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Soluble transferrin receptor and risk of type 2 diabetes in the obese and non-obese

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Abstract

Background: Studies evaluating the relationship between soluble transferrin receptor (sTfR), a biomarker inversely related to body iron stores, and risk of type 2 diabetes mellitus (T2DM) are scarce and inconclusive. Furthermore, sTfR concentrations have been observed to be significantly higher in obese than in non-obese individuals. Therefore, the aim of the present study was to assess the relationship between sTfR and the risk of T2DM in obese and non-obese subjects.

Design: A nested case-control study of 153 cases of newly diagnosed diabetic subjects, 73 obese and 80 non-obese, and 306 individually matched-controls, 138 obese and 166 non-obese, who did not develop T2DM for a median 6-year follow-up (interquartile range: 3.9-6.5) was conducted using data from the PREDIMED (PREvention with MEDiterranean Diet) cohort (http://www.controlled-trials.com/ISRCTN35739639). Cases and controls were matched for age (≤67 vs. >67years), gender, dietary intervention group, and BMI (≤27 vs. >27kg/m^2).

Results: Waist circumference is the main determinant of sTfR concentrations in the whole sample (β=0.476, P<0.001), in the obese (β=0.802, P<0.001) and the non-obese (β=0.455, P=0.003). Furthermore, sTfR is directly associated with the risk of T2DM in obese individuals (OR=2.79; 95% CI:1.35-5.77, P=0.005) and inversely associated in non-obese individuals (OR=0.40; 95% CI:0.20-0.79, P=0.015).

Conclusions: The association between sTfR levels and risk of T2DM in a population at high cardiovascular risk depend on the presence or absence of obesity. While in non-obese subjects elevated sTfR levels are associated with a decreased risk of developing T2DM, in obese subjects the risk increases. This suggests that obesity alters the relationship between sTfR and T2DM incidence.

Clinical trial registration number: ISRCTN35739639

Keywords: Soluble transferrin receptor, body iron stores, type 2 diabetes mellitus, obesity, nested case-control, PREDIMED.

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic diseases worldwide. In 2015 it was estimated that 415 million people suffered from diabetes, and if the recent increases continue, by 2040 some 642 million people will have diabetes [1].

Obesity [2], inflammation [3], diet [4], age and family history of diabetes [1] are known risk factors for T2DM. Increasing evidence suggests that high body iron stores (B-IronS) are associated with an increased risk of T2DM. This relationship has been found especially with ferritin [5–14], but not with soluble transferrin receptor (sTfR). sTfR represents a significant advanced in the evaluation of body iron status, since its measurements are unaffected by the acute-phase response, such as ferritin, [15] and sTfR levels have been reported to have low biological variability [16]. sTfR is the truncated form of the transferrin receptor, which is responsible for the cellular uptake of iron. Its expression is determined by the cellular demand for iron [17]. Interestingly, low B-IronS causes overexpression of transferrin receptor

and sTfR levels, while high B-IronS results in decreased sTfR levels, i.e. it is inversely related to B-IronS.

Studies evaluating the relationship between sTfR and risk of T2DM are scarce and inconclusive. In a general German population from the EPIC-Potsdam [9] and Cooperative Health Research in the Region of Augsburg (KORA) [14] cohorts, and in Spanish population at high cardiovascular risk from the PREDIMED cohort [13], serum ferritin, but not sTfR, was associated with a higher risk of T2DM. In a sample of obese participants from the Diabetes Prevention Program (DPP) cohort [7] an association was also found between elevated serum ferritin levels and an increased risk of T2DM. However, far from confirming the results of serum ferritin, they observed that high levels of sTfR (indicative of low B-IronS) were directly related to the risk of T2DM [7]. The authors of this study hypothesised that the sTfR was a biomarker of some other factor, unrelated to iron metabolism, that was causally related to T2DM [7]. Curiously, a recognised risk factor for T2DM such as obesity [2], seems to influence sTfR homeostasis. Several studies have observed a significantly higher levels of sTfR in obese children [18], adolescents [19], adults [20], pre [21] and postmenopausal women [22] and even in obese men with hyperferritinaemia [23] than in the respective non-obese groups.

Given the discrepancy in the findings on the association between sTfR and the risk of T2DM in the obese and the general population, coupled with the increased levels of sTfR observed in the obese, the objective of the present study was to assess the associations between sTfR plasma concentrations and the incidence of T2DM in obese and non-obese subjects.

Material and methods

Study design

The present nested case-control study was conducted within the framework of the PREvention with MEDiterranean Diet (PREDIMED) trial, described in detail elsewhere [24]

(www.predimed.es) (controlled-trials.com:ISRCTN35739639). The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent, and the protocol was approved by the institutional review boards of the participating centres. The present study was conducted in accordance with the STROBE statement along with references to STROBE statement [25] and the broader EQUATOR guidelines [26].

Subjects

For the present study we used data from 1378 Caucasian individuals who were non-diabetic at baseline, recruited from three Spanish centres (Reus-Tarragona, Pamplona and Barcelona-Clinic). An additional sample of blood obtained exclusively in these three centres were used to carry out the study. Eligible participants were men and women aged 55-80 initially free of CVD but with at least three of the following cardiovascular risk factors: current smoking, hypertension, dyslipidaemia, overweight/obesity, or family history of early-onset coronary disease. All participants who self-reported baseline-prevalent T2DM, because of a previous diagnosis or treatment with antidiabetic agents, were also excluded from the present analysis. We selected all the 153 individuals who developed T2DM during a median follow-up of 6.0 years (interquartile range: 3.9-6.5), of whom 73 were obese (BMI≥30 kg/m^2) and 80 non-obese (BMI<30 kg/m^2). In order to increase the statistical power of the study, for every incident diabetic, two controls were matched for gender, dietary intervention group (MeDiet+virgin olive oil, MeDiet+nuts or control group), age (≤67 or >67 years) and BMI (≤27 or >27 kg/m2), taking the respective median as the cut-off. The result was 306 controls, 139 obese subjects and 167 non-obese subjects. The effective sample size was 459 participants.

Ascertainment of T2DM

Whenever cases of new-onset T2DM were identified during follow-up in accordance with the criteria of the American Diabetes Association (ADA) [27], reports were sent to the

PREDIMED Clinical Events Committee, whose members were blinded to treatment allocation. Only when a second test had been conducted with the same criteria and repeated within the following three months was a new case of diabetes definitively confirmed by the adjudication committee.

Biochemical determinations

Blood samples were collected after an overnight fast. The aliquots of serum and EDTA plasma were immediately processed, coded, and shipped to a central laboratory in a portable cooler (4ºC), and stored at -80ºC until analysis. The time between blood sampling and freezing was less than one hour. Fasting glucose, total triglycerides, and total and HDL-cholesterol were measured by standard enzymatic methods. LDL-cholesterol was calculated using the Friedewald equation. Fasting insulin was measured in duplicate by ELISA (Ezhi-14K, Millipore, St. Charles, USA). The assay has a sensitivity of 1 µU/mL, and an intra- and inter-assay CV of analysis of ≤ 5.9% and < 10.3%, respectively. The homeostasis model assessment (HOMA) index was calculated for each individual. High-sensitivity C-reactive protein (hs-CRP) was measured using a highly sensitive immunoassay (Helica Biosystems Inc., Santa Ana, CA, USA). The assay has a sensitivity of 0.2 µg/L, and an intra- and inter-assay CV of analysis of ≤3.7% and <4.8%, respectively. Serum ferritin (Ferritin Elecsys, Roche diagnostics, Mannheim, Germany) and sTfR (Access sTfR 0QC, Beckman Coulter, USA) were measured by immunochemiluminescence. The assay has a sensitivity of 0.05 µg/L for ferritin, and 0.05 nmol/L for sTfR. The intra- and inter-assay CV of analysis was ≤2% and <3.5%, respectively, for ferritin, and ≤5% and ≤8%, respectively, for sTfR.

Other measures

At baseline, a general questionnaire on sociodemographics and lifestyle characteristics was administered, and anthropometric variables were measured. A previously validated semiquantitative 137-item food frequency questionnaire [28] was also applied. Nutrients and

energy intake were quantified according to the Spanish food composition tables [29]. Leisure-time physical activity was assessed with a validated questionnaire [30]. Blood pressure was measured in triplicate using a calibrated semiautomatic oscillometer (Omron HEM-705CP, Hoofddorp, Netherlands) [31].

Statistical methods

Variables with a non-normal distribution were log-transformed to normalize the distributions. Qualitative and quantitative variables were compared using the χ^2 test or Student t-test, respectively.

A multiple linear stepwise regression analysis was applied to evaluate the influence on sTfR concentrations of such factors as age, gender, educational level, civil status, smoking, physical activity, intervention group, BMI or waist circumference, total energy intake, meat and processed meat, dairy products, alcoholic beverages, coffee, monounsaturated and polyunsaturated fatty acids, magnesium, total cholesterol, hs-CRP, serum ferritin, fasting insulin and glucose.

To analyse the relationship between sTfR and risk of T2DM in obese and non-obese subjects several logistic regression models were applied. An unadjusted model was fitted with gender-adjusted tertiles of sTfR as independent variable. The model was re-fitted with adjustment for sociodemographics and lifestyle variables which included age (years), education level (primary/secondary/tertiary), marital status (married/unmarried/divorced-widower), smoking (smoker/non-smoker), physical activity (<200 metabolic equivalents (METs)-minutes per day/≥200METs-minutes per day), family history of diabetes (yes/no) and total cholesterol (mg/dL). Adjustments were also made for confounding dietary variables related to the risk of T2DM [4] such as total energy intake (kcal/day), red meat (g/day), dairy products (g/day), coffee (mL/day), alcoholic beverages (cc/day), saturated, monounsaturated and polyunsaturated fatty acids (g/day), magnesium (mg/day), as well as hs-CRP (mg/L) and

waist circumference (cm). Subsequently, fasting insulin (μU/mL) and glucose (mg/dL) were introduced. The linear trend across tertiles was tested by assigning the median value to each category and entering these new variables in the logistic regression model as continuous variables.

All data analyses were conducted with the SPSS 22.0 for Windows package. A value of $p<0.05$ was considered statistically significant.

Results

Of the 459 subjects, two individuals for whom sTfR values were unavailable were removed from the study, leaving 457 in the final analyses. There was no significant interaction between sTfR and gender ($P>0.05$) in the final logistic model, but interaction ($P<0.05$) was observed between sTfR and obesity (as a dichotomic variable), which justified analysing obese and non-obese subjects separately.

Participants were adults, most of whom were married and had a primary level of education (table 1). Two of them were supplemented with iron. In the non-obese group, incident diabetics had higher levels of fasting glucose and serum ferritin, increased insulin resistance and waist circumference and a greater family history of diabetes than non-incident diabetics. In the obese group, incident diabetics also had significantly higher fasting glucose, insulin resistance and waist circumference, which are risk factors for T2DM. However, there were no differences in iron status. As expected, we also observed that obese subjects had higher levels of hs-CRP than non-obese subjects.

Positive correlations (Figure 1) were observed between sTfR concentrations and waist circumference ($r=0.143$, $P=0.002$) or BMI ($r=0.113$, $P=0.016$) in the whole sample, and in the obese group ($r=0.210$, $P=0.002$) ($r=0.169$, $p=0.015$), respectively, but not in the non-obese group. A correlation between sTfR and hs-CRP ($r=0.098$, $P=0.038$) was also found in the whole sample.

By using multiple linear stepwise regressions, we found that serum ferritin, waist circumference and the consumption of coffee and alcoholic beverages were major determinants of sTfR concentrations (table 2). The association between waist circumference and sTfR was stronger in obese subjects (β=0.802, P<0.001) than non-obese subjects (β=0.455, P=0.003). When BMI was added to the regression instead of waist circumference, only serum ferritin and BMI significantly predicted sTfR in obese subjects (β=0.030, P=0.017) but not in non-obese subjects.

In the crude logistic regression model (table 3), when the highest tertile was compared with the lowest tertile, the OR for the association of sTfR with the risk of T2DM was 0.61 (95% CI: 0.36-1.05, P=0.081) in non-obese subjects, and 1.36 (95% CI: 0.78-2.38, P=0.238) in obese subjects. After adjustment for sociodemographic, lifestyle, anthropometric, dietary and biochemistry variables of the risk of T2DM, the trend improved in non-obese subjects (OR=0.39, 95% CI:0.21-0.74. P=0.005) and in obese subjects (OR=2.15, 95% CI: 1.11-4.17, P=0.020). When family history of diabetes and fasting insulin were added to the model the relationship in both groups did not alter substantially. Finally, after adjusting for fasting glucose, obese subjects presented an OR of 2.79 (95% CI: 1.35-5.77, P=0.005), while non-obese subjects had an OR of 0.40 (95% CI: 0.20-0.79, P=0.015). The same final logistic regression model was applied to assess the relationship between serum ferritin and the sTfR:ferritin ratio, categorised according to gender-adjusted tertiles, and the risk of T2DM. The OR for ferritin and the risk of T2DM was 2.39 (95% CI: 1.20-4.75, P= 0.014) in non-obese subjects and 0.65 (95% CI: 0.33-1.31, P=0.386) in obese subjects. Meanwhile, the OR for the sTfR:ferritin ratio and the risk of T2DM was 0.43 (95% CI: 0.21-0.87, P= 0.026) in non-obese subjects and 1.51 (95% CI: 0.75-3.05, P= 0.444) in obese subjects.

Discussion

In this nested case-control study conducted in an adult Mediterranean population at high cardiovascular risk, we observed that the nature of the association between sTfR and risk of T2DM depends on the presence or absence of obesity. While in the non-obese group, high sTfR levels were associated with a decreased risk of T2DM, in the obese group the risk increased.

Among the strengths, the longitudinal design increases the quality of scientific evidence provided by other designs. The control of potential confounding factors was improved by matching case of incident T2DM for age, gender, BMI and intervention group with two non-incident diabetics. Moreover, sTfR was determined by immunochemoluminescence, a highly sensitive and specific method, T2DM diagnosed using the ADA criteria [27], and the median period of follow-up of 6.0 years (interquartile range: 3.9 to 6.5) was longer than most previous studies that analysed the relationship between sTfR and the risk of T2DM [7,9].

Among the weaknesses, the study population was adult with several cardiovascular risk factors, and it is not clear whether the results can be extrapolated to the general population or other populations. Despite the lengthy follow-up period, up to eight years, some control subjects may have presented a pre-diabetic state that could have influenced the relationship between iron excess and risk of T2DM. This hypothetical situation would not detract from the results, since the differences between cases and controls would be mitigated.

The transferrin receptor is involved in the process of cellular uptake of iron, so its expression is determined by the cellular demand for iron. Its truncated form, sTfR, is located in serum in proportion to the transmembrane protein and, therefore, to cell iron demands [17]. This is the reason why sTfR is believed to be a good biomarker of iron deficiency [15].

When we use this biomarker to assess the relationship between iron status and risk of T2DM in non-obese subjects, we observed that high sTfR levels, and high sTfR:ferritin ratios, which

are indicative of low B-IronS, are also associated with a lower risk of this metabolic disease. Our result is consistent with previous studies that found a relationship between low B-IronS, measured as sTfR:ferritin ratio, and risk of T2DM [9,13,14]. In this regard, it has been observed that blood donors [32] and subjects undergoing phlebotomy [33] are more likely to prevent T2DM because of the corresponding decrease in B-IronS. It is speculated that oxidative stress [34,35] is the mechanism by which excess iron is associated with a higher incidence of T2DM. On the contrary, reduced B-IronS may entail a lower degree of oxidative stress, which in turn may decrease risk of T2DM.

We also observed that elevated sTfR levels are associated with a higher risk of T2DM in obese subjects. This finding is consistent with a previous study conducted by Rajpathak et al. in a sample of obese subjects from the USA DPP cohort [7]. Rajpathak et al. suggested that sTfR was affected by some other factor that was causally related to the incidence of T2DM. This factor may be obesity, a known risk factor for T2DM [2]. Other studies in a general German population from the EPIC-Potsdam [9] and KORA [14] cohorts, and in a Spanish population at high cardiovascular risk from the PREDIMED cohort [13] found no association between sTfR and risk of T2DM. The lack of relationship may be caused by the presence of both obese and non-obese subjects in all these three samples [9,13,14]. This would conceal the opposite association between sTfR and T2DM in obese and non-obese subjects that we observed.

Conversely, the Finnish KIHD cohort [10] of middle-aged men found that both high and low sTfR concentrations were associated with an increased risk of developing T2DM, while medium values were associated with a decreased risk. However, no significant differences in sTfR levels in each quintile of serum ferritin were observed, and they even seemed to increase as serum ferritin values increased, even though the two biomarkers are known to behave differently. These data suggest that, in addition to the iron status of the individuals,

some other factor may have influenced the levels of sTfR and, therefore, the association found between sTfR and T2DM.

The associations observed between sTfR and the risk of T2DM in both obese and non-obese subjects did not alter substantially after adjusting for family history of diabetes and glucose metabolism variables such as fasting insulin and glucose (table 3). However, these relationships did become significant after adjustment for waist circumference, as well as other variables. When we evaluated the effect of obesity on the concentration of sTfR (table 2) we found that waist circumference and/or BMI increased the sTfR levels in the whole sample, in both obese and non-obese subjects, as had also been noted by Lecube et al. in postmenopausal women [22]. These results suggest that obesity has an effect on sTfR homeostasis that would explain the results in obese subjects found in the DPP cohort [7] and in our study. We also observed that B-IronS, estimated by ferritin, and alcohol and coffee consumption significantly decreased sTfR levels. Alcohol consumption has been associated with higher body iron stores [36]. Elevated alcohol consumption and body iron stores may reduce cellular iron demands and, therefore, sTfR levels. Meanwhile, coffee consumption has been related to an inhibition of the synthesis of sTfR [37], which is consistent with our findings.

The mechanism that might mediate the relationship between sTfR and the risk of T2DM in obese subjects is not well-established. There is strong evidence that obesity, a chronic low-grade inflammatory condition, increases sTfR concentrations [18–23]. It has also been noted that obesity-induced inflammation increases hepcidin synthesis [18,19,21]. Hepcidin is a key hormone in iron homeostasis. It blocks iron release from enterocytes and the reticuloendothelial system by degrading the iron exporter ferroportin [20,38,39]. In this context, there may be a functional iron deficiency, defined as the lack of iron in the tissues even though the level of B-IronS is correct [39]. The expression of cellular transferrin

receptors may increase because of this functional iron deficiency secondary to obesity, which is a main risk factor for T2DM [2], but not necessarily to a real iron deficiency. Hence, high sTfR levels may reflect of a chronic pathological state such as obesity, and, therefore, they may be related to a greater risk of developing T2DM.

To conclude, in a Mediterranean population at high cardiovascular risk, there were opposite associations between sTfR levels and risk of T2DM in obese and non-obese subjects were quite different. In non-obese subjects, high sTfR levels, indicative of low B-IronS, are associated with a decreased risk of T2DM. Conversely, in obese subjects, high sTfR concentrations are associated with an increased risk of T2DM.

Our results suggest that obesity may alter the relationship between sTfR and T2DM, so sTfR is an unreliable biomarker for assessing iron status in an obese population. Further studies are warranted to confirm these results.

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Disclosure statement

Authors have no conflict of interest affecting the conduct or the reporting of the work submitted.

Author contributions

JCF performed the literature search, performed the statistical analyses and interpretation of the results and drafted the manuscript. VA was responsible for designing the study, directing and performing the statistical analyses, interpreting the results, and drafting the manuscript. NA coordinated the biochemical analyses, contributed to the interpretation of the results, and revised the manuscript. JB conceived and participated in the design of the PREDIMED study, coordinated the fieldwork and revised the manuscript. JDE participated in the design of the PREDIMED study, participated in the fieldwork, and revised the manuscript. MAG and RE conceived and participated in the design of the PREDIMED study, coordinated the fieldwork and revised the manuscript. MF and DC participated in the design of the study and revised the manuscript. JS conceived and participated in the design of the PREDIMED study and the present work, coordinated the fieldwork, participated in the interpretation of the results, and revised the manuscript.

All authors read and approved the final manuscript.

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Table 1. General characteristics of incident and non-incident diabetics participants in both obese and non-obese subjects.

Baseline Variable	NON-OBESE n=247		*P*-value	OBESE n=212		*P*-value
	Non-incident T2DM	Incident T2DM		Non-incident T2DM	Incident T2DM	
	n = 167	n = 80		n = 139	n = 73	
Sociodemographic data						
Gender (% men)[c]	52.7	51.2	0.832	40.3	42.5	0.759
Age (years) [b]	66.0 ± 6.2	66.1 ± 5.8	0.952	66.7 ± 5.9	66.6 ± 6.0	0.970
Education level (% primary) [c]	60.5	65.0	0.351	81.3	74.0	0.303
Civil status (% married) [c]	77.8	75.0	0.868	80.6	78.1	0.800
Family history of T2DM (%) [c]	20.4	33.8	0.022	29.5	38.4	0.191
Centre (% Pamplona) [c]	62.3	46.2	<0.001	50.4	37.0	<0.001
Anthropometry						
Weight (kg) [b]	72.1 ± 9.4	73.0 ± 9.1	0.469	82.5 ± 9.8	84.5 ± 10.3	0.164
BMI (kg/m^2) [b]	27.6 ± 1.7	27.8 ± 1.8	0.443	32.5 ± 1.8	32.9 ± 2.0	0.127
Waist circumference (cm) [d]	93.7 ± 1.1	96.9 ± 1.1	0.010	103.5 ± 1.1	106.3 ± 1.1	0.020
Lifestyle						
Smoking (% smoker) [c]	23.4	33.8	0.084	18.0	17.8	0.974
Physical activity (% Sedentary) [c]	43.7	36.2	0.265	48.2	46.6	0.822
Diet						
Dairy products (g/day) [b]	335.4 ± 209.8	323.7 ± 212.6	0.685	390.3 ± 213.2	387.3 ± 248.0	0.926
Meat and processed meat (g/day) [b]	132.7 ± 50.3	135.8 ± 47.9	0.641	142.3 ± 52.8	139.3 ± 51.4	0.686
Red meat (g/day) [b]	45.6 ± 26.1	45.7 ± 27.3	0.985	49.2 ± 28.6	50.3 ± 30.0	0.805
Fish (g/day) [b]	63.4 ± 33.7	69.8 ± 32.2	0.153	69.7 ± 35.1	67.4 ± 31.5	0.649
Eggs (g/day) [b]	20.7 ± 11.4	19.5 ± 11.3	0.433	20.8 ± 10.4	19.5 ± 11.0	0.396
Legumes (g/day) [b]	18.0 ± 7.8	17.0 ± 8.4	0.349	17.7 ± 6.8	17.1 ± 6.6	0.569
Vegetables (g/day) [b]	281.8 ± 105.4	291.8 ± 119.6	0.502	279.9 ± 102.6	292.0 ± 122.4	0.472
Fruits (g/day) [b]	351.1 ± 179.2	329.2 ± 184.6	0.374	336.4 ± 187.3	358.6 ± 191.6	0.832
Grains and starches (g/day) [b]	277.9 ± 104.7	277.8 ± 111.0	0.997	263.7 ± 103.5	267.1 ± 118.7	0.540
Oils and fats (g/day) [b]	60.0 ± 23.3	60.9 ± 26.3	0.789	57.3 ± 23.3	59.3 ± 23.4	0.182
SFA (mg/day) [b]	26.8 ± 9.6	26.7 ± 9.2	0.941	26.3 ± 8.2	28.0 ± 9.0	0.149
MUFA (mg/day) [b]	53.8 ± 14.5	53.0 ± 15.2	0.689	52.3 ± 14.4	53.3 ± 15.3	0.637
PUFA (mg/day) [b]	16.5 ± 6.1	17.8 ± 7.3	0.159	15.3 ± 8.8	16.0 ± 5.8	0.437
Coffee (cc/day) [b]	35.2 ± 51.6	25.2 ± 44.2	0.117	28.0 ± 42.6	20.6 ± 35.2	0.099
Alcoholic beverages (cc/day) [b]	154.6 ± 208.2	188.3 ± 229.8	0.250	113.3 ± 182.8	193.0 ± 386.2	0.362
Energy (Kcal/day) [b]	2409.4 ± 568.0	2391.5 ± 624.5	0.822	2308.1 ± 549.7	2385.5 ± 650.5	0.362
Calcium (mg/day) [b]	957.9 ± 347.9	940.9 ± 333.8	0.718	999.2 ± 320.7	1053.7 ± 409.3	0.325
Magnesium (mg/day) [b]	364.0 ± 76.8	364.6 ± 92.6	0.954	358.0 ± 81.5	369.2 ± 92.2	0.386
Iron (mg/day) [b]	16.3 ± 3.5	16.5 ± 4.2	0.665	15.7 ± 3.5	16.2 ± 4.1	0.432
Biochemestry						
Total Cholesterol (mmol/L) [b]	5.8 ± 1.0	5.7 ± 0.9	0.248	5.8 ± 0.9	5.7 ± 1.2	0.664
LDL-Cholesterol (mmol/L) [d]	3.5 ± 0.03	3.5 ± 0.03	0.660	3.5 ± 0.03	3.4 ± 0.03	0.110
HDL-Cholesterol (mmol/L) [d]	1.4 ± 0.03	1.4 ± 0.03	0.296	1.4 ± 0.03	1.3 ± 0.03	0.036
Triglycerides (mmol/L) [d]	1.4 ± 0.02	1.4 ± 0.02	0.742	1.4 ± 0.02	1.6 ± 0.02	0.005
Fasting insulin (pmol/L) [d]	28.5 ± 12.3	31.3 ± 11.8	0.264	34.0 ± 12.5	41.0 ± 13.2	0.034
HOMA-IR [d]	0.9 ± 1.8	1.2 ± 1.8	0.002	1.2 ± 1.8	1.7 ± 1.9	<0,001
Fasting Glucose (mmol/L) [d]	5.1 ± 0.06	6.0 ± 0.07	<0.001	5.3 ± 0.07	6.3 ± 0.07	<0.001
sTfR (mg/L) [d]	3.7 ± 1.4	3.5 ± 1.4	0.269	3.6 ± 1.4	3.9 ± 1.5	0.190
Ferritin (µg/L) [d]	98.2 ± 2.7	131.2 ± 2.5	0.028	114.4 ± 2.3	123.8 ± 2.6	0.542
sTfR:ferritin ratio [d]	12.9 ± 3.0	9.3 ± 2.7	0.024	11.0 ± 2.6	10.6 ± 2.9	0.785
hs-CRP (mg/L) [d]	1.1 ± 3.9	1.2 ± 4.2	0.519	1.7 ± 4.2	1.6 ± 4.2	0.583

[a] Education level (primary/secondary/tertiary), civil status (married/unmarried/divorced), center (Barcelona, Pamplona, Reus-Tarragona), smoking (smoker/not smoker), physical activity (200METs•min/day/≥200METs•min/day), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), HOMA-IR (Homeostasis assessment model for insulin resistance), sTfR (soluble transferrin receptor, 1mg/L=13.55nmol/L), hs-CRP (high-sensitivity C-reactive protein).

[b] Mean ± SD; [c] %; [d] geometric mean ± SD.

Table 2. Variables predicting sTfR levels in the whole sample, in non-obese and in obese subjects.

	Regression coefficient	Standard error	P-value
Whole sample			
Waist circumference (cm)	0.476	0.106	<0.001
Serum ferritin (µg/L)	-0.086	0.012	<0.001
Alcoholic beverages (cc/day)	-0.0001	0.0000	0.002
Coffee (cc/day)	-0.0005	0.0002	0.046
$F_{4, 454}$= 22.343, R^2_c= 15.8%, P< 0.001			
Non-obese			
Waist circumference (cm)	0.455	0.151	0.003
Serum ferritin (µg/L)	-0.078	0.015	<0.001
Alcoholic beverages (cc/day)	-0.0002	0.0001	0.004
Coffee (cc/day)	-0.001	0.0003	0.004
$F_{4, 245}$= 14.377, R^2_c= 17.9%, P< 0.001			
Obese			
Waist circumference (cm)	0.802	0.205	<0.001
Serum ferritin (µg/L)	-0.092	0.018	<0.001
Alcoholic beverages (cc/day)	-0.0001	0.0001	0.030
$F_{3, 208}$= 13.506, R^2_c= 15.3%, P< 0.001			

Model: gender (male=0, female=1), age (years), education level (primary=0, secondary/tertiary=1), civil status (married=0, unmarried=1), smoking (non-smoking=0, smoking=1), physical activity (sedentary=0, non-sedentary=1), intervention group (Mediterranean diet supplemented with nuts or olive oil group/control group), waist circumference (cm), total energy intake (Kcal/day), meat and processed meat (g/day), dairy products (g/day), coffee (cc/day), alcoholic beverages (cc/day), magnesium (g/day), saturated, monounsaturated and polyunsaturated fatty acids (g/day), cholesterol levels (mg/dL), hs-CRP (mg/L), fasting insulin (µU/mL), fasting glucose (mg/dL), serum ferritin (µg/L).

Table 3. Odds ratio and 95% confidence interval (95%CI) for risk of type 2 diabetes by tertiles of sTfR, SF and sTfR:ferritin ratio, and adjusted for gender.

	NON-OBESE GROUP				OBESE GROUP			
	T1	**T2**	**T3**	***P*-trend**	**T1**	**T2**	**T3**	***P*-trend**
sTfR men (mg/L)	<1.12	1.12-1.36	≥1.36		<1.13	1.13-1.35	≥1.35	
sTfR women (mg/L)	<1.15	1.15-1.35	≥1.35		<1.13	1.13-1.37	≥1.37	
Geometric median sTfR (mg/L)	0.98±1.12	1.24±1.06	1.62±1.16		0.99±1.09	1.24±1.06	1.69±1.17	
Cases (n)	32	26	22		22	23	28	
Controls (n)	50	56	60		47	50	41	
Crude Model (sTfR)	1	0.71 (0.42-1.19)	0.61 (0.36-1.05)	0.081	1	0.95 (0.53-1.70)	1.36 (0.78-2.38)	0.238
Model 1	1	0.58 (0.31-1.07)	0.39 (0.21-0.73)	0.004	1	1.33 (0.70-2.54)	2.14 (1.10-4.15)	0.021
Model 1 + FHD	1	0.56 (0.30-1.04)	0.39 (0.21-0.74)	0.005	1	1.30 (0.68-2.48)	2.15 (1.11-4.17)	0.020
Model 1 + FHD + fasting insulin	1	0.55 (0.30-1.02)	0.38 (0.20-0.72)	0.004	1	1.25 (0.66-2.40)	2.13 (1.10-4.12)	0.020
Model 1 + FHD + fasting insulin + fasting glucose	1	0.52 (0.27-1.00)	0.40 (0.20-0.79)	0.015	1	1.52 (0.76-3.03)	2.79 (1.35-5.77)	0.005

Crude Model: unadjusted. Model 1: Centre (Pamplona/Barcelona/Reus-Tarragona), intervention group (Mediterranean diet supplemented with nuts or olive oil group/control group), education level (primary/secondary/tertiary), civil status (married/unmarried/divorced or widower), smoking (smoker/no-smoker), physical activity (200METs•min/day/≥200METs•min/day), meat and processed meat (g/day), dairy products (g/day), coffee (cc/day), alcoholic beverages (g/day), energy intake (kcal/day), magnesium (mg/day), saturated, monounsaturated and polyunsaturated fatty acids (mg/day), total cholesterol (mg/dL), high-sensitivity C-reactive protein (ng/mL), waist circumference (cm).

Family history of DM (yes/no), fasting insulin (μU/mL) and fasting glucose (mg/dL).

Figure 1: Age- and gender-adjusted partial correlations between sTfR and BMI and waist circumference.