on leptin.

The marathon run is suitable to study the role of energy expenditure *per se* on leptin regulation. First of all, runners are not in a fasting state, because limited food and water intake are allowed at precise times both before and during the run. Second, it is the most strenuous type of exercise that an individual may accomplish, with more than 42 km normally run in less than 3.5 h and with a net energy expenditure of over 2800 Cal (31, 32). This means that in a short period of time the runner would have expended as much energy as in 24 h of a very active day. In the present work, the high energy expenditure in a short time was accompanied by a significant reduction in serum leptin levels. Although sta-

tistically significant, we are very cautious about the biological meaning of small differences in leptin such as those reported here before and after the marathon run. The only reason to reinforce the relevance of the figures is the hemoconcentration that occurs after the run (33), a fact that should enhance and never reduce the serum concentration of all circulating proteins with a long half-life such as leptin. The marathon is an exercise of such intensity that only perfectly trained individuals are able to participate and finish it. Thus, the athletes studied here were under a normal training schedule and well into energy balance before the run. The relevant state of negative energy balance induced by the run may well be the cause of the observed marathon-mediated leptin decline.

An exercise-mediated reduction in leptin values has been observed in previous reports, but without reaching statistical significance (22, 34). There is no clear explanation for these controversial results, but in a previous report about long distance runners (22) leptin values were corrected by applying a mathematical formula for hemoconcentration (35). Considering that changes in leptin are small, and hematocrit is not a finely measurable variable, the effects of two uncertainties may have been summed. There is no proof at the moment that such small changes in leptin levels may have a biological meaning, or what hormonal or metabolite signals are implicated in this reduction (17, 36, 37). However, the present report shows that a high level energy expenditure may acutely regulate serum leptin levels in man, and although scarce, these changes would become very relevant if the individual is in an environment requiring repetitive vigorous exercise.

In conclusion, 1) compared with matched sedentary subjects, leptin levels are reduced in male marathon runners in parallel with the strong reduction in total body fat; 2) expressed as the ratio of leptin per kg body fat, no differences were observed between marathonians and controls; and 3) after an energy expenditure of 2800 Cal in the marathon run, a significant reduction in leptin levels occurred. Strong changes in energy expenditure may acutely regulate serum leptin levels in man.

Acknowledgments

The expert technical assistance of Ms. Mary Lage is gratefully acknowledged. The voluntary participation of the marathon team Grupo 10 and of Drs. A. Prada and M. Nieto from the Instituto de Deportes del Ayuntamiento de Sevilla, Spain, is gratefully acknowledged.

References

1. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425–432.
2. **Pelleymounter MA, Cullen MJ, Baker MB, et al.** 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 269:540–543.
3. **Halaas JL, Gajiwala KS, Maffei M, et al.** 1995 Weight-reducing effects of the plasma proteins encoded by the obese gene. Science. 269:543–546.
4. **Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P.** 1995 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 269:546–549.
5. **Considine RV, Sinha MK, Heiman ML, et al.** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 334:292–295.
6. **Lönnqvist F, Arner P, Nordfors L, Schalling M.** 1995 Overexpression of the obese (*ob*) gene in adipose tissue of human obese subjects. Nat Med. 1:950–953.
7. **Hamilton BS, Paglia D, Kwan AYM, Deitel M.** 1995 Increased obese mRNA expression in omental fat cells from massively obese humans. Nat Med. 1:953–956.
8. **Grinspoon S, Gulick T, Askari H, et al.** 1996 Serum leptin levels in women with Anorexia Nervosa. J Clin Endocrinol Metab. 81:3861–3863.
9. **Casanueva FF, Dieguez C, Popovic V, Peino R, Considine RV, Caro JF.** 1997 Serum immunoreactive leptin concentrations in patients with anorexia nervosa before and after partial weight recovery. Biochem Mol Med. 60:116–120.
10. **Ferron F, Considine RV, Peino R, Lado IG, Dieguez C, Casanueva FF.** 1997 Serum leptin concentrations in patients with anorexia nervosa bulimia nervosa and non-specific eating disorders correlate with the body mass index but are independent of the respective disease. Clin Endocrinol (Oxf). 46:289–293.
11. **Carro E, Señaris R, Considine RV, Casanueva FF, Dieguez C.** 1997 Regulation of *in vivo* growth hormone secretion by leptin. Endocrinology 138:2203–2206.
12. **Ahima RS, Dushay J, Flier SN, Prabakaran K, Flier JS.** 1997 Leptin accelerates the timing of puberty in normal female mice. J Clin Invest. 99:391–395.
13. **Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF.** 1997 Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones and pubertal stage. J Clin Endocrinol Metab. 82:2849–2855.
14. **Butte NF, Hopkinson JM, Nicolson MA.** 1997 Leptin in human reproduction: serum leptin levels in pregnant and lactating women. J Clin Endocrinol Metab. 82:585–589.
15. **Señaris R, Garcia-Caballero T, Casabiell X, et al.** 1997 Synthesis of leptin in human placenta. Endocrinology. 138:4501–4504.
16. **Kohrt WM, Landt M, Birge SJ.** 1996 Serum leptin levels are reduced in response to exercise training but not hormone replacement therapy in older women. J Clin Endocrinol Metab. 81:3980–3985.
17. **Sinha MK, Opentanova I, Ohannesian JP, et al.** 1996 Evidence of free and bound leptin in human circulation. J Clin Invest. 98:1277–1282.
18. **Licinio J, Mantzoros C, Negrao AB, et al.** 1997 Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med. 3:575–579.
19. **Saad MF, Damani S, Gingerich RL, et al.** 1997 Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab. 82:579–584.
20. **Laughlin GA, Yen SSC.** 1997 Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab. 82:318–321.
21. **Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL.** 1997 Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab. 82:561–565.
22. **Hickey MA, Considine RV, Israel RG, et al.** 1996 Leptin is related to body fat content in male distance runners. Am J Physiol. 271:E938–E940.
23. **Kushner RF, Schoeller DA.** 1986 Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr. 44:417–424.
24. **Segal KR, Van Loan M, Fitzgerald PI, Hodgdon JA, Van Itallie TB.** 1988 Lean body mass estimation by bioelectrical impedance analysis: a four site cross-validation study. Am J Clin Nutr. 47:7–14.
25. **di Prampero PE.** 1986 The energy cost of human locomotion on land and in water. Int J Sports Med. 7:55–72.
26. **Nicklas BJ, Ryan AJ, Treuth MM, et al.** 1995 Testosterone, growth hormone and IGF-I responses to acute and chronic resistive exercise in men aged 55–70 years. Int J Sports Med. 16:445–450.
27. **Ahima RS, Prabakaran D, Mantzoros M, et al.** 1996 Role of leptin in the neuroendocrine response to fasting. Nature. 382:250–252.
28. **Friedman JM.** 1997 The alphabet of weight control. Nature. 385:119–120.
29. **Sinha MK, Ohannesian JP, Heiman ML, et al.** 1996 Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J Clin Invest. 97:1344–1347.
30. **Leal-Cerro A, Considine RV, Peino R, et al.** 1996 Serum immunoreactive-leptin levels are increased in patients with Cushing's syndrome. Horm Metab Res. 28:711–713.
31. **Brueckner JC, Atchou G, Capelli C, et al.** 1991 The energy cost of running increases with the distance covered. Eur J Appl Physiol. 62:385–389.
32. **Hausswirth C, Bigard AX, Berthelot M, Thomaidis M, Guezennec CY.** 1996 Variability in energy cost of running at the end of a triathlon and a marathon. Int J Sports Med. 17:572–579.
33. **Boudou P, Fiet J, Laureaux C, Patricot MC, et al.** 1987 Variations de quelques constituants plasmatiques et urinaires chez les marathoniens. Ann Biol Clin. 45:37–45.
34. **Racette SB, Coppack SW, Landt M, Klein S.** 1997 Leptin production during moderate-intensity aerobic exercise. J Clin Endocrinol Metab. 82:2275–2277.
35. **Dill DB, Costill DL.** 1974 Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. J Appl Physiol. 37:247–248.
36. **Kolaczynski JW, Nyce MR, Considine RV, et al.** 1996 Acute and chronic effects of insulin on leptin production in humans: studies *in vivo* and *in vitro*. Diabetes. 45:699–701.
37. **Wabitsch M, Jensen PB, Blum WF, et al.** 1996 Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes. 45:1435–1438.