Circulating soluble transferrin receptor concentration decreases after exercise-induced improvement of insulin sensitivity in obese individuals

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Abstract

Background: Circulating soluble transferrin receptor (sTfR) has been recently found to be associated negatively with insulin sensitivity.

Objective: To evaluate circulating sTfR concentration after changing insulin sensitivity in obese individuals.

Design: Circulating sTfR concentration was evaluated after diet-induced weight loss in obese women (diet (D) group, n=8); after diet-induced weight loss plus resistance training (D+RT group, n=11); and after follow-up without weight loss (control (C) group, n=7).

Results: After 16 weeks, insulin sensitivity (HOMA (Homeostasis Model Assessment) value) significantly improved in parallel to weight loss (- 7.3%) and reduced total fat mass (evaluated using magnetic resonance imaging) in the D group. Thigh muscle mass decreased significantly (P=0.03). Serum sTfR concentration did not change significantly. In the D+RT group, weight loss (- 8.7%) and improvement of insulin sensitivity were of similar magnitude. Thigh muscle mass was preserved (P=0.8). Serum sTfR concentration decreased significantly (P=0.001). Interestingly, higher the thigh muscle

volume after weight loss, higher the decrease in circulating sTfR concentration. We also found that higher the increases in leg force at week 16, higher the decrease in circulating sTfR concentration in all individuals as a whole. No significant changes were observed in insulin sensitivity, sTfR concentration or thigh muscle mass in the C group.

Conclusion: These findings suggest a long-term regulation of serum sTfR concentration by exercise-induced improvement of insulin sensitivity in obese individuals.

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Introduction

Insulin is an anabolic hormone that stimulates the cellular uptake of many nutrients, including amino acids, cations and iron. Iron circulates in a form bound to transferrin and is taken up from the blood by a high-affinity specific transferrin receptor (TfR) (Finch, 1994; Davis, Corvera, & Czech, 1986; Yokomori et al., 1991). Insulin is known to cause a rapid and marked stimulation of iron uptake by different cell types, redistributing TfRs from an intracellular membrane compartment to the cell surface (Finch, 1994; Davis et al., 1986; Yokomori et al., 1991). Iron uptake by insulin parallels its effects on glucose transport (Tanner & Lienhard, 1989).

As a result of the externalization of TfR during the endocytic cycle, a soluble form of TfR (sTfR) can be detected in serum. Circulating sTfR concentrations are proportional to cellular expression of the membrane-associated TfR (Baynes & Cook, 1996). Serum sTfR concentration is closely related to cellular iron demands.

In an earlier paper, we provided evidence according to which serum sTfR concentration was linked to insulin resistance (Fernandez-Real et al., 2007). We observed that the lower the insulin sensitivity, the higher the sTfR concentration. Furthermore, insulin sensitivity, independently of age, obesity and iron status, contributed independently to the variance of circulating sTfR concentration (Fernandez-Real et al., 2007). As insulin injection increases serum sTfR concentration in animal models (Clairmont & Czech, 1990), hyperinsulinaemia may contribute to the inappropriately high sTfR concentration detected in individuals with altered glucose tolerance.

Serum sTfR concentrations seemed to be not acutely regulated by insulin, were given the unaltered serum sTfR levels during an oral glucose tolerance test (Fernandez-Real et al., 2007). Recently, resistance training (RT) has been shown to improve insulin sensitivity in individuals with type 2 diabetes (Ibanez et al., 2005). Taking into account these observations, 26 obese women were randomized to follow a structured RT program and a hypocaloric diet (n=11), compared with hypocaloric diet alone (n=8) and a group of individuals in whom no action was implemented (n=7). We aimed to

evaluate whether the improvement of insulin sensitivity affects serum sTfR concentration.

Subjects and methods

A total of 26 sedentary, non-smoking, obese (body mass index, 30-40 kg m^{-2}) women, aged 40-60 years, participated in this study. Before inclusion in the study, all candidates were thoroughly screened. None of the participants received any medication, and blood disorders, including anemia, were excluded in all participants. This project was approved by the ethical committee of the Regional Health Department. Participants were randomized to into three groups: a Control group (C; n=7); a diet group (D; n=8) with a caloric restriction of 500 kcal per day; and a diet and resistance training group (D+RT; n=11) with the same caloric restriction as group D and a 16-week supervised resistance training program of 2 sessions per week. During the 16 weeks of the study, the participants maintained their customary recreational physical activities (for example, walking). The baseline characteristics of the participants are presented in Table 1. Three women in the control group, three in the D group and four in the D+RT group were post-menopausal. In the remaining women, the participants were studied in the follicular phase (days 3-9 of the menstrual cycle).

Diet

Diet was designed in both D and D+RT groups to reduce 500 kcal per day, according to an earlier evaluation of the habitual physical activity of each participant by an accelerometer (TriTrac-R3D System, Software Version 2.04; Madison, WI, USA). This diet was designed to elicit a 0.5 kg weight loss per week. The C group was asked to maintain body weight. Throughout the 16-week intervention period, the body weight was recorded every 2 weeks in both D and D+RT groups. Each participant of the intervention groups participated in a series of 1-h seminars (every 2 weeks), wherein the dietician taught proper food selection and preparation, eating behavior, control of portion sizes, and modification of binge eating and other adverse habits. The average compliance with the diet classes and the exercise sessions was above 95%.

Resistance training program

The strength training program was a combination of heavy resistance and 'explosive' strength training. The participants were asked to report to the training facility twice per week for 16 weeks to perform dynamic resistance exercises, for 45-60 min per session. A minimum of 2 days elapsed between two consecutive training sessions. Each training session included two exercises for the leg extensor muscles (bilateral leg press and bilateral knee extension exercises), one exercise for the arm extensor muscle (the bench press) and four to five exercises for the main muscle groups of the body. Only resistance machines (Technogym, Gambettola, Italy) were used throughout the training period. In all the individual exercise sessions performed, one of the researchers was present to direct and assist each participant toward performing the appropriate work rates and loads. Lower and upper body maximal strength was assessed at weeks 0 and 16 by using 1-RM (repetition maximum) actions.

Magnetic resonance

The volumes of visceral and abdominal subcutaneous adipose tissue were measured by magnetic resonance. Magnetic resonance imaging was carried out with a 1T magnet (Magnetom Impact Expert, Siemens) using body coil. The participants were examined in a supine position with both arms positioned parallel along the lateral sides of the body. The procedure was carried out on the upper part of the body, followed by lower part acquisition after repositioning of the participant. We obtained a spoiled T1-weighted gradient-echo sequence with repetition time of 127 ms and echo time of 6 ms. Each half-body volume was scanned using two stacks, each containing 10 contiguous 10mm thick slices. Each stack was acquired in 20 s and interleaved slice order was used. An FOV (field of view) of 500 mm was used, and all the stacks were acquired with breath holding. Depending on the height of the person, this resulted in a total of 31-40 axial images per person. The total investigation time was about 5 min.

Magnetic resonance imaging of the both thighs was then obtained. T1-weighted sequence was used with a repetition time of 645 ms and a spin echo time of 20 ms. The field of view was 500 x 500 mm and the matrix was 512 x 192. The slices were 10 mm thick, with no gap between the slices. The thighs were scanned using two stacks,

each containing 15 contiguous 10 mm thick slices; the scan was performed axially from articular boundary of lowest external femoral condyle. The images were retrieved from the scanner according to a DICOM (Digital Imaging and Communications in Medicine) protocol. The acquired axial magnetic resonance images were transferred to an external personal computer running Windows XP. The level of each abdominal image was labeled using sagittal scout images, referred to as the distal level. We used a specially designed image analysis software (SliceOmatic 4.3, Tomovision Inc, Montreal, Canada) for quantitative analysis of the images.

The experimental design was approved, from an ethical and scientific standpoint, by the Hospital Ethics Committee and volunteers gave their informed consent to participate in the study.

Analytical determinations

In all studies, blood samples were collected after an overnight fast in the morning to avoid potential confounding influences due to hormonal rhythmicity. Total serum triglycerides were measured through the reaction of glycerol-phosphate oxidase and peroxidase. Intra-assay and interassay coefficients of variation were <4%.

Serum sTfR was measured using a double monoclonal sandwich enzyme immunoassay (ELISA) (Biovendor, Palacheho tr., Brno, Czech Republic). Intra- and inter-assay coefficients of variation were <4.5%.

Serum adiponectin levels were measured by a commercially available enzyme-linked immunoassay, ELISA kit (LINCO Research, Missouri, USA). The intra- and inter-assay coefficients of variation were <8%. The lowest level of adiponectin that can be detected by this assay is 0.78 ng ml^{-1}. No significant cross-reactivity with other cytokine or hormone molecules was detected.

Resting blood samples were drawn at weeks 0 and 16. The participants reported to the laboratory and sat quietly for 10-15 min before giving a blood sample. Basal glycemia was analyzed using an enzymatic hexokinase method (Roche Diagnostics, Mannheim, Germany). Serum insulin levels were measured in duplicates by monoclonal immunoradiometric assay (INSI-CTK Irma, DiaSorin, Madrid, Spain). Intra-assay and

inter-assay coefficients of variation were >5%. To estimate insulin resistance, the HOMA (Homeostasis Model Assessment) index was calculated as fasting insulin concentration (μU ml^{-1}) x fasting glucose concentration (mmol l^{-1})/22.5.

Statistical analysis

Descriptive results of continuous variables are expressed as mean (s.d.). Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test and then variables were given a base-10 log transformation, if necessary. These parameters (HOMA value, triglycerides, sTfR) were analyzed on a log scale and tested for significance on that scale. The anti-log-transformed values of the means (geometric mean) are reported in Table 1. The relation between variables was tested using Pearson's test (with log-transformed values) and multiple linear regression analysis. We used paired and unpaired t-tests and ANOVA (analysis of variance) test with Bonferroni's post hoc analysis for comparisons of quantitative variables. The statistical analyses were carried out using the program SPSS (v.12.0 for Windows; Chicago, IL, USA).

Table 1. Antropometrical and biochemical characteristics of study subjects

	Control			Diet			Diet + exercise			P^a
n	**7**			**8**			**11**			
Age	**51.5 ± 7.2**			**51.6 ± 6.6**			**47.7 ± 6.5**			0.36
	Pre	**Post**	***P***	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***	-
IBM (kg m^{-2})	34.58 ± 3.90	34.38 ± 3.55	**0.52**	34.11 ± 3.89	31.62 ± 3.92	**0.009**	34.27 ± 2.78	31.33 ± 2.00	**<0.0001**	0.96
Weight (kg)	88.68 ± 12.74	88.15 ± 11.74	**0.506**	87.22 ± 18.01	80.81 ± 16.64	**0.012**	88.08 ± 12.63	80.36 ± 9.11	**<0.0001**	0.98
WHR: Wais-to-hip-ratio	0.91 ± 0.01	0.89 ± 0.02	**0.26**	0.93 ± 0.02	0.91 ± 0.03	**0.08**	0.92 ± 0.03	0.88 ± 0.03	**0.026**	0.126
Thigh muscle volumen (cm^3)	46667 ± 7736	46465 ± 7167	**0.74**	48966 ± 10883	47226 ± 11609	**0.039**	46867 ± 9021	46470 ± 8839	**0.87**	0.87
Thigh fat volumen (cm^3)	88332 ± 19339	89127± 19090	**0.59**	87641 ± 21716	72697 ± 18365	**0.004**	104923 ± 16539	87479 ± 14200	**<0.0001**	0.11
Visceral adipose tissue (cm^3)	33708 ± 1228	3329 ± 1187	**0.65**	3243 ± 1085	2557 ± 1171	**0.007**	3211 ± 1232	2528 ± 1039	**0.0001**	0.96
Total adipose tissue (cm^3)	16833 ± 4183	16623 ± 3785	**0.53**	16973 ± 4787	13333 ± 4840	**0.001**	18205 ± 3337	13810 ± 2201	**<0.0001**	0.73
Arm force (1-RM, kg)	34.28 ± 6.56	34.81 ± 6.38	**0.20**	30.31 ± 5.25	29.21 ± 5.86	**0.175**	32.95 ± 6.96	43.63 ± 7.61	**<0.0001**	0.47
Leg force (1-RM, kg)	187.28 ± 30.47	188.71 ± 31.25	**0.17**	182.87 ± 39.80	205.37 ± 67.07	**0.09**	175.00 ± 33.68	274.54 ± 64.00	**<0.0001**	0.75
HOMA	4.00 ± 1.54	3.01 ± 1.50	**0.08**	3.93 ± 2.61	2.92 ± 2.20	**0.066**	3.19 ± 1.53	2.13 ± 1.07	**0.029**	0.605
Fasting Triglycerides (mg per 100 ml)	127 ± 31.4	117.4 ± 30.6	**0.15**	131.3 ± 44.3	140.2 ± 45.8	**0.38**	109.1 ± 29.7	99.4 ± 22.2	**0.26**	0.35
Adiponectin	13.1 ± 3.2	12.6 ± 2.1	**0.44**	12.3 ± 4.8	12.6 ± 4.2	**0.63**	14.1 ± 4.5	12.9 ± 3.5	**0.106**	0.66
Soluble transferrin receptor (ng ml^{-1})	1.56 ± 0.3	1.49 ± 0.3	**0.39**	1.56 ± 0.4	1.46 ± 0.3	**0.23**	1.51 ± 0.5	1.27 ± 0.4	**0.001**	0.72

Abbreviations: BMI, body mass index, HOMA, Homeostasis Model Assessment; 1-RM, one-repetition maximum. [a] ANOVA *P* for baseline characteristics among groups.

Results

Baseline characteristics were similar in the three groups (ANOVA *P*, Table 1). Circulating sTfR concentration correlated positively with HOMA value in all participants as a whole (r = 0.36, *P* = 0.04).

Diet led to weight loss (- 7.3%) and reduced total fat mass, and thigh muscle and adipose mass in the D group. Insulin sensitivity (HOMA value) tended to improve. Serum sTfR concentration did not change significantly (Figure 1). In the D+RT group, weight loss (- 8.7%) and improvement of insulin sensitivity were of similar magnitude. In contrast to the D group, thigh muscle mass was preserved, and leg strength and force increased significantly (Figure 2). In this D+RT group, serum sTfR concentration decreased significantly (Figure 1).

No significant changes were observed in the different parameters evaluated (Table 1) in the C group, although HOMA value tended to decrease, possibly in the context of observation.

Interestingly, higher the thigh muscle volume, higher the decrease in circulating sTfR (Figure 2). Similarly, higher the change in leg force at weeks 8 and 16 (Figure 2) and higher the absolute value of arm force (panel d, Figure 2), higher the decrease in circulating sTfR in all participants as a whole. There was no significant relationship between sTfR concentration and adiponectin or adiponectin changes.

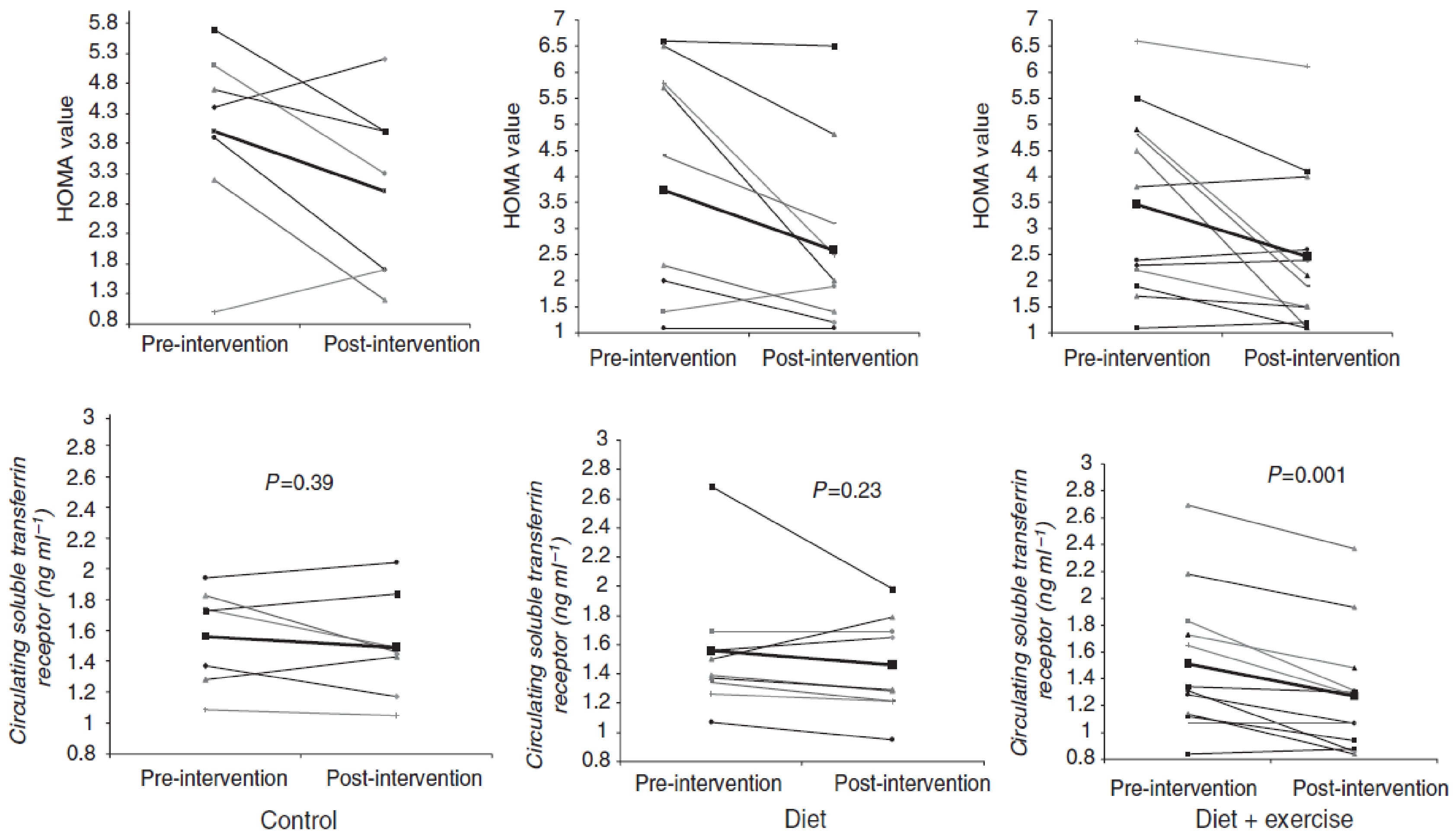

Figure 1. Homeostasis Model Assessment (HOMA) value (upper panels) and serum soluble transferrin receptor (sTfR) concentration (lower panels) before and after each intervention period.

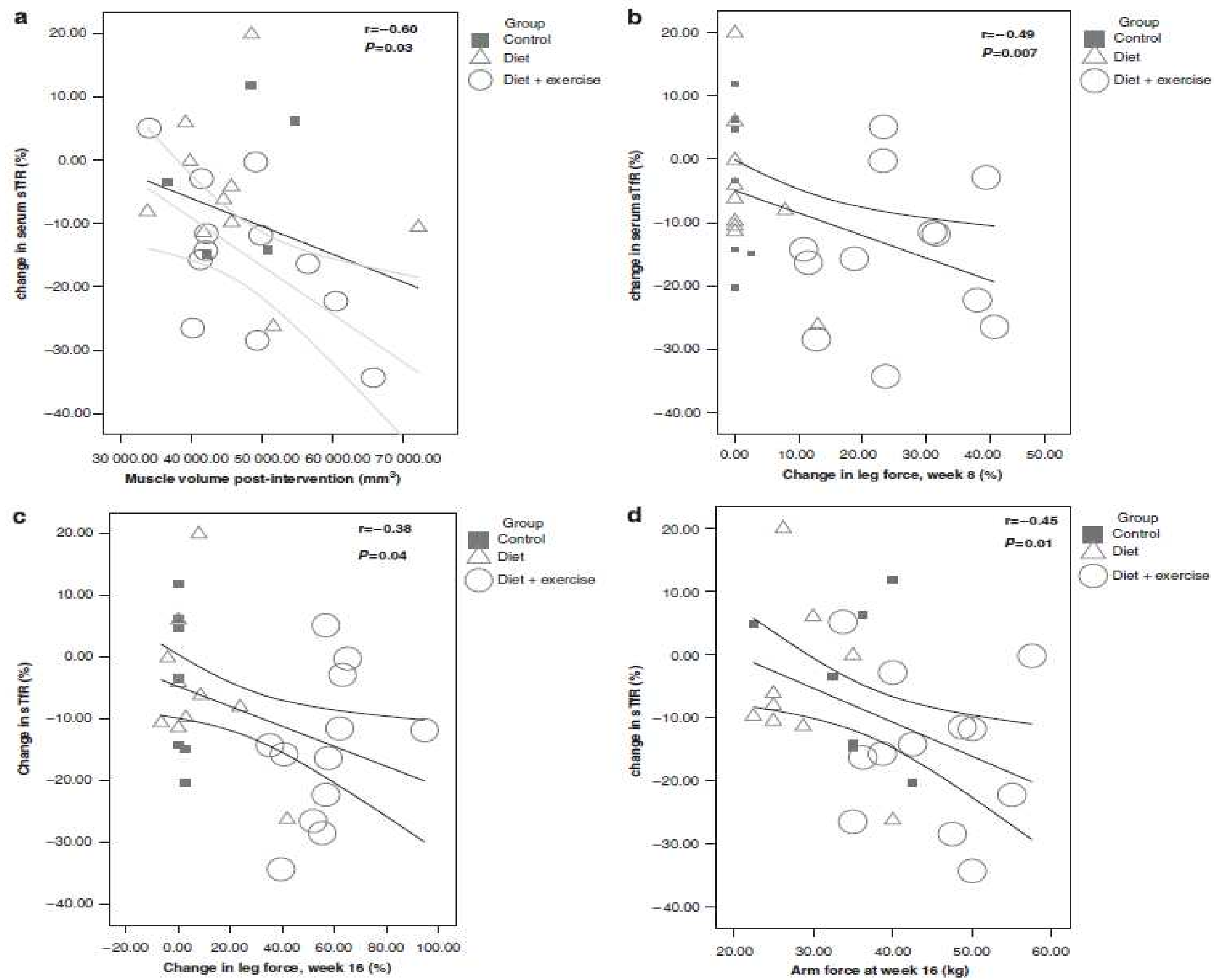

Figure 2. Changes in circulating soluble transferrin receptor (sTfR) according to thigh muscle volume (a), the change in leg force at weeks 8 and 16 (b and c), and the absolute value of arm force (d).

Discussion

The main unprecedented findings in this manuscript are: (1) circulating sTfR decreased significantly after improvement of insulin sensitivity in middle-aged women; (2) this decrease was observed only in the D+RT group despite similar weight loss and improvement of insulin sensitivity than in the D group; (3) the decrease of sTfR was correlated with muscle volume, and leg and arm force (Figure 2). In this sense, the preservation of the muscle volume in the D+RT group compared with decreased muscle volume in the D group was remarkable. The leg and arm force improved only in the D+RT group in which sTfR decreased significantly. Figure 1 discloses the relatively stable values of sTfR in the control and the D groups (except for one participant in this latter group), and the uniform decrease of sTfR in almost all participants in the D+RT group.

The reason for these disparate effects of improved insulin sensitivity induced by diet vs diet plus exercise on circulating sTfR concentrations is currently unknown. However, the maximal effects of insulin and muscular contraction on glucose transport are known to be additive in mammalian skeletal muscle, strongly suggesting that these stimuli act through separate pathways. Contracting myofibers need to obtain glucose very rapidly to cope with the energy demands that increase dramatically when contraction is initiated.

Coderre *et al.* (1995) reported the isolation of distinct insulin- and exercise-sensitive GLUT4 intracellular pools.

Interestingly, the transferrin receptor defines two distinct contraction and insulin-responsive GLUT4 vesicle populations in *in vitro* studies on skeletal muscles (Lemieux, Han, Dombrowski, Bonen, & Marette, 2000). Insulin did not stimulate transferrin receptor recruitment from the GLUT4-containing intracellular fraction to the plasma membrane in skeletal muscle. In contrast, muscular contraction stimulated the recruitment of the transferrin receptor from the same GLUT4-containing intracellular fraction to the plasma membrane (Lemieux et al., 2000). The sTfR is a soluble truncated monomer of tissue receptor, lacking its first 100 amino acids, which circulates in the form of a complex of transferrin and its receptor.

The sTfR is produced by proteolysis, mediated by a membrane-associated serine protease that occurs mostly at the surface of exosomes within the multivesicular intracellular body before exocytosis. The bulk of sTfR measured in serum is proportional to the mass of cellular TfR (Baynes & Cook, 1996). It is thus possible that the exercise-induced stimulation of TfR translocation and recycling endosomes fulfill the dual functions of providing both glucose and iron to contracting myofibers (Lemieux et al., 2000), leading to decreasing serum sTfR concentration in this context. Insulin (improved insulin action by diet alone) failed to induce TfR translocation or caused only a marginal redistribution of the receptor in skeletal muscle (Lemieux et al., 2000).

The activation of TfR recycling in contracted muscle may be important to maintain the levels and activities of iron containing proteins involved in the respiratory capacity of muscle mitochondria. In fact, skeletal muscle represents about 40% of body mass and contains 10-15% of body iron, which is mainly located in myoglobin. Skeletal muscle plays a functional role in oxygen storage, transport and use, and iron is a key component of myoglobin and heme groups of cytochromes.

We are not aware of any study evaluating sTfR in parallel to insulin sensitivity. The concentration of sTfR increased immediately after exercise, as found in several studies (Schumacher, Schmid, Konig, & Berg, 2002; Rocker et al., 2002; Nikolaidis, Michailidis, & Mougios, 2003; Deruisseau et al., 2004; Malczewska, Stupnicki, Blach, & Turek-Lepa, 2004; Robach et al., 2006; Duca et al., 2006; Di, Stel, Banfi, Gonano, & Cauci, 2008). The study of the long-term concentration of serum sTfR was carried out in a group of elite rugby players during a competitive season (Banfi, Del, Mauri, Corsi, & Melegati, 2006). The sTfR concentration increased during the competition period and decreased at the end of the season.

We did not find changes in circulating adiponectin. Plasma adiponectin has been measured in response to acute exercise bouts as well as moderate to long-term training programs in a variety of populations, as recently reviewed (Berggren, Hulver, & Houmard, 2005). Neither an acute bout of stationary cycling nor a 3-week exercise intervention in patients with type 2 diabetes, nor a 6-m exercise intervention led to significant changes in plasma adiponectin (Berggren et al., 2005).

There is a considerable body of evidence gathered from studies over the past half a century indicating that regular physical activity reduces the risk of cardiovascular disease (Gill & Malkova, 2006). Regular physical activity is particularly beneficial in individuals with insulin-resistant conditions, such as obesity, type 2 diabetes and the metabolic syndrome (Holloszy, 2005). Although the post-exercise increase in muscle insulin sensitivity has been characterized in considerable detail, the basic mechanisms underlying this phenomenon remain a mystery (Holloszy, 2005; Cartee & Holloszy, 1990).

In summary, our findings hint at a long-term regulation of serum sTfR concentration by insulin sensitivity. These findings need to be considered when evaluating iron status in obesity (Lecube et al., 2006; Lecube, Hernandez, Pelegri, & Simo, 2008).

Conflict of interest

The authors declare no conflict of interest.

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