Sterol Regulatory Element-Binding Factor 2 (SREBF-2) Predicts 7-Year NAFLD Incidence and Severity of Liver Disease and Lipoprotein and Glucose Dysmetabolism.

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Sterol regulatory binding factor (SREBF)-2 predicts 7-year NAFLD incidence and severity of liver disease, lipoprotein and glucose dysmetabolism

RUNNING TITLE: SREBF-2 and NAFLD

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Abstract

We aimed at prospectively assessing the impact of sterol regulatory element-binding protein(SREBP)-2 polymorphism on the risk of developing non-alcoholic fatty liver disease (NAFLD) and on liver histology, lipoprotein and glucose metabolism in biopsy-proven NAFLD. We followed-up 175 nonobese nondiabetic participants in a population-based study, without NAFLD or metabolic syndrome at baseline, characterized for SREBF-2 rs133291 C/T polymorphism, dietary habits, physical activity, adipokines, C-reactive protein (CRP), and endothelial adhesion molecules. NAFLD patients underwent liver biopsy, an OGTT with Minimal Model analysis to yield glucose homeostasis parameters, and an oral fat tolerance test with measurement of plasma lipoproteins, adipokines, and cytokeratin-18 fragments. After 7 years, 27% subjects developed NAFLD and 5% developed diabetes. SREBF-2 predicted incident NAFLD and diabetes and CRP and endothelial adhesion molecule changes. In biopsy-proven NAFLD patients, SREBF-2 predicted NASH (OR: 2.92, 2.08-4.18; p=002), the severity of tissue insulin resistance, of β-cell dysfunction, and of oral fat intolerance (characterized by higher postprandial lipemia, cholesterol enrichment of triglyceride-rich lipoproteins and of oxLDLs, and by HDL-C fall, adipokine imbalance, and postprandial apoptosis activation). SREBF-2 polymorphism predisposes to NAFLD and to associated cardio-metabolic abnormalities and affects liver histology, glucose and lipid metabolism in biopsy-proven NAFLD.

Keywords: NAFLD, SREBP-2, cholesterol, postprandial lipemia, lipogenesis

Introduction

Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease, encompasses a histological spectrum, ranging from simple steatosis (SS) to steatosis plus necroinflammation (nonalcoholic steatohepatitis, NASH), which can be differentiated only by liver biopsy. While SS has a benign hepatological course, NASH can progress to end-stage liver disease and is projected to be the leading cause of liver transplantation by 2020(1-2)[1, 2]; furthermore, both histological subtypes confer an increased risk of type 2 diabetes (T2DM) and cardiovascular disease (CVD) independently of classical risk factors, through mechanisms potentially involving adipokine and lipoprotein dysregulation. The scant prospective data on factors predisposing to NAFLD and associated cardio-metabolic disorders connected baseline metabolic syndrome, insulin resistance/hyperinsulinemia and weight gain to incident ultrasonographic NAFLD(3, 4)[3, 4]; however, not every insulin resistant or obese subject develops NAFLD, suggesting other genetic or environmental factors promote liver disease in insulin resistant subjects.

Among environmental factors, dietary fat excess has been extensively connected to NAFLD experimentally, while such evidence in humans remains controversial(5)[5].

A genetic predisposition to NAFLD and NASH is indisputably present, though the exact pathway involved remains unclear. Altered cholesterol metabolism, resulting in hepatic cholesterol accumulation, has been recently linked to liver injury and NASH development in experimental and human NASH, independently of obesity(6-10)[6, 7, 8, 9, 10]. Sterol regulatory element-binding factor (SREBF)-2 gene codes for SREBP-2, the master nuclear transcription factor co-ordinately regulating genes involved in cellular cholesterol biosynthesis, uptake, and excretion(11)[11]. Hepatic SREBP-2 up-regulation parallels the severity of liver disease in animal and human NAFLD (6-8, 10): SREBF-2 is therefore an ideal candidate for modulating the genetic susceptibility to NAFLD and NASH.

The functional single nucleotide polymorphisms (SNPs) rs133291 C/T in SREBF2 gene has been linked to serum LDL-cholesterol(12)[12]. but there are no human data on the impact of this SNPs on the risk of developing NAFLD and associated metabolic abnormalities.

We hypothesized that SREBF-2 SNP may not only predispose to NAFLD development, but also affect the severity of liver disease and of NAFLD-associated glucose and lipid dysmetabolism.

We aimed at: 1) assessing the role of SREBF-2 on the risk of developing NAFLD in healthy subjects at baseline 2) elucidating the impact of SREBF-2 on the severity of liver histology and on glucose and lipid homeostasis in biopsy-proven NAFLD.

Methods

Subjects. Based on limited data on NAFLD incidence(4-6)[3,4,] **Errore. Il segnalibro non è definito.** and on SREBF-2 SNP prevalence(15)[15], considering a type I error of 0.05 and a type II error of 0.20, allowing for a 10% drop-out rate, at least 162 subjects were needed at the end of the study to detect a significant difference in the prevalence of SREBF-2 SNP between NAFLD and controls.

Among 1658 Caucasian participants in a metabolic survey in the province of Asti (North-Western Italy) in 2004-2005 (previously described(13)[13]), 193 randomly selected subjects gave informed consent to be included in the study. At baseline, all were in good general health, with normal findings on medical history, physical examination, blood count, and chemical screening battery. Subjects with diabetes, obesity, metabolic syndrome, insulin resistance, CVD, significant alcohol consumption (as defined below), transaminase elevation or known liver disease (as defined below) were excluded from the study, which was approved by the local Ethics Committee and conformed to the Helsinki Declaration.
Baseline data collected during the period 2004–2005 included: measurements of weight, height, and waist circumference, blood pressure following a uniform protocol; dietary and physical activity record (see below), and routine biochemistry. Frozen serum samples collected from all participants at entry were stored at -80°C for genetic and biochemical analyses. From January to December 2011, participants in the baseline survey were submitted to follow-up evaluation. All anthropometric, dietary and physical activity records and biochemical analyses were repeated at the end of follow-up.

Definitions. NAFLD was diagnosed by persistently (>6 months) elevated aminotransferases (defined by ALT ≥30 U/L in men and ≥20 U/L in women, according to recently proposed cut-offs that enhance sensitivity for steatosis detection(14, 15)[14, 15)] and ultrasonographic diagnosis of steatosis based on standardized criteria (namely, parenchimal brightness, liver-to-kidney contrast, and deep beam attenuation(18)[16]), made by 2 experienced radiologists blinded to clinical data of participants.

Exclusion criteria were: a history of alcohol consumption >20 g/d (men) and >10 g/d (females) as assessed by a detailed inquiry of patients and relatives and by a validated questionnaire filled in daily for one week by the patients; positive serum markers of viral hepatitis B-C; other competing causes of hepatic steatosis (detailed in *online Appendix).* Diabetes was defined by a fasting plasma glucose (FPG) ≥126 mg/dl or 2-h plasma glucose ≥200mg/dl during an OGTT or by drug treatment for elevated plasma glucose(17)[17]; obesity was defined by a BMI≥30 kg. Insulin resistance was defined by a homeostasis model assessment of insulin resistance (HOMA-IR) index ≥2, a cut-off closely correlating with clamp measures in Northern Italian subjects and with OGTT-derived indices of insulin sensitivity in our subjects with NAFLD(18, 19)[18, 19]. Metabolic syndrome (MS) was defined according to the joint IDF/NHLBI/AHA/IAS/IASO statement(20)[20] (Table 1).

Dietary and physical activity record

Subjects were instructed to fill in a 7-day validated diet record during an individual training session with a nutritionist; a list of foods was designed, sizes were specified for each item different portion according to the EPIC study(21, 22)[21, 22]. The recorded period included a complete week. The diet record was analyzed using the WinFood database (Medimatica, Teramo, Italy) according to the table of food consumption of the Italian National Institute of Nutrition and Food Composition Database for Epidemiological Study in Italy(23, 24) [23, 24].

Patients were interviewed about smoking habits and alcohol consumption, and completed the Minnesota-Leisure-Time-Physical-Activity questionnaire(25)[25]. The physical activity level was calculated as the product of duration and frequency of each activity (in h/week), weighed by an estimate of the metabolic equivalent of the activity, and summed for activities performed.

Proinflammatory markers and adipokines

Serum C-reactive protein (CRP) was determined via a high sensitivity latex agglutination method on HITACHI 911 Analyser (Sentinel Ch., Milan). The kit had a minimum detection of less than 0.05 mg/L, and a measurable concentration range up to 160 mg/L. The intra-assay and inter-assay variation coefficients were, respectively, 0.8–1.3 and 1.0–1.5%.

Serum adiponectin was measured by a sandwich ELISA (R&D Systems Europe,

Abingdon, UK); the kit has a sensitivity of 0.25 pg/ml in a 50-µl sample size and a range of 3.9 to 250 ng/ml. The intra- and interassay CVs were 3.4 and 5.8%, respectively.

Resistin was measured by a biotin-labeled antibody- based sandwich enzyme immunoassay (BioVendor Laboratory Medicine, Brno, Czech Republic). The intra-assay e inter-assay CVs were respectively 2.8–3.4% and 5.5– 6.8%

Circulating markers of endothelial dysfunction.

Serum soluble adhesion molecules E-selectin and intercellular adhesion molecule (ICAM)-1 were measured as validated markers of endothelial dysfunction, subclinical atherosclerosis and early cardiovascular risk(28)[26]. E-selectin and ICAM-1 levels were measured by a solid phase ELISA (R&D SYSTEM, Minneapolis, MN, USA). Minimum detectable doses and intra- and inter-assay CVs were, respectively, <0.1 ng/mL, 4.7-5.0 %, 7.4-8.8%, 0.17-1.26 pg/mL, 2.3-3.6%, 5.5-7.8%, and <0.1 ng/mL, 4.7-5.0%, 7.4-8.8%.

Genetic analyses.

The functional SNP rs133291 C/T in the intron region of SREBF-2 gene were assessed by the real-time allele discrimination method, using TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster city, CA). The -493 G/T microsomal triglyceride transfer protein (*MTP*) gene polymorphism and apoE genotype, affecting both lipid metabolism and the risk of NAFLD(27)[27], were also assessed: -493 G/T MTP gene polymorphism was assessed by PCR-RFLP using a two-step nested PCR. ApoE genotype was determined by PCR amplification of genomic DNA using specific oligonucleotide primers.

Impact of SREBPF-2 on liver histology in established NAFLD.

To investigate the impact of SREBPF-2 genotype on liver disease severity in NAFLD, within 1 month of the follow-up examinations patients with NAFLD were offered liver biopsy and each pathological feature was read and scored by a single pathologist (ED) blinded to the patient clinical-biochemical characteristics and scored according to the NASH Clinical Research Network criteria[28](28). NASH was defined according to Brunt criteria[29](29).

Impact of SREBPF-2 on glucose homeostasis in established NAFLD.

Biopsy-proven NAFLD patients and an equal number of subjects without NAFLD at the end of follow-up (controls), matched for age, sex, BMI, and SREBPF-2 genotype, underwent a standard OGTT. Minimal Model analysis of plasma glucose, insulin and C-peptide during the OGTT yielded the following parameters of glucose homeostasis.

Prehepatic insulin delivery was estimated as the suprabasal (Δ) 30-min AUC of C-peptide divided by the 30-min increase in circulating glucose. The hepatic insulin extraction (He), as percentage of secreted hormone, was estimated by [1 – (AUC insulin/AUC C-pep)].

Insulin sensitivity was estimated from a model of glucose clearance, which provides the oral glucose insulin sensitivity (OGIS), an index of whole body insulin sensitivity. Muscle and hepatic insulin resistance index were calculated from OGTT as previously proposed and validated against clamp in nondiabetic subjects[30], [31](30, 31). Adipose tissue insulin resistance (IR) index was calculated as fasting free fatty acids (FFA) x fasting insulin(32)[32].

The following indexes of β-cell function were also calculated: the insulinogenic index (IGI), computed as the suprabasal serum insulin increment divided by the corresponding plasma glucose increment in the first 30 min (ΔI30/ΔG30), and the CP-genic index (CGI), computed as Δ C-pep30/ΔG30, previously validated against measures of β-cell functions derived from frequently-sampled intravenous glucose tolerance test (FIVGTT)[33](33). Two integrated indexes of β-cell function, i.e. disposition index (DI) and adaptation index (AI), were also calculated. DI and AI relate β-cell ability to adapt insulin secretion to changes in insulin sensitivity and represent integrated parameters of β-cell function. We and others previously validated DI and AI against frequently sampled intravenous glucose tolerance test (FIVGTT) MINIMAL MODEL parameters in NAFLD and nondiabetic subjects[34], [35] (34, 35); these indexes also accurately predicted future T2DM in the general population[36](36).

Subjects with newly-diagnosed diabetes were excluded, to avoid the confounding effect of T2DM on liver disease and on glucose metabolism.

Impact of SREBF-2 on dietary fat tolerance in biopsy-proven NAFLD.

As postprandial lipemia is a CVD risk factor potentially operating in NAFLD and lipotoxicity substantially contributes to NASH pathogenesis (1, 9, 37) [1, 37], biopsy-proven NAFLD patients and matched controls underwent also an oral fat tolerance test (OFTT). The fat load consisted of a mixture of dairy cream (35% fat) and egg yolk for a total energy content of 766 kcal. The total amount of fat was based on the subject's body surface area (78.3 g fat 55.6 % saturated fatty acids, 29.6 % monounsaturated fatty acids, 4.2 % polyunsaturated fatty acids, 0.5 g cholesterol per m^{-2})(32). The fat load was consumed during a period of 5 min; subjects kept fasting on the test morning and strenuous activity was forbidden since 24 hours before the test, since exercise can reduce postprandial lipemia. Blood samples were drawn every 2 hours for 10 hours and the following parameters were assessed at each time-point:

1)plasma total cholesterol (Chol), triglyceride (Tg), HDL-C, apolipoprotein A1, and FFA were measured by automated enzymatic methods.

2)triglyceride-rich lipoproteins (TRLP) subfractions: TRLP were isolated through preparative ultracentrifugation and their total Tg and Chol content were subsequently measured. One aliquot of plasma was brought to densities of 1006 g/L by adding a KBr solution (d=1330 g/L) and centrifuged at 105,000 g for 21h at 10°C in a Beckman L8-70M ultracentrifuge. Tg and total Chol concentration was then determined in lipoprotein fraction enzymatically. The first higher amount of blood (10 ml) was drawn for subfractionating TRLPs by ultracentrifugation on a discontinuous density gradient. Separated plasma was brought to a density of 1.10 g/mL by adding solid KBr. The density gradient was prepared by adding to 4 mL of this plasma 3 mL of a 1.065 g/mL solution containing 0.05% KBr/NaCl plus EDTA (pH 7.4); 3 mL of a similar solution at 1.020 g/mL; 3 mL of physiological saline at 1.006 g/mL. The sample was ultracentrifuged in a Beckman L8-70M centrifuge at 20°C in stages allowing the separation of two VLDL fractions with decreasing Sf values: VLDL 1: Sf>100; VLDL 2: Sf =20-100. The automated methods mentioned above were used to determine Chol and Tg on the two fractions.

VLDL apoB48 and apoB100 were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using 3,9% gel according to Battula et al.[38](38) Nondelipidated lipoprotein samples were reduced in SDS sample buffer for 4 minutes at 96°C. Samples were applied to the gel and run at 40 mA in 0,025M Tris, 0,192M glycine and 0,1% SDS. Gel was stained with Silver Stain (Bio Rad). Since the

chromogenicity of apoB48 is similar to that of apo B100[39] (39) a protein standard was prepared from LDL isolated by sequential ultracentrifugation and used to quantify apoB100 and apoB48. The bands were quantified by densitometry using Gel Doc equipment (Bio Rad). Density values were assigned to the apo B100 bands of the standard LDL and a standard curve was constructed. The values were recalculated by linear regression.

To isolate LDL, blood was centrifuged for 30' at 2500 rpm and at 4°C in a J6B centrifuge (Beckman Instruments, Palo Alto, CA), and the obtained plasma was brought to a density of 1.10 g/mL by the addition of solid KBr. The density gradient was prepared manually according to Redgrave[40] (40) by adding to 4 mL of this plasma 3 mL of a 1.065 g/mL solution containing 0.05% KBr/NaCl plus EDTA (pH 7.4), 3 mL of a similar solution at 1.020 g/mL, 3 mL of physiological saline at 1.006 g/mL. The sample was ultracentrifuged in a SW4O rotor in a Beckman L8-70M centrifuge at 39,000 rpm for 18 h at 20°C to isolate LDL.

3) LDL conjugated dienes, validated markers of oxidized low-density lipoproteins (oxLDLs), were determined as follows. Capillary electrophoresis was performed as described by Stocks et al.[41](41).The cathode and anode electrolytes and the capillary run buffer were 40 mM methylglucamine-Tricine, pH 9.0. LDL samples were injected by low pressure for 4 seconds. Dimethylformamide was injected as an electroendosmotic flow(EOF) marker for 1 second. A voltage of 24 KV was applied ramping over 0.8 min. Migration of LDL particle was monitored at 200 and 234 nm. The amount of conjugated dienes was obtained from the percentage of the height of LDL peak at 234 nm related to the height of LDL peak at 200 nm.

4)circulating, adipokines (resistin, adiponectin), and cytokeratin-18 (CK-18) fragments (a validated marker of hepatocyte apoptosis) were determined (2). CK-18 fragments were measured with the M30-Apoptosense ELISA kit, a one step in vitro immunoassay for the quantitative determination of the apoptosis-associated CK18Asp396 neo-epitope in serum (PEVIVA AB, Bromma, Sweden), has a sensitivity of 25 U/L in a 25-μL sample size and a range of 75 to 1000 U/L. The intra- and inter-assay CVs are less than 8%.

Statistical analysis

Participants with and without follow-up visits were compared to determine the appropriateness of an analysis based on participants with complete data only.

Hardy–Weinberg equilibrium was assessed using $\chi 2$ test. Comparisons of genotype frequencies between NAFLD developers and non-NAFLD developers were performed using 2 x 2 contingency tables with $\chi 2$ analysis.

Subjects with and without NAFLD at the end of the follow-up were compared for baseline values and for changes during follow-up(Delta-values). Normality was evaluated by Shapiro-Wilk test and non-normal values were log-transformed for regression analysis.

Fisher or chi-square test were used to compare categorical variables, as appropriate.

Differences across groups were analyzed by ANOVA and then by Bonferroni correction, when variables were normally distributed; otherwise the Kruskal-Wallis test, followed by the post hoc Dunn test, was used to compare nonparametric variables. To adjust for multiple comparison testing, the Benjamini-Hochberg False Discovery Rate correction was applied to raw p-values; significance was set at an adjusted p-value threshold of 0.05[42](42)

Analysis of lifestyle, anthropometric and metabolic parameters and of genetic polymorphisms was made using Spearman correlation test. Genetic polymorphisms were modelled as an additive effect, that is, quantitative predictor variables reflecting the number of risk alleles (0, 1, or 2).

Based on results of univariate analysis, a logistic regression model was used to identify independent predictors of incident NAFLD/NASH, of incident T2DM and of markers of subclinical atherosclerosis at the end of follow-up. Variables were selected from parameters which differed between NAFLD developers and non-NAFLD developers at baseline and during follow-up (Delta-values). Quartiles of continuous variables were considered for this analysis.

The area under the curve (AUC) and incremental AUC (IAUC) of parameters measured during the oral fat test and the OGTT in patients with established NAFLD and controls were computed by the trapezoid method. Univariate and multiple regression analysis were used to identify independent predictors of glucose and lipid homeostasis parameters and of different histological features in biopsy-proven NAFLD.

Data were expressed as mean±SEM. (STATISTICA software, 5.1, Statsoft Italia, Padua, Italy).

Results

After a mean follow-up period of 7.1±0.3 years, data on 175 participants were available for the follow-up examinations. The 18 (9.3%) subjects lost at follow-up did not significantly differ in baseline anthropometric, lifestyle, clinical or biochemical feature from the other participants included in the analysis. At the end of follow-up, 27% subjects developed NAFLD.

Subjects characteristics at baseline and at the end of follow-up. SREBPF-2 CC carriers were 66% in non-NAFLD developers vs. 45% in NAFLD developers (p=0.029), heterozygous CT carriers were 32% in non-NAFLD developers vs. 42% in NAFLD developers (p=0.328) and TT carriers were 1% in non-NAFLD developers vs. 13% in NAFLD developers (p=0.010; Table 1). The genotype frequencies were in Hardy-Weinberg equilibrium (p>0.05 for all) and the allele frequencies were consistent with published reports in Caucasian population.

At baseline, there was no significant difference in any other genetic, anthropometric, lifestyle, clinical or biochemical feature between NAFLD developers and non-NAFLD developers (Table 1).

At the end of follow-up, NAFLD patients gained significantly more weight, became more insulin resistant, had higher fasting plasma insulin and C-reactive protein and lower plasma adiponectin levels than non-NAFLD developers; fourteen (29%) NAFLD patients vs. 20(16%) non-NAFLD developers had elevated fasting plasma glucose (p=0.041), and 6(13%) NAFLD patients vs. 2 (2%) non-NAFLD developers had T2DM (p=0.010)(Table 1). There was no significant difference in any other anthropometric, clinical or biochemical feature between NAFLD developers and non-NAFLD developers during follow-up. Physical activity and dietary habits did not change during follow-up (Table 1, *online Appendix* Table 1-2).

Liver biopsy, performed in 40 NAFLD patients comparable to the whole NAFLD cohort, demonstrated NASH in 19 (48%) subjects (details on single histological features reported in *online Appendix).*

Predictors of incident NAFLD and of NASH at the end of follow-up

Incident NAFLD was independently predicted by SREBF-2 SNP (OR, 95%CI: 2.01, 1.47-3.31; p=009) and by Delta-BMI (OR, 95%CI: 1.21, 1.08-2.47; p=0.028)

NASH was independently predicted by SREBF-2 SNP (OR, 95%CI: 2.92, 2.08-4.18; p=002)

Predictors of incident T2DM and of markers of subclinical atherosclerosis at the end of follow-up.

Incident T2DM was predicted by SREBF-2 SNP (OR, 95%CI: 1.91, 1.16-3.98; p=0.011) and Delta-BMI (OR, 95%CI: 1.20, 1.05-2.71; p=0.040).

SREBF-2 predicted also predicted the upper serum CRP (OR 2.52, 1.76-3.38; 0.008), E-selectin (OR 1.62, 1.12-3.80; 0.010) and ICAM-1 (OR 1.57, 1.15-3.92; 0.019) quartiles at the end of follow-up.

Impact of SREBF-2 SNPs on liver histology, glucose homeostasis and oral fat tolerance in biopsy-proven NAFLD patients and matched controls.

To assess the relationship of SREBF-2 to liver disease, glucose and lipid homeostasis, biopsy-proven NAFLD patients and controls were grouped according to SREBF-2 genotypes. Due to the low prevalence of SREBF-2 TT carriers and to the overlapping clinical characteristics with heterozygous carriers, they were combined with the heterozygote genotype.

Liver histology. In biopsy-proven NAFLD patients, CT/TT carriers showed more severe histological steatosis, necroinflammation, NAS score and fibrosis score, and a higher prevalence of NASH (80% vs. 15%; p=0.0002) than the CC genotype (*online Appendix* Table 2)..

In both NAFLD patients and controls, SREBF-2 CT/TT allele carriers had higher fasting insulin, CRP, E-selectin and ICAM-1 than CC genotype, while they did not differ in any other clinical, biochemical or dietary features.

Glucose homeostasis. In both NAFLD patients and controls, SREBF-2 CT/TT carriers displayed more severe hepatic, muscle and adipose tissue insulin resistance and pancreatic β-cell dysfunction than CC carriers(Table 2). SREBF-2 SNP independently predicted insulin resistance in different tissues and pancreatic β-cell dysfunction (Table 4),

Oral fat tolerance test. Following fat ingestion, CT/TT carriers showed higher postprandial lipemia (accounted for by a higher level of large TRLPs of both intestinal and hepatic origin), FFA and oxLDLs

elevation than CC carriers; CT/TT carriers had also a marked postprandial increase in cholesterol content of large TRLPs, coupled with a deeper HDL-C and apoA1 fall, a postprandial adipokine imbalance, characterized by lower adiponectin and higher resistin levels, and higher circulating CK-18 fragments(Table 3; Figures 1-2).

SREBF-2 independently predicted Tg and cholesterol increase in TRLPs, the fall in HDL-C and apoA1 levels, and adipokine and CK-18 fragment responses postprandially (Table 4)..

Discussion

Main findings of this study are the following:

1) SREBF-2 SNPs predicted 7-yr incidence of NAFLD in nonobese nondiabetic patients without metabolic syndrome at baseline. SREBF-2 also predicted incident NASH and T2DM, and changes in markers of endothelial dysfunction at the end of follow-up.
2) SREBF-2 extensively affected tissue insulin sensitivity, pancreatic β-cell function, and lipoprotein and adipokine responses to fat ingestion.

Data from cellular and human models and recent cross-sectional human studies suggested altered cholesterol metabolism may be central for NAFLD development, with the magnitude of hepatic cholesterol overload paralleling liver disease severity(6-10): these studies demonstrated NAFLD is characterized by increased hepatic cholesterol synthesis and uptake and reduced cholesterol excretion, favoring hepatic cholesterol accumulation, mitochondrial oxidative injury and endoplasmic reticulum (ER) stress, which eventually lead to steatosis, hepatocyte apoptosis, inflammation and NASH (6-10, 43-46). Another set of experiments connected adipocyte cholesterol dysregulation to adipose tissue dysfunction, a key feature of obesity-associated disorders, including NAFLD[43], [44](43, 44).

The nuclear transcription factor SREBP-2 is a key regulator of cellular cholesterol homeostasis: its activation promotes hepatocyte cholesterol accumulation by coordinately activating cholesterol biosynthesis and uptake and repressing cholesterol excretion(6-10). Cross-sectional human studies found an increased hepatic expression of SREBP-2 and of its target genes, with the degree of SREBP-2 activation paralleling

the severity of hepatic cholesterol overload and liver histology(8-10). However, to date it was unclear whether increased SREBP-2 activation might be causally related to NAFLD or rather the consequence of obesity-associated hyperinsulinemia and chronic low-grade inflammation, which are almost universal in NAFLD and up-regulate SREBP-2 expression (6, 7). Our data point to a crucial role of genetically determined SREBP-2 activation, which may predispose initially lean, insulin sensitive subjects to NAFLD development and may extensively modulate liver injury, glucose and lipid metabolism in established NAFLD. We also expanded the knowledge of the mechanisms linking SREBP-2 activation to liver and cardio-metabolic disease, as SREBF-2 polymorphism extensively modulated tissue insulin sensitivity, pancreatic β-cell function, and dietary fat tolerance. Adipose tissue dysfunction, in particular, is characterized by a resistance to the anti-lipolytic action of insulin and by a pro-inflammatory pattern of adipokine secretion, resulting in an increased circulating levels of lipotoxic FFA and pro-inflammatory adipokines, and is emerging as a key determinant of liver disease and associated cardio-metabolic abnormalities in NAFLD (32, 45, 46) [32, 45, 46]. Experimental data demonstrated inappropriate SREBP-2 activation promotes adipocyte cholesterol overload and cholesterol-induced adipose tissue dysfunction, which were reversed by unloading adipocytes of cholesterol (43, 44). Consistent with these findings, we connected a genetic variant in SREBF-2 expression with adipose tissue insulin resistance and a pro-inflammatory pattern of adipokine secretion. We also disclosed a novel mechanism whereby SREBF-2 promotes ectopic cholesterol accumulation following fat ingestion, i.e. an enhanced postprandial lipoprotein-mediated cholesterol delivery to peripheral tissues coupled with an impaired reverse cholesterol transport: despite comparable values in fasting conditions, SREBF-2 CT/TT carriers showed in fact an increased cholesterol content in large TRLPs and oxLDLs and a fall in HDL-C and apoA1 levels, the latter being responsible for reverse cholesterol transport. Beside promoting adipose tissue dysfunction, increased SREBP-2 expression can also contribute to the increased cardiovascular risk in NAFLD, as suggested by the independent relationship between SREBF-2 and changes in CRP and endothelial adhesion molecules. Underlying mechanisms must be elucidated by future studies, but increased postprandial lipemia and postpradial dysregulation of lipoprotein-mediated cholesterol fluxes may play a role.

Finally, SREBF-2 may also affect the increased risk of T2DM in NAFLD by affecting both tissue insulin resistance and pancreatic β-cell function (47)[47]), an effect at least in part mediated by postprandial cholesterol-rich TRLPs and oxLDL accumulation (Table 4).

Collectively, our findings suggest SREBF-2 SNP extensively affects liver disease, glucose and lipid metabolism in NAFLD, and could also explain the apparent discrepancy between animal studies, where a high fat/high cholesterol diet constantly promotes NASH, and human data, which variably found an excessive dietary fat and cholesterol intake in NAFLD patients(5)[5]: according to our model, SREBF-2 polymorphism would enhance susceptibility to the lipotoxic effects of dietary cholesterol even in the absence of excessive dietary intakes.

In conclusion, we showed polymorphism in SREBF-2 gene, coding for nuclear transcription factor SREBP-2, predicts NAFLD incidence and the severity of liver disease and of associated glucose and lipid dysmetabolism. Present findings may have important clinical and research implications: first, SREBF-2 SNP may help selecting NAFLD patients at higher risk of progressive liver disease and of cardio-metabolic complications for tight monitoring and experimental treatments. Secondly, interventions reducing cellular cholesterol overload may ameliorate liver disease in NAFLD, regardless of coexisting hypercholesterolemia, as suggested by preliminary human trials with statins and ezetimibe, known inhibitors of cholesterol synthesis and absorption, respectively (48-50)[48, 49, 50] . Lastly, future research should assess whether a genetically-determined, SREBF-2-mediated maladaptive response to a chronic, daily, repetitive stimulus like fat ingestion may link chronic overfeeding to NAFLD development and whether strategies targeting SREBF-2 expression, including antisense nucleotides, may improve liver and cardio-metabolic disease in NAFLD patients more effectively than selective modulation of individual steps in cholesterol metabolism. Strengths of our study are the careful selection and thorough characterization of participants and the histological characterization of NAFLD, a limitation is the small number of patients, which mandates confirmation of our findings in larger cohorts.

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Author contributions.

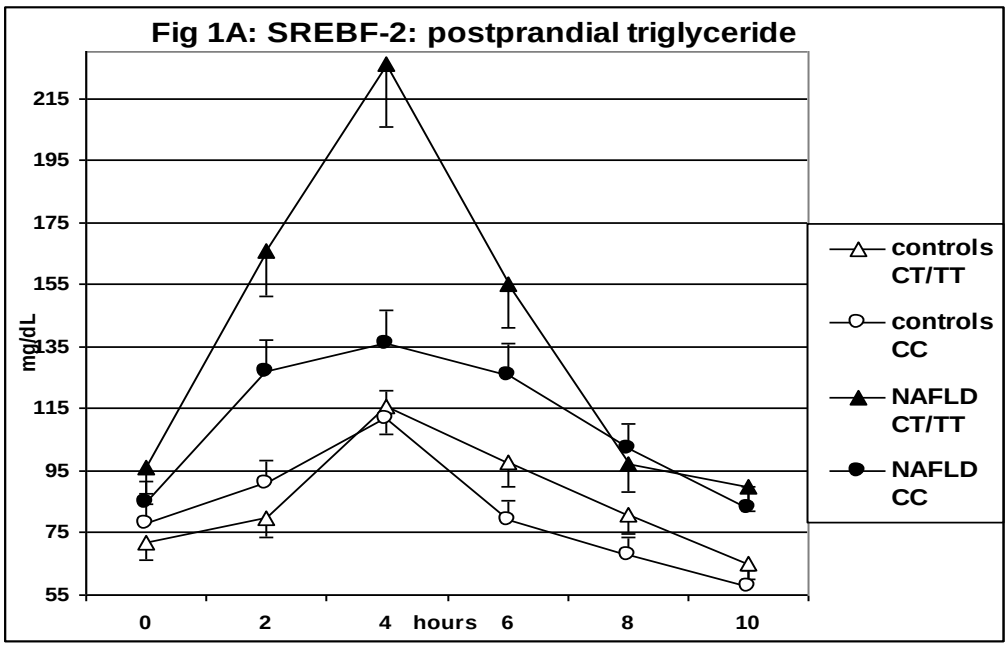
Giovanni Musso: conceived and designed the study, analyzed the results, drafted the article, gave final approval

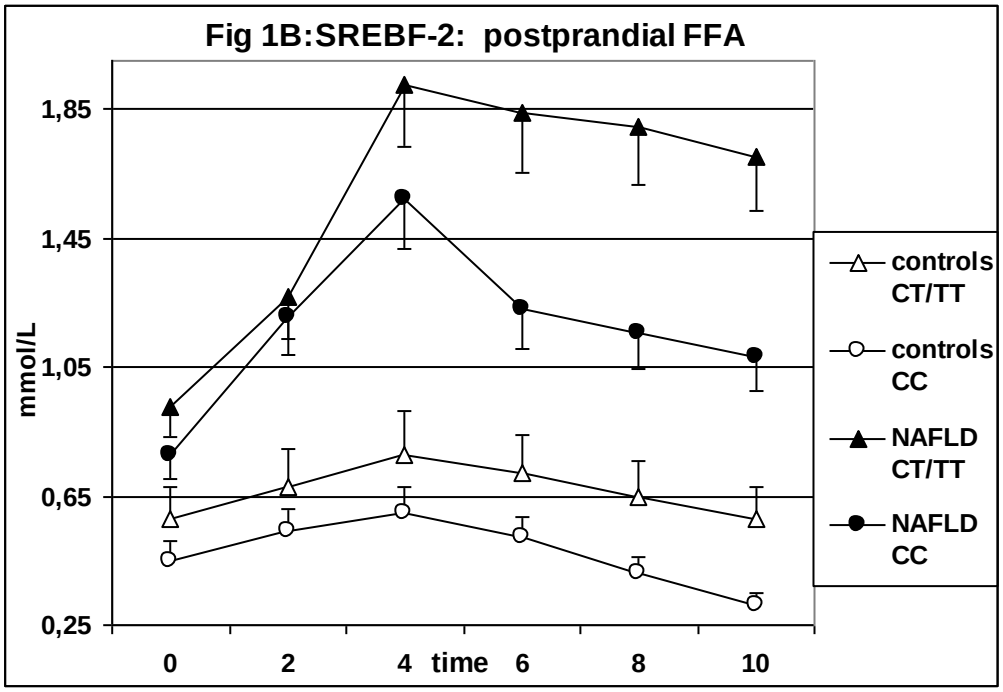
Maurizio Cassader: acquired data, critically analyzed the results, contributed to the draft of the article, gave final approval

Franco De Michieli: acquired data, critically analyzed the results, contributed to the draft of the article, gave final approval

Roberto Gambino: acquired data, critically analyzed the results, contributed to the draft of the article, gave final approval

Figure 1. Oral fat tolerance test: postprandial responses in plasma triglyceride, free fatty acids (FFA), VLDL1-Chol, VLDL2-Chol, LDL conjugated dienes, and HDL-C. Data are presented as mean±SEM.





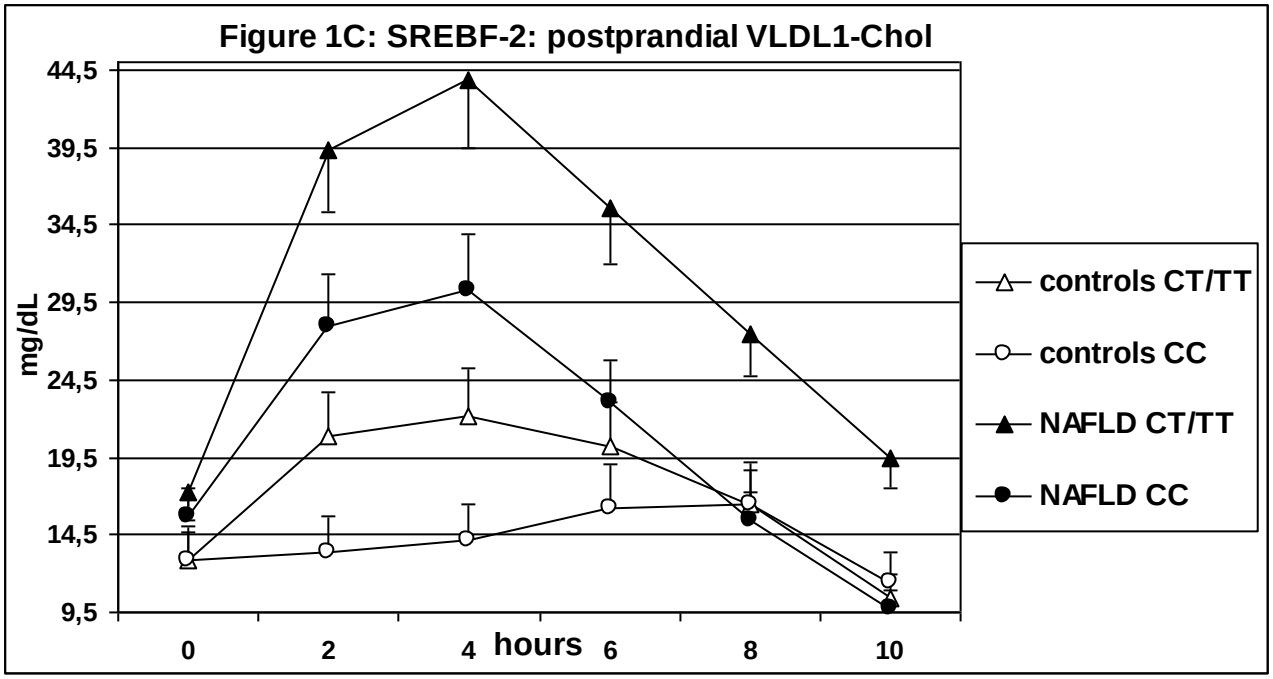


Figure 1C: SREBF-2: postprandial VLDL1-Chol
44,5
39,5
34,5
29,5
24,5
19,5
14,5
9,5
mg/dL
0
2
4
hours
6
8
10
controls CT/TT
controls CC
NAFLD CT/TT
NAFLD CC

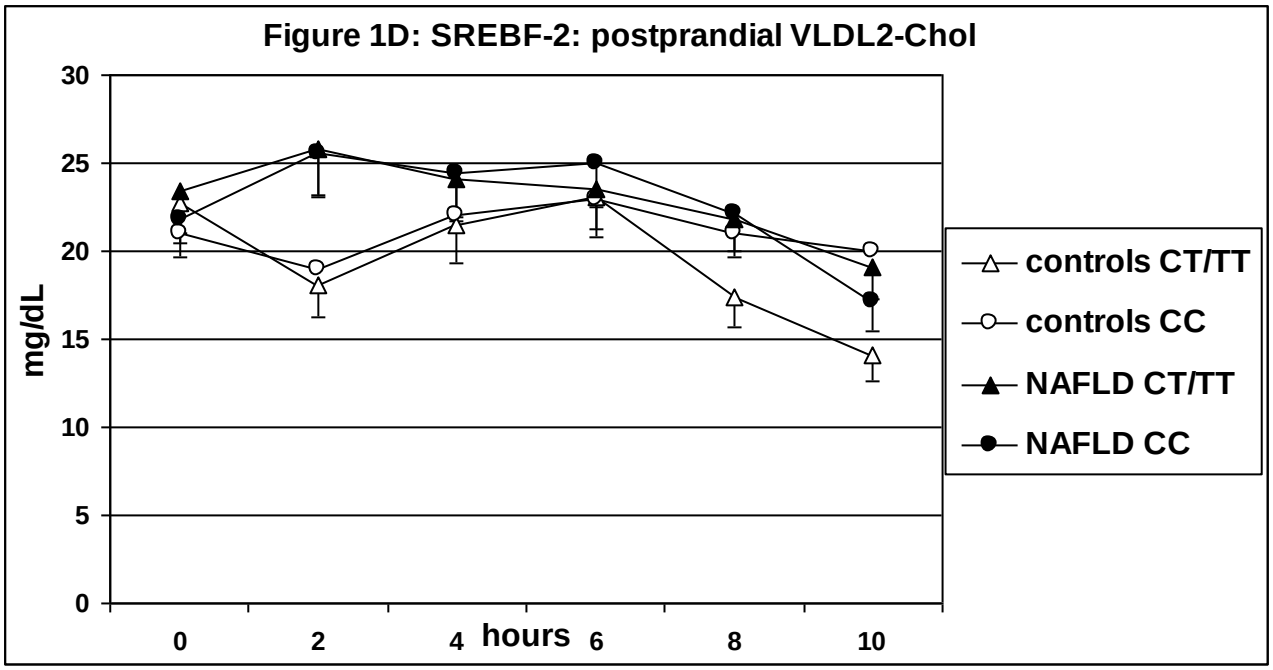


Figure 1D: SREBF-2: postprandial VLDL2-Chol
30
25
20
15
10
5
0
mg/dL
0
2
4
hours
6
8
10
controls CT/TT
controls CC
NAFLD CT/TT
NAFLD CC

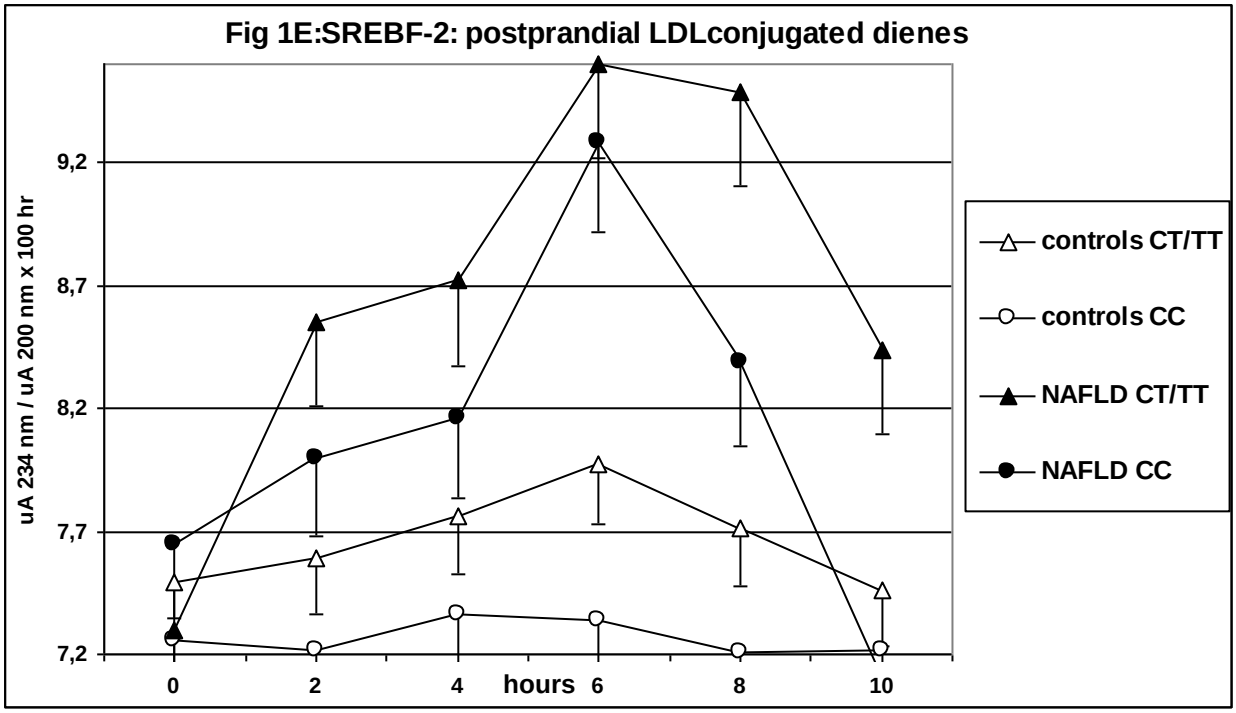


Fig 1E:SREBF-2: postprandial LDLconjugated dienes
uA 234 nm / uA 200 nm x 100 hr
9,2
8,7
8,2
7,7
7,2
0
2
4
hours
6
8
10
controls CT/TT
controls CC
NAFLD CT/TT
NAFLD CC

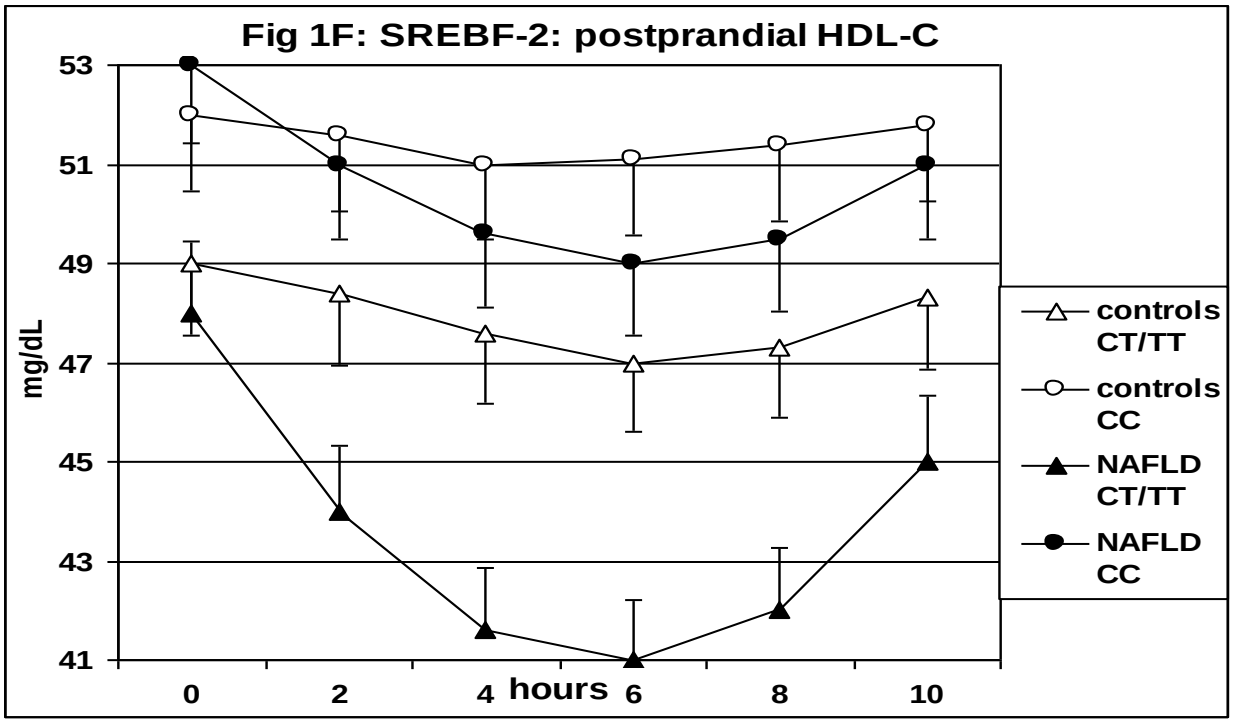
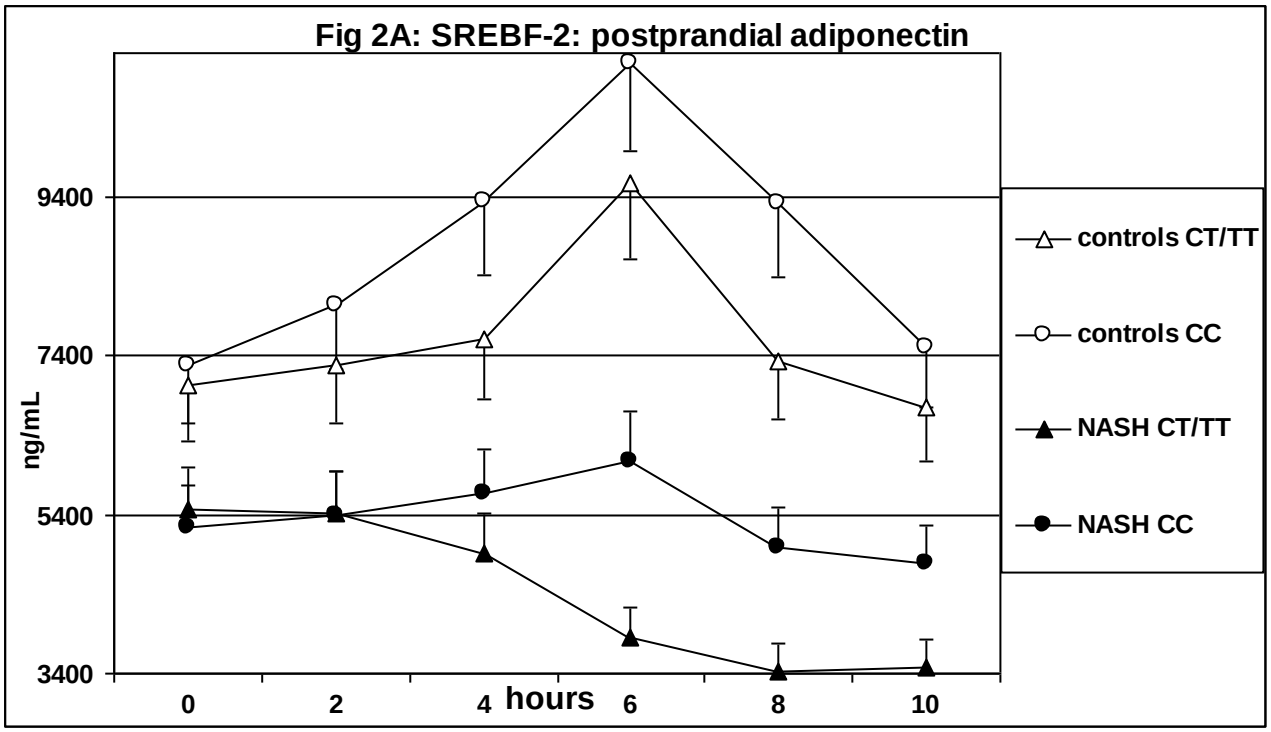
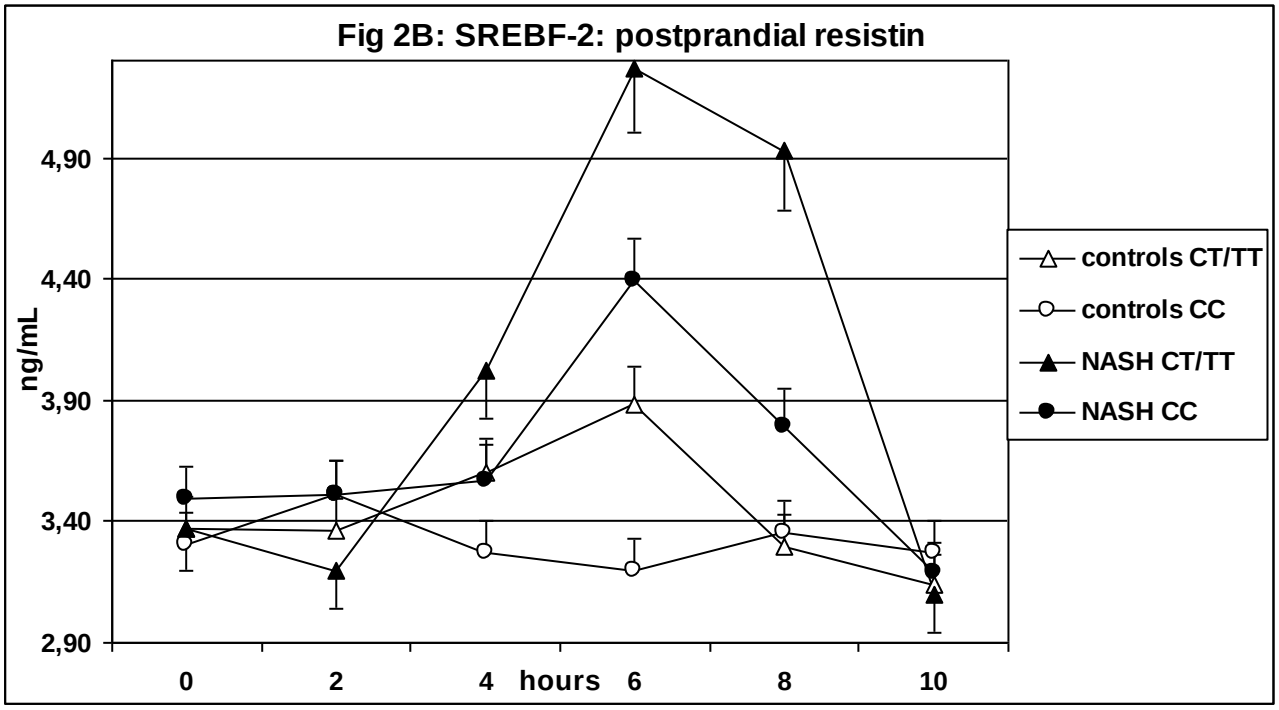


Fig 1F: SREBF-2: postprandial HDL-C
mg/dL
53
51
49
47
45
43
41
0
2
4
hours
6
8
10
controls CT/TT
controls CC
NAFLD CT/TT
NAFLD CC

Figure 2. Oral fat tolerance test: postprandial responses in serum adiponectin, resistin and cytokeratin-18 (CK-18) fragments. Data are presented as mean±SEM.





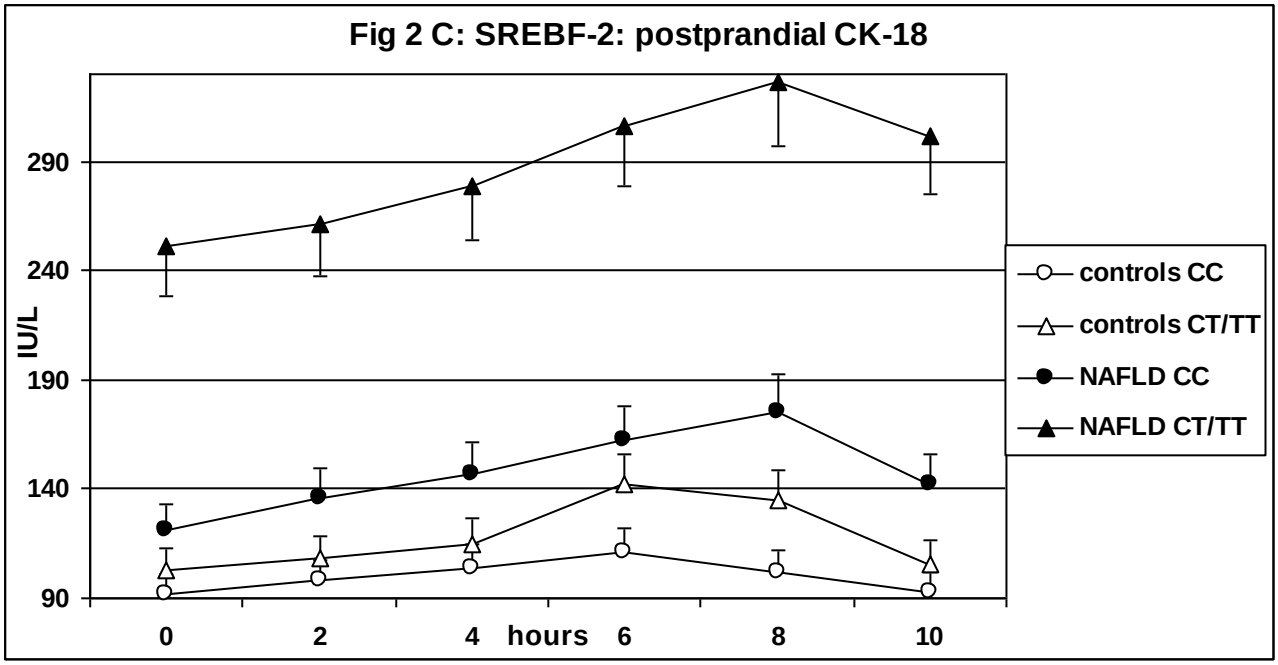


Fig 2 C: SREBF-2: postprandial CK-18
290
240
190
140
90
IU/L
0
2
4
hours
6
8
10
controls CC
controls CT/TT
NAFLD CC
NAFLD CT/TT

Table 1 Baseline, end-of-follow-up and changes during follow-up (Delta) values in main clinical and biochemical features of study subjects(n=175), grouped according to the development or not of NAFLD at the end of follow-up.

	Baseline		P	End-of-follow-up		P	Changes during follow-up (Delta values)		P
Parameter	**Non-NAFLD developers (n=127)**	**NAFLD developers (n=48)**		**Non-NAFLD developers (n=127)**	**NAFLD developers (n=48)**		**Non-NAFLD developers (n=127)**	**NAFLD developers (n=48)**	
Age(yr)	50±1	50±1	0.401	57±1	57±1	0.412	7±1	7±1	0.396
Sex(%M)	65	65	0.892	-	-	-	-	-	-
BMI(kg/m^2)	26.1±0.3	26.1±0.5	0.897	26.2±0.2	26.9±0.5	0.189	0.1±0.2	0.8±0.3	0.012
Waist (cm)	91.4±0.8	90.4±1.2	0.392	95.1±1.0	93.5±1.2	0.397	3.4±0.7	3.1±1.0	0.450
Sys BP (mmHg)	131±2	132±2	0.671	134±2	134±2	0.971	2±2	2±2	0.951
Dia BP (mmHg)	82±1	81±1	0.634	82±1	81±2	0.980	1±2	1±2	0.701
Family history of T2DM(%)	17	21	0.684	-	-	-	-	-	-
Smoking status:									
never(%)	64	66	0.812	65	66	0.934	1	1	0.779
former(%)	22	16	0.552	23	20	0.837	2	4	0.689
current(%)	14	18	0.872	12	14	0.939	-2	-4	0.690
Education:									
Primary school	68	66	0.872	-	-	-	-	-	-
Secondary school	23	24	0.901						
University	9	10	0.831						
METS(h/week)	20.8±1.0	22.1±1.7	0.258	20.0±1.6	21.2±1.6	0.382	-0.8±1.3	-0.9±1.5	0.791
CRP(mg/L)	1.7±0.2	1.8±0.4	0.845	1.9±0.2	2.9±0.4	0.017	0.3±0.2	1.2±0.3	0.015
E-selectin (ng/mL)	20.6±3.5	22.5±3.7	0.781	23.5±3.8	42.8±4.3	0.007	3.5±1.4	20.1±4.7	0.0003
ICAM1-1 (ng/mL)	198.5±8.4	201.1±8.1	0.818	215.6±9.2	258.9±9.8	0.005	16.1±8.4	58.9±8,1	0.001
Adiponectin (ng/mL)	7231±292	7170±697	0.960	7115±241	5330±491	0.001	-145±52	-1819±513	0.0001
Resistin(ng/mL)	3.34±0.15	3,15±0.25	0.841	3.61±0.31	4.11±0.43	0.382	0.4±0.2	0.5±0.2	0.791
Glucose(mg/dL)	94±2	95±2	0.501	96±3	96±3	0.873	2±2	3±2	0.315
Insulin(μU/mL)	7.4±0.5	7.5±0.6	0.302	8.6±0.5	10.6±1.0	0.09	1.1±0.6	5.5±0.4	0.03
HOMA-IR (mmol/l x μU/ml)	1.6±0.2	1.6±0.3	0.279	2.0±0.2	2.5±0.3	0.14	0.2±0.1	0.7±0.2	0.038
Total C(mg/dL)	185±4	186±6	0.998	195±4	202±6	0.172	10±1	18±3	0.130
LDL-C(mg/dL)	104±3	103±5	0.762	126±3	132±6	0.217	21±4	29±6	0.277
HDL-C(mg/dL)	61±1	62±2	0.468	51±1	52±2	0.692	-9±1	-10±2	0.749
Tg(mg/dL)	111±4	112±8	0.912	109±4	123±12	0.243	-3±4	10±8	0.086

AST(IU/L)	17±1	17±1	0.691	19±1	45±4	0.0008	2±1	23±2	0.0001
ALT(IU/L)	19±1	20±1	0.568	20±2	74±6	0.0007	2±1	58±2	0.0001
GGT(IU/L)	19±2	21±3	0.599	22±1	69±4	0.0000	3±1	51±2	0.0001
T2DM(%)	0	0	0.999	2	13	0.009	-	-	-
Obesity (%)	0	0	0.999	7	16	0.139	-	-	-
Met Sy(%)	0	0	0.999	15	18	0.314	-	-	-
abdominal obesity(%)	23	24	0.875	38	42	0.607	14	18	0.561
hypertension (%)	41	45	0.712	55	58	0.911	15	13	0.533
high FPG(%)	12	15	0.611	17	29	0.041	4	12	0.039
high Tg(%)	6	10	0.475	11	16	0.474	6	6	0.981
low HDL(%)	0	4	0.179	24	35	0.115	24	31	0.437
n-criteria for Met Sy	1.0±0.2	1.1±0.2	0.793	1.5±0.2	1.8±0.2	0.376	0.5±0.1	0.7±0.2	0.323
SREBF-2(%)									
CC	84(66%)	22(45%)	0.019	-	-	-	-	-	-
CT	41(32%)	20(42%)	0.357						
TT	2(1%)	6(13%)	0.007						
ApoE (%)									
2-3	15	14	0.812						
3-3	64	66	0.802						
3-4	21	20	0.936						
MTP(%)									
TT	15	8	0.272	-	-	-	-	-	-
TG	33	36	0.898						
GG	52	56	0.689						

The P values value refer to comparison between groups at baseline, at the end of follow-up and delta-values, respectively. Data are expressed as mean±SEM.

Abbreviations: BP: blood pressure; C: cholesterol; CRP: C-reactive protein; Dia: diastolic; HOMA-IR: homeostasis model assessment of insulin resistance; ICAM: intercellular adhesion molecule; METS: Metabolic equivalent of activity (h/week); MTP: microsomal triglyceride transfer protein; SREBF: sterol regulatory element-binding factor; Sys: systolic; T2DM: type 2 diabetes mellitus; Tg: triglycerides;

Met Sy: metabolic syndrome according to the joint statement of AHA, IDF and NHLBI, requires the presence of ≥3 of the following criteria:

-abdominal obesity: waist circumference≥102 cm(males) and ≥88 cm(females)

-high triglycerides: ≥150 mg/dL (1.7 mmol/L) or on drug treatment for elevated triglycerides

-low HDL-C: <40 mg/dL (1.0 mmol/L) (males) or <50 mg/dL (1.3 mmol/L) (females) or on drug treatment for reduced HDL-C

-hypertension: systolic BP≥130 and/or diastolic BP≥85 mm Hg or on drug treatment

-high fasting plasma glucose (FPG): FPG ≥100 mg/dL (5.6 mmol/L) or on drug treatment for elevated glucose.

Table 2. **OGTT-derived indexes of glucose homeostasis in patients with NAFLD and controls grouped according to SREBF-2 genotype.**

	Controls			**NAFLD**		
	CC (n=20)	**CT/TT (n=20)**	**P**	**CC (n=20)**	**CT/TT (n=20)**	**P**
OGIS (ml · min^{-1} · m^{-2})	493.1±21.4	435.2±13.3	**0.033**	398.2± 12.4	365.4±10.6	**0.030**
Hepatic IR	1679±173	2781±197	**0.005**	3478±280	4899±372	**0.009**
Muscle IS	0.024±0.002	0.019±0.002	**0.048**	0.018±0.002	0.012±0.001	**0.013**
Adipose IR	21.9±4.1	36.3±5.2	**0.035**	53.1±7.7	85.3±9.6	**0.013**
Hepatic extraction(%)	73±12	70±11	0.826	75±15	68±14	0.412
Insulinogic Index (IGI) ($\mu U_{insulin} \cdot g^{-1}_{glucose}$)	186±23	111±18	**0.025**	155±21	78±15	**0.008**
Disposition Index (DI) ($\mu U_{insulin} \cdot g^{-1}_{glucose} \cdot ml^{-1} \cdot m^{-2}$)	91698±4293	65685±3982	**0.004**	61535±3514	29250±2015	**0.0008**
Cp-genic Index(CGI) ($ng_{C\text{-}pep} \cdot g^{-1}_{glucose}$)	641±36	528±29	**0.031**	524±36	392±29	**0.011**
Adaptation Index (AI) ($ng_{C\text{-}pep} \cdot g^{-1}_{glucose} \cdot ml^{-1} \cdot m^{-2}$)	315027±19812	254040±16915	**0.030**	208028±15012	147000±13912	**0.009**

Data are presented as mean ± SEM. Differences were considered statistically significant at $p<0.05$.

Statistically significant P values are written in bold characters.

Abbreviations: OGIS: oral glucse insulin sensitivity index; IR: insulin resistance; IS: insulin sensitivity:

IGI: insulinogenic index= Δinsulin30'/Δglucose30' during the OGTT; CGI (Cpgenic index)= ΔC-peptide30'/Δglucose30' during the OGTT;

Disposition Index and Adaptation Index were computed by multiplying IGI or CGI x OGIS, respectively.

Hepatic extraction is the % secreted insulin extracted by the liver.

Table 3. **Oral fat load parameters in patients with NAFLD and controls grouped according to SREBF-2 genotype.**

	Controls			NAFLD		
Parameter	**CC (n=20)**	**CT/TT (n=20)**	**P**	**CC (n=20)**	**CT/TT (n=20)**	**P**
Fasting Tg(mg/dL)	72±11	78±10	0.915	85±10	96±13	0.531
IAUC Tg (mg/dL x hr)	56±12	167±26	**0.006**	291±44	514±67	**0.010**
Fasting FFA (Mol/L)	0.45±0.23	0.58±0.28	0.798	0.78±0.31	0.93±0.36	0.713
IAUC FFA (Mol/L x hr)	0.40±0.11	1.02±0.25	**0.037**	4.42±0.64	7.00±0.79	**0.013**
Fasting VLDL1-Tg (mg/dL)	37±9	46±10	0.953	32±8	27±8	0.825
IAUC VLDL1-Tg (mg/dL x hr)	297±29	638±62	**0.002**	828±77	1279± 107	**0.005**
Fasting VLDL2-Tg (mg/dL)	46±7	53±8	0.795	42±4	46±5	0.792
IAUC VLDL2-Tg (mg/dL x hr)	89±10	115±13	0.312	166±79	179±74	0.902
Fasting VLDL1-Ch (mg/dL)	12,8±2.4	11.7±2.01	0.953	15.7±3.4	17.2±3.7	0.315
IAUC VLDL1-Ch (mg/dL x hr)	16.4±4.1	45.3±7.4	**0.006**	73.9±12.3	156.9±19.4	**0.0008**
Fasting VLDL2-Ch (mg/dL)	21.0±2.5	22.7±2.9	0.997	21.9±2.9	23.4±3.2	0.738
IAUC VLDL2-Ch (mg/dL x hr)	1.2±1.1	3.0±1.8	0.401	1.5±1.6	1.9±1.6	0.899
Fasting VLDL1 ApoB48 (mg/dL)	1.89±0.43	2.01±0.57	0.791	1,79±1.95	2.40±1.38	0.459
IAUC VLDL1 ApoB48 (mg/dL x hr)	1.35±0.93	5.24±0.90	**0.012**	8.09±1.38	16.02±2.82	**0.003**

Fasting VLDL2 ApoB48 (mg/dL)	0.52±0.29	0.80±0.41	0.392	0.97±0.51	1.36±0.52	0.749
IAUC VLDL2 ApoB48 (mg/dL x hr)	2.81±0.47	3.39±0.71	0.367	2.56±0.49	4.92±1.23	0.219
Fasting VLDL1 ApoB100 (mg/dL)	3.59±1.12	4.82±1.88	0.512	5.32±1.51	5.98±2.01	0.901
IAUC VLDL1 ApoB100 (mg/dL xhr)	2.01±0.42	6.21±1.03	**0.012**	9.01±1.43	19.21±2.93	**0.003**
Fasting VLDL2 ApoB100 (mg/dL)	1.51±0.75	1.90±0.43	0.999	3.01±0.49	4.23±0.93	0.302
Fasting LDL C.D. (uA 234 nm/uA 200 nm x 100)	7.26±1.72	7.49±1.80	0.823	7.65±2.18	7.30±2.04	0.911
IAUC LDL C.D. (uA 234 nm/uA 200 nm x 100 x hr)	0.22±0.10	2.31±0.23	**0.006**	5.89±1.01	15.42±3.78	**0.0009**
Fasting HDL-C(mg/dL)	52±2	49±2	0.329	53±2	49±1	0.138
IAUC HDL-C(mg/dL x hr)	-4±1	-16±2	**0.002**	-30±3	-49±4	**0.0009**
Fasting apoA1 (mg/dL)	121±9	116±8	0.399	115±9	92±7	0.183
IAUC apoA1 (mg/dL x hr)	-11±6	-20±10	**0.012**	-26±13	-100±24	**0.001**
Fasting adiponectin (ng/mL)	7598±982	6982±739	0.499	5231±4053	5453±4577	0.968
IAUC adiponectin (ng/mL x hr)	13508±1190	7156±894	**0.002**	1924±969	-10798±636	**0.0008**
Fasting resistin(ng/mL)	3.3±0.9	3.4±1.0	0.902	3.5±1.0	3.4±0.9	0.943
IAUC resistin (ng/mL x hr)	0.2±0.1	1.1±0.3	**0.012**	3.5±0.9	7.6±1.5	**0.027**
Fasting CK-18(I.U./L)	82±8	103±11	0.319	131±15	252±31	**0.006**
IAUC CK-18 (I.U./L x hr)	95±12	179±15	**0.031**	298±19	387±26	**0.011**

Oral fat load parameters of patients with NAFLD and controls according to SREBF-2 genotype. Data are presented as mean ± SEM. Statistically significant P values are written in bold characters.

Abbreviations: IAUC: incremental area under the curve; FFA: free fatty acids; Tg: triglyceride; C.D. : conjugated dienes; Ch: cholesterol;

Table 4 Multiple regression analysis: statistically significant predictors of parameters related to glucose and lipid metabolism in biopsy-proven NAFLD subjects and matched controls (n=80).

Glucose homeostasis			
Dependent variable	**Independent variables**	**β (95% CI)**	**P**
OGIS	SREBF-2	-0.47(-0.52, -0.42)	0.009
	IAUC VLDL1-Ch	0.38(0.33, 0.42)	0.020
Hepatic IR	SREBF-2	0.45 (0.40, 0.53)	0.006
Adipose tissue IR	SREBF-2	0.51(0.46-0.56)	0.008
	IAUC VLDL1-Ch	0.41 (0.36, 0.47)	0.012
Muscle IS	SREBF-2	-0.42(-0.37,-0.47)	0.012
Insulinogenic Index(IGI)	IAUC adiponectin	0.49 (0.42-0.58)	0.013
	IAUC VLDL1-Ch	-0.48(-0.43,-0.54)	0.010
	IAUC LDL C.D.	-0.50(-0.45, -0.56)	0.009
Disposition Index (DI)	SREBF-2	-0.44 (-0.50, 0.39)	0.010
	IAUC VLDL1-Ch	-0.51 (-0.56, -0.46)	0.005
	IAUC LDL C.D.	-0.48 (-0.42, -0.54)	0.010
CP-genic Index (CGI)	SREBF-2	-0.46 (-0.53, -0.41)	0.009
	IAUC VLDL1-Ch	-0.50 (-0.45, -0.51)	0.004
Adaptation Index (AI)	SREBF-2	-0.45 (-0.50, -0.40)	0.010
	IAUC VLDL1-Ch	-0.49 (-0.44,-0.54)	0.009
Oral fat tolerance test			
IAUC triglycerides	SREBF-2	0.50(0.44-0.57)	0.009
IAUC FFA	Adipose IR index	0.48 (0.43, 0.54)	0.010
	IAUC adiponectin	0.51(0.46-0.57)	0.005
IAUC VLDL1-Tg	SREBF-2	0.49(0.42-0.57)	0.007
IAUC VLDL1-Ch	SREBF-2	0.52(0.47-0.57)	0.007
IAUC VLDL1-apoB100	SREBF-2	0.51(0.46-0.57)	0.002
IAUC VLDL1-apoB48	SREBF-2	0.50(0.45-0.55)	0.008
IAUC LDL conjugated dienes	IAUC VLDL1-Tg	0.49(0.43-0.55)	0.010

IAUC HDL-C	SREBF-2 IAUC VLDL1-Tg	0.51(0.46-0.57) 0.49(0.44-0.54)	0.008 0.009
IAUC apoA1	SREBF-2	0.53(0.49-0.58)	0.003
IAUC adiponectin	SREBF-2 Fasting adiponectin	0.50 (0.45, 0.56) 0.47(0.42-0.54)	0.009 0.014
IAUC resistin	SREBF-2 IAUC VLDL1-Ch	0.48 (0.43, 0.52) 0.46(0.42-0.50)	0.011 0.010
IAUC CK-18 fragments	SREBF-2 IAUC adiponectin Fasting CK-18 fragments	0.46 (0.41-0.51) 0.49(0.43-0.55) 0.45(0.40-0.51)	0.011 0.010 0.021

Abbreviations: OGIS: oral glucose insulin sensitivity index; IR: insulin resistance; IS; insulin sensitivity;

FFA: free fatty acids; VLDL: very low density lipoprotein;CK-18: cytokeratin-18;

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