CLINICAL STUDY

Free leptin index and thyroid function in male highly trained athletes

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Abstract

Objective: Exercise training may cause changes in thyroid function. This thyroid response may be due to exercise-induced modulation of energy metabolism but also of the adipocytes endocrine function. In particular, the role of leptin and of circulating soluble leptin receptor (sOB-R) was unexplored. The aim of this study was to assess the relationships between thyroid function, whole body energy metabolism, and adipokines – mainly leptin and its receptor, sOB-R.

Methods: We measured serum TSH, free tri-iodothyronine (FT₃), free thyroxine, leptin, and sOB-R and assessed energy homeostasis by means of indirect calorimetry, in 27 highly trained athletes and 27 sedentary, healthy men.

Results: TSH–FT₃ ratio was lower in athletes ($P < 0.03$), either in sustained power or anaerobic power-sprint athletes ($n = 13$) or marathon runners ($n = 14$). Whole body respiratory quotient was lower in athletes. Fasting serum sOB-R was higher and leptin lower in athletes than controls. Also serum adiponectin, resistin, and retinol binding protein-4 concentrations were different in athletes than in controls. The ratio between leptin and sOB-R, the free leptin index (FLI), was lower in athletes than in controls (0.025 ± 0.014 vs 0.085 ± 0.049 ; $P < 0.001$). In multivariate analysis, FLI retained independent association with TSH–FT₃ ratio.

Conclusion: Male, elite athletes had lower TSH–FT₃ ratio and FLI than controls while FLI was independently associated with TSH–FT₃ ratio supporting the hypothesis that the level of biologically active leptin is involved in the adaptive response of thyroid function in professional athletes.

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Introduction

Regulation of the metabolic adaptation during prolonged physical training by the thyroid hormones is considered of importance but remains controversial. Some authors reported no major effect of exercise training on serum TSH levels in professional athletes (1) and sedentary individuals (2) suggesting that the influence of chronic exercise is likely to be small; in contrast other authors suggested long-term exercise training may lead to downregulation of thyroid hormone concentrations (3–5). Among the candidate factors, this response may be induced by the exercise-induced negative energy balance as well as signaling alterations in the hypothalamus–pituitary–thyroid and hypothalamus–adipocyte–leptin axes (5). With this respect, leptin is an adipocyte-secreted hormone that plays an important role as a signal of energy availability in energy-deficient states (6), and during training

situations both leptin and TSH levels were shown to decrease in parallel (7).

In this context, our study aimed to assess simultaneously whole body energy metabolism, thyroid function and circulating leptin concentration in professional, elite track runners and to compare them to sedentary age- and body mass index (BMI)-matched subjects. Leptin may bind to several membrane-bound isoforms, but a soluble form of the leptin receptor (sOB-R) is also generated by cleavage of the membrane-bound isoform (8). sOB-R is therefore an important regulator of leptin activity (9). The balance between the serum leptin and sOB-R concentration, known as free leptin index (FLI), is the ratio between the two concentrations and was recently reported to be associated with metabolic features in insulin resistant subjects (10). For this reason we decided to test whether the habitual, long lasting habit to perform heavy physical activity was associated not only with changes

in the serum leptin levels, but also with its soluble receptor and with FLI. We also measured in the same population the serum concentration of other adipokines presumably involved in the regulation of energy metabolism and insulin action such as adiponectin, resistin, retinol binding protein-4 (RBP-4), and visfatin.

Methods

Subjects

Twenty-seven elite track runners (sustained power or anaerobic power-sprint athletes $n=13$; marathon runners $n=14$) participating in national and international competitions were recruited within the Federazione Italiana Atletica Leggera Lombardia Section. They were compared with 27 sedentary young lean men. Matching of age and BMI was 1:1 and was performed within five units of age and one unit of BMI. These healthy individuals were recruited in the outpatient services of the Center of Nutrition/Metabolism of the San Raffaele Scientific Institute. Body weight was stable for at least 6 months. The clinical and laboratory features of the subjects are summarized in Table 1. All subjects were in good health as assessed by medical history, physical examination, hematological, and urinalysis. Informed consent was obtained from all subjects after explanation of purposes, nature, and

potential risks of the study. The protocol was approved by the Ethical Committee of the San Raffaele Scientific Institute.

Experimental protocol

Subjects were instructed to consume an isocaloric diet (containing at least 250 g carbohydrates and 70–90 g protein/day) and to abstain from exercise activity for 3 days before the studies. Subjects were admitted to the Metabolic Room of the Section of Nutrition/Metabolism of the San Raffaele Scientific Institute at 0730 h after a 10-h period overnight fast. Indirect calorimetry was performed continuously, while lying quietly, for 30 min with a ventilated hood system (Sensor Medics 2900, Metabolic Measurement Cart, Milano, Italy). The mean coefficients of variation (CV) within the session for both O_2 and CO_2 measurements were below 3%. Subsequently, a blood sample was obtained for the assessment of fasting serum glucose, insulin, free fatty acids (FFA), total cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, leptin, sOB-R, adiponectin, resistin, visfatin, RBP-4, high sensitivity C reactive protein (hsCRP), TSH, free tri-iodothyronine (FT_3), and free thyroxine (FT_4). Body fat was assessed the morning prior to the indirect calorimetry by means of bioelectrical impedance using a Body Impedance Analyzer (Akem, RIL System, Detroit, MI, USA) in the 27 control subjects and in 19 athletes.

Analytical determinations

Glucose concentration was measured with standard glucose oxidase method on a glucose analyzer (Beckman Glucose Analyzer 2, Inc., Fullerton, CA, USA). Blood samples for FFA assessment were collected in prechilled tubes containing 0.1% EDTA. Tubes were immediately placed in ice and plasma was immediately processed by centrifugation at 4 °C. Plasma was then frozen and stored at -70 °C and FFA determinations were performed within 0–5 working days as suggested by Zambon *et al.* to obtain plasma FFA determination (microfluorometric assay) avoiding lipoprotein lipase reaction in blood (11). Serum triglycerides, total cholesterol, and high density lipoprotein (HDL)-cholesterol were measured as previously described (enzymatic method on an Hitachi 747) (12). Low density lipoprotein-cholesterol was calculated using the Friedewald formula. Serum resistin was measured by ELISA kit (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic). The sensitivity of the assay was 0.2 ng/ml of sample. The intra-assay CV was $<3.5\%$ and inter-assay was $<7\%$. Serum levels of insulin (sensitivity 2 μ U/ml; intra- and interassay CV <3.1 and 6% respectively) and leptin (sensitivity 0.5 ng/ml; intra- and interassay CV <5 and 9% respectively) were measured with RIA (Linco Research, St Charles, MO, USA) following the manufacturer's assay protocols. Serum adiponectin was

Table 1 Anthropometric, laboratory, and metabolic characteristics of study subjects.

	Athletes	Normals
Number	27	27
Age (years)	28 ± 11	30 ± 6
Body weight (kg)	68 ± 7	68 ± 5
Height (cm)	178 ± 6	177 ± 6
BMI (kg/m^2)	21.5 ± 1.1	21.8 ± 1.2
Body fat (%)	$8.1 \pm 0.4^{*a}$	11.6 ± 0.8
Plasma glucose (mg/dl)	85 ± 7	85 ± 7
Serum insulin (μ U/ml)	$7.7 \pm 4.2^\dagger$	10.6 ± 4.9
Serum C-peptide (ng/ml)	$1.24 \pm 0.65^*$	1.96 ± 0.91
HOMA2-%S	$113 \pm 51^\dagger$	83 ± 41
HOMA2-%B	$114 \pm 42^\dagger$	143 ± 55
Serum FFA (mmol/l)	0.63 ± 0.24	0.53 ± 0.22
Total cholesterol (mg/dl)	152 ± 38	164 ± 31
HDL-cholesterol (mg/dl)	57 ± 10	55 ± 13
Triglycerides (mg/dl)	62 ± 22	72 ± 38
hsCRP (mg/dl)	1.11 ± 0.96	0.82 ± 0.77
Free T_3 (pg/ml)	5.4 ± 0.9	5.3 ± 0.9
Free T_4 (pg/ml)	18.2 ± 3.9	18.5 ± 3.4
TSH (μ U/ml)	$0.90 \pm 0.65^\dagger$	1.27 ± 0.76
TSH/free T_3 ratio	$0.16 \pm 0.11^\dagger$	0.25 ± 0.17
REE (kcal/die)	1903 ± 324	1796 ± 214
Percentage of predicted REE (%)	112 ± 16	107 ± 11
RQ	$0.77 \pm 0.06^\dagger$	0.83 ± 0.11

* $P < 0.01$ versus normals; $^\dagger P < 0.05$ versus normals (2-tails independent *t*-test). REE, resting energy expenditure; RQ, respiratory quotient.

^aAssessment of body fat was obtained in 19 athletes.

measured by ELISA kit (B-Bridge International, Inc., Sunnyvale, CA, USA) with a sensitivity of 25 pg/ml. Serum high sensitive C-reactive protein was measured using an ELISA kit (Diagnostic Biochem Canada Inc., London, Ontario, Canada) with a sensitivity of 10 ng/ml and intra- and interassay CV <5 and <7% respectively. Serum sOB-R was measured by ELISA kit (BioVendor Laboratory Medicine, Inc.) with a sensitivity of 0.04 ng/ml and intra- and interassay CV <7 and <10% respectively. Serum FT₃ (sensitivity 0.3 pg/ml; intra- and interassay CV <3.5 and 9% respectively) and FT₄ (sensitivity 1 pg/ml; intra- and interassay CV <5 and 10% respectively) were measured with ELISA (Diagnostic Biochem Canada Inc.) following the manufacturer recommendations. TSH was measured by immunofluorimetric method. Finally, urine nitrogen was measured by the Kjeldhal method as previously reported (13).

Calculations

Insulin sensitivity was estimated by means of the updated computer model homeostasis model assessment (HOMA)-2 indexes (14) available from www.OCDem.Ox.ac.uk. Resting energy expenditure (REE) was calculated by the Weir's standard equation from the oxygen consumption rate and the carbon dioxide production rates measured by means of indirect calorimetry (excluding the first 10 min of data acquisition), and from the urinary nitrogen excretion. Predicted REE was calculated using the Harris-Benedict equations (15). The percentage of predicted REE was calculated as the ratio predicted to be measured (16). The balance between leptin and sOB-R, the FLI, was determined by calculating the ratio between the serum concentrations of leptin and sOB-R as previously described (10).

Statistical analysis

Data in text, tables, and figures are mean $\pm$ s.d. Analysis was performed using the SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA). When parameters (HOMA%-IS, serum triglycerides) showed a skewed distribution (Kolmogorov-Smirnov test of normality), they were log-transformed before the analysis. Comparison between groups was performed using 2-tailed independent samples *t*-test or Kruskal-Wallis non-parametric test depending on the distribution of the data, and a *P* value <0.05 was considered to be statistically significant. Two-tailed Pearson's correlation was performed to establish partial correlation coefficients between variables and also in this case, non-parametric correlation coefficient was obtained using Spearman's ρ when appropriate. To ascertain the relative contribution of independent factors on the TSH-FT₃ ratio, we used stepwise regression analysis (using *F* ratio-to-remove of 4 and *F* ratio-to-enter of 3.996) including the parameters that in univariate analysis that had a *P* value <0.1.

Results

Clinical features

Anthropometric and laboratory features of the elite athletes and controls are summarized in Table 1. The two study groups were not different for age and BMI but athletes had a lower body fat mass. Fasting serum glucose concentration was also not different, while serum insulin and C-peptide concentrations were lower in athletes than in the control group. As a consequence, the surrogate index of insulin sensitivity based on fasting glucose and insulin concentration (HOMA2-%S) was higher in athletes than controls and the surrogate index of β -cell sensitivity (HOMA2-%B) was lower in athletes than controls. With respect to lipid metabolism the lipoprotein profile was not different between groups as well as the fasting serum FFA concentration (*P*=0.102). hsCRP, as a surrogate marker of low-grade inflammation was not different between groups (*P*=0.45).

Parameters of thyroid function

Parameters of thyroid function are summarized in Table 1. Fasting serum FT₃ and FT₄ concentrations were not different in athletes in comparison to sedentary. Serum TSH was lower in athletes when compared to sedentary as well as the TSH/FT₃ ratio.

Whole body energy metabolism

Parameters of whole body energy metabolism are summarized in Table 1. REE rate and percentage of predicted REE were not different between groups. The respiratory quotient (RQ) was lower in athletes when compared to sedentary. REE corrected for the kg of LBM was also not different between groups (30.5 ± 2.0 vs 29.9 ± 1.1 kcal/kg of LBM; *P*=0.201).

Adipokines concentration

Fasting serum adipokines concentrations are summarized in Fig. 1. Fasting serum leptin concentration was lower in athletes than sedentary (1.39 ± 0.51 vs 3.33 ± 1.50 ng/ml; *P*=0.001) meanwhile sOB-R was higher in the athletes than sedentary (59 ± 13 vs 47 ± 20 ng/ml; *P*=0.019). The balance between leptin and sOB-R, the FLI was lower in athletes than in sedentary (0.025 ± 0.014 vs 0.085 ± 0.049 ; *P*<0.001). Serum adiponectin (8.4 ± 2.9 vs 6.4 ± 2.8 μ g/ml; *P*=0.014) and resistin (4.3 ± 0.8 vs 3.7 ± 1.1 ng/ml; *P*=0.048) were higher in athletes than sedentary. RBP-4 was lower in athletes than in sedentary (30 ± 7 vs 34 ± 7 μ g/ml; *P*=0.025) meanwhile serum visfatin (15 ± 9 vs 12 ± 5 ng/ml; *P*=0.28) was not different between groups.

Figure 1 Serum adipokines and sOB-R concentrations in study groups. Fasting serum concentrations of leptin, sOB-R, adiponectin, resistin, RBP-4, and visfatin are summarized. Black columns represent the athletes, white columns the sedentary control subjects. Mean $\pm$ s.d. * $P < 0.05$ versus normal sedentary controls (two-tailed independent samples *t*-test).

Correlation analysis

The age- and BMI-adjusted Pearson's correlation showed that among the parameters of energy metabolism the TSH/FT₃ ratio correlated with RQ, and showed a trend with the percentage of predicted REE (Table 2). The analysis showed a significant correlation with the sOB-R and FLI, but not with leptin, adiponectin, resistin, retinol-binding protein 4, and visfatin (Table 2). The TSH/FT₃ ratio was also significantly associated with fasting serum triglycerides, and showed a trend with HDL-cholesterol and fasting serum insulin (Table 2). No significant association was detected with fasting serum glucose, C-peptide, FFA, and hsCRP concentrations. There was clearly no significant correlation between the TSH/FT₃ ratio and fat mass in the two subgroups separately and when the correlation analysis was performed in the entire cohort (fat mass was available in only 19 of the 27 elite athletes) a significant association remained undetectable ($r = -0.21$; $P = 0.201$); nevertheless we cannot exclude that a hypothetical analysis with all the athletes included in the plot could be able to generate a trend for a significant association.

As summarized in Table 2, when stepwise regression analysis model including the independent variables with P value < 0.1 in univariate analysis was performed, FLI and serum triglycerides retained independent association with the TSH/FT₃ ratio.

Discussion

It is believed that long-term overtraining may lead to downregulation of hypothalamic hormonal responses; in particular in professional athletes adaptation of the

thyroid function may be secondary to the catabolic training condition and/or hormonal feedback from the depleted adipose tissue, with leptin acting as a major endocrine modulator.

In this background, our study is the first to explore the relationship between parameters of thyroid function and leptin taking simultaneously into account the serum soluble leptin circulating receptor (sOB-R) concentrations in male, elite, professional athletes. We found that adaptation of the thyroid function was not only in parallel with lower serum leptin concentrations but also with higher sOB-R and that the FLI was independently associated with the thyroid function. In these subjects we also assessed the serum levels of other adipokines, driving us to the conclusion that even if their levels may be also different than in sedentary, they are not associated as tightly as FLI with the thyroid function.

The impact of exercise on thyroid hormone metabolism is somewhat controversial as well as the impact on leptin and other adipokines (17). In longitudinal studies, this was probably due to differences in the intensity, duration, and form of exercise as well as on the baseline individual fitness and metabolic conditions and on gender (18). For this reason we decided to explore the issue limiting initially our focus on a group of male, elite professional (long lasting involvement in heavy exercise training program) athletes and comparing them to a control group of sedentary normal-weight subjects. We found that in the fasting and resting state they had a subtle but significant lower serum TSH level which was more evident when the TSH level was normalized to the FT₃ (Table 1).

We measured resting whole body energy metabolism by means of indirect calorimetry and found that it was not different in athletes than in the sedentary controls. This finding is in agreement with a work by Befroy *et al.* in which resting energy metabolism was assessed by means of ³¹P-magnetic resonance spectroscopy (MRS) of the skeletal muscle (19). Correlatively we noticed that the fasting oxidative metabolism of these athletes was shifted towards fatty acids oxidation as reflected by the lower RQ (Table 1). The percentage of predicted REE and the RQ were correlatively associated with the TSH-FT₃ ratio (Table 1). This association in univariate analysis was not retained when the multivariate analysis was performed.

An additional aim of the present work was to evaluate the relationship among the thyroid function and serum leptin not in isolation, as generally reported, but taking simultaneously into consideration the serum levels of other adipokines also known to be potentially involved in the regulation of insulin sensitivity, body fat distribution, and energy metabolism. Not surprisingly, we found that serum leptin was lower in the athletes and adiponectin was higher in athletes due to higher insulin sensitivity (as reflected by the higher HOMA2%-S). Serum resistin was lower as we have

Table 2 Results of the correlation and multiple regression analysis between TSH/free tri-iodothyronine (FT₃) ratio, metabolic parameters, and serum adipokines concentrations.

	Variable	<i>r</i>	β	<i>P</i>
Partial correlation analysis (age-, and BMI-adjusted)				
Metabolic parameters	Triglycerides	0.46	–	0.001
	RQ	0.32	–	0.037
	Percentage of predicted REE	–0.28	–	0.073
	HDL-cholesterol	–0.25	–	0.076
	Insulin	0.24	–	0.094
	HOMA2-%S	–0.13	–	0.41
	Glucose	–0.07	–	0.64
	sOB-R	– 0.44	–	0.003
	FLI	0.40	–	0.004
	Leptin	0.25	–	0.11
	Resistin	0.23	–	0.14
	Adiponectin	–0.21	–	0.19
sOB-R, FLI and adipokines	Visfatin	0.07	–	0.67
	Retinol binding protein 4	–0.00	–	0.98
Stepwise multiple regression analysis (including age, BMI, and dependent variables with <i>P</i> < 0.1 in univariate analysis)				
Step 1	FLI	0.46	0.46	0.001
Step 2	FLI + triglycerides	0.56	0.36/0.33	0.001
Excluded variables at step 2				
	sOB-R	–0.27	–0.30	0.061
	Age	–0.25	–0.21	0.086
	Percentage of predicted REE	–0.22	–0.19	0.13
	HDL-cholesterol	–0.14	–0.12	0.35
	BMI	–0.05	0.05	0.73
	RQ	–0.03	–0.03	0.83
	Insulin	–0.02	–0.02	0.88

r, the correlation coefficient; β , the standardized regression coefficient. Bold indicates *P* < 0.05.

previously reported (12) as well as the RBP4 as reported by others (20). In the present work we report two novel findings in this area; serum visfatin was not different between the athletes and sedentary subjects (Fig. 1) and most importantly sOB-R was higher in the athletes (Fig. 1). The leptin receptor can be spliced into different isoforms but the soluble form, sOB-R is of particular interest because it constitutes the secretory (soluble) form of the receptor which is directly released into the circulation where, by binding leptin, it controls leptin bioavailability (9). Only very recently has its importance been taken into consideration when the balance between the serum leptin and sOB-R concentration, known as FLI, was reported to be associated with a number of features of the metabolic syndrome cross-sectionally (10). With respect to all the above-described adipokines, only the FLI retained an independent association when tested in the multivariate, stepwise regression analysis. This finding would suggest that sOB-R and leptin share an important combined endocrine effect in modulating the thyroid function in regularly exercising human subjects. Along with FLI the fasting serum triglycerides concentration also retained an independent association with the thyroid function in the stepwise regression analysis, recalling that signaling from metabolic surrogate markers of the nutritional status are also relevant in regulating the thyroid function in energy deprivation

and catabolic conditions such as intense exercise training programs.

An important limitation of the present work is the lack of the assessment of the fitness level in a quantitative fashion. The collection of VO_{2max} data, for example, would have allowed us to establish whether the adaptation of the TSH/FT₃ ratio and more importantly of FLI could be significantly related to the fitness level making our observation more robust. In addition a more detailed analysis of body fat distribution using techniques able to give insights also about topography of body fat could be useful to further clarify the relationships between the metabolic and endocrine changes in the athletes which were characterized in general terms by a lower body fat mass. In particular, the knowledge of the waist circumference as a surrogate marker of visceral adiposity could have been useful to establish whether the changes in leptin and sOB-R observed in the athletes were directly linked to the exercise habit of these individuals or whether they were secondary to the different and visceral adiposity. This suspect could be generated by the observation that in the stepwise multiple regression analysis also serum triglycerides concentration (as a potential marker reflecting the amount of visceral adiposity) fitted the model along with the FLI (Table 2).

These data represent the first report suggesting a potential role for the circulating bioavailable leptin,

rather than leptin *per se*, in exercise physiology. Additional studies are warranted to firmly establish not only a role for FLI as a surrogate biomarker of adaptation to exercise but also as a surrogate biomarker in other metabolic conditions associated with energy deprivation and excess, as well as insulin resistance and cardiovascular disease. For example the effects of the acute performance of physical exercise and also of the long-term exercise training program, not only in fit individuals but also in insulin resistant states, should be established.

In conclusion, this study demonstrated that exercise-induced parameters of thyroid function in male, elite professional athletes were independently associated with the FLI which is based on the fasting serum leptin and sOB-R concentrations, supporting the hypothesis that leptin bioavailability rather than the crude serum leptin concentration is involved in the adaptive response of thyroid function in professional athletes.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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