Original Article

Polymorphisms rs2167444 and rs508384 in the *SCD1* Gene Are Linked with High ApoB-48 Levels and Adverse Profile of Cardiometabolic Risk Factors

(apolipoprotein B-48 / cardiometabolic risk factors / metabolic syndrome components / small-dense LDL / oxidative stress / fatty acid desaturases / *SCD1* polymorphisms)

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Abstract. Elevated plasma concentration of apolipoprotein B-48 (apoB-48) is an independent risk factor of cardiovascular disease. Stearoyl-CoA desaturase-1 (SCD1) is a rate-limiting lipogenic enzyme and a key regulator of fuel metabolism. The aim of this study was to analyse associations between clinical, biochemical, and genetic factors and different apoB-48 levels in subjects at increased cardiometabolic risk. We examined 220 subjects exhibiting at least one metabolic syndrome (MetS) component. In conjunction with basic clinical, anthropometric and laboratory measurements, we analysed various polymorphisms of stearoyl-CoA desaturase-1 (*SCD1*). Subjects were divided into two groups according to the median apoB-48 level: (1) high apoB-48 (≥ 7.9 mg/l, N = 112) and (2) low apoB-48 (< 7.9 mg/l, N = 108). Neither group differed significantly in anthropometric measures. High plasma apoB-48 levels were associated with increased systolic blood pressure (+3 %; P < 0.05), MetS prevalence (59.8 vs. 32.4 %; P < 0.001), small-dense LDL frequency (46.4 vs. 20.4 %; P < 0.001), triglycerides (+97 %; P < 0.001), non-HDL-cholesterol (+27 %; P < 0.001), and lower concentrations of HDL-cholesterol (–11 %; P < 0.01). This group was further characterized by a higher HOMA-IR index (+54 %; P < 0.001) and increased concentrations of conjugated dienes (+11 %; P < 0.001) and oxidatively modified LDL (+ 38 %; P < 0.05). Lower frequencies of SCD1 minor genotypes (rs2167444, rs508384, P < 0.05) were observed in subjects with elevated plasma concentrations of apoB-48. Elevated plasma concentrations of apoB-48 are associated with an adverse lipid profile, higher systolic blood pressure, insulin resistance, and oxidative stress. Lower proportions of minor SCD1 genotypes (rs2167444, rs508384) implicate the role of genetic factors in the pathogenesis of elevated levels of apoB-48.

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Abbreviations: ALT – alanine aminotransferase, apoB-48 – apoprotein B-48, BMI – body mass index, BP – blood pressure, CM – chylomicrons, CMR – chylomicron remnants, DM – diabetes mellitus, D6D – D6 desaturase, D9D – D9 desaturase, FA – fatty acid, GGT – γ-glutamyl transferase, HDL – high-density lipoproteins, HOMA-IR – homeostatic index of insulin resistance, IDL – intermediate-density lipoprotein, LDL – low-density lipoproteins, MetS – metabolic syndrome, NEFA – non-esterified fatty acids, ox-LDL – oxidized LDL, PAD – peripheral artery disease, SCD1 – stearoyl-CoA desaturase-1, sd-LDL – small dense LDL, SNP – single-nucleotide polymorphism, TG – triglycerides, TRL – triglyceride-rich lipoproteins, VLDL – very low-density lipoproteins, WC – waist circumference.

Introduction

Apolipoprotein B-48 (apoB-48) is a specific structural component of chylomicrons (CM) and their remnants (CMR). In humans, the apoB-48 protein is synthesized only in enterocytes and co-linear with 2152 aminoterminal residues of apoB-100. ApoB-48 mRNA is synthesized as a result of post-transcriptional enzymatic deamination, whereby cytidine in the CAA codon (for glutamine) is exchanged for uracil, creating a premature stop codon (UAA) in apoB RNA editing. The apoB mRNA editing enzyme consists of several factors. Among the most important of these are catalytic deaminase APOBEC-1 and APOBEC-1 complementation factor (Fazio and Linton, 2015). CM particles and CMR always contain one molecule of apoB-48. Accordingly, plasma concentrations

of apoB-48 provide information about the number of lipoprotein particles of intestinal origin (Sakai et al., 2003; Nakajima and Tanaka, 2018).

Triglyceride-rich lipoproteins (TRL), such as CM and CMR as well as VLDL and their remnants (intermediate density lipoprotein, IDL), containing apoB-100, are important, independent risk factors of atherosclerotic cardiovascular disease (Nakajima and Tanaka, 2018). Many experimental and clinical studies have proved that CMR directly or indirectly correlate with initiation and progression of atherosclerosis due to their accumulation in the arterial wall, enhancement of systemic inflammation, platelet activation, and thrombus formation. Remnant lipoproteins can directly penetrate the arterial wall, infiltrate the sub-endothelial space, and accelerate foam cell formation. The adverse effects of CMR increase instability and progression of atherosclerotic plaque (Masuda and Yamashita, 2017). The apoB-48 concentration correlates with intima-media thickness of the common carotid artery endothelial dysfunction and with coronary heart disease. Fasting serum apoB-48 levels are reported to be an independent predictor of peripheral artery disease (PAD) in patients with type 2 diabetes. Out of several risk factors, such as LDL-cholesterol, HDL-cholesterol, HBA1c, diabetes mellitus (DM) duration, smoking, and systolic blood pressure, only apoB-48 has been identified as an independent predictor of PAD (Chan et al., 2012; Lapice et al., 2012; Mancera-Romero et al., 2013). High fasting serum apoB-48 concentration is understood to be a strong, independent predictor of both new onset and chronic coronary artery disease, irrespective of the LDL-cholesterol level (Masuda et al., 2012; Mori et al., 2013). ApoB-48 has been shown to be a marker associated with coronary lesion progression in post-percutaneous coronary intervention patients undergoing LDL-lowering therapy (Mori et al., 2013). Other studies report higher fasting apoB-48 concentrations in men compared to women, and high apoB-48 levels in subjects with obesity, dyslipidaemia, MetS, hypothyroidism, chronic kidney disease , and DM type 2 (DM2T) (reviewed in Masuda and Yamashita, 2017; Nakajima and Tanaka, 2018).

Stearoyl-coenzyme A desaturase 1 (SCD1) (EC:1.14. 19.1) is a main regulator of fuel metabolism, body weight control, and central lipogenic enzyme activity (Uto, 2016). In humans and other species, SCD1 is a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids from their saturated fatty acid precursors. Two isoforms in the human genome (1 and 5) are mapped to chromosome 10q24.31. The SCD1 isoform is highly expressed in lipogenic tissues, mainly in the liver and adipose tissue, while isoform SCD5 is primarily expressed in the brain and pancreas (Uto, 2016). SCD1 and Δ 9 desaturase (D9D) mainly catalyse conversion of palmitic (C16:0) and stearic (C18:0) acids to palmitoleic (C16:1n-7) and oleic (C18:1n-9) acids (Tan et al., 2015; AlJohani et al., 2017). The *SCD1* gene expression is regulated by several intrinsic (genetic, hormonal) and extrinsic factors (diet, alcohol consumption, smoking, physical activity) (Poloni et al., 2015; Kamal et al., 2018). Experimental studies in mice (either with naturally occurring *scd1* or transgenic *scd1* deletions) show that *scd1* deficiency leads to reduced body weight adiposity, increased insulin sensitivity, and resistance to diet-induced obesity. These studies also report up-regulation of several genes controlling lipid oxidation and down-regulation of lipid synthesis genes (Paton and Ntambi, 2009). On the other hand, over-expression of *SCD1* is associated with numerous metabolic disorders, such as obesity, DM2T, arterial hypertension, dyslipidaemia, and non-alcoholic fatty liver disease (Koeberle et al., 2016; Kamal et al., 2018).

To measure SCD1 activity, human studies use surrogate marker D9D (ratios of palmitoleic (16:1n-7) to palmitic (16:0) acid or oleic (18:1n-9) to stearic (18:0) acid) using various biological matrices, such as plasma lipids, erythrocyte membranes, and adipose tissue. Increased activity of D9D (representing SCD1) has been reported in obesity-related conditions, such as DM2T, MetS, arterial hypertension, malignancies, and depressive disorders (Warensjö et al., 2008; Assies et al., 2010; Vareka et al., 2012; Mahendran et al., 2014). Fatty acid (FA) metabolism and composition are subject to genetic regulation and can be modified by many factors including gender, age, ethnicity, health status, and diet (Saponaro et al., 2015).

Several studies have shown that some gene polymorphisms in the *SCD1* gene are associated with various pathological conditions. British authors found a borderline association between a single-nucleotide polymorphism (SNP) of *SCD1* (rs41290540) and the risk of DM2T (Liew et al., 2004). Swedish authors reported an association of four SNPs of SCD1 (rs10883463, rs7849, rs2167444, and rs508384) with body mass index (BMI), waist circumference (WC), and insulin sensitivity. Their rare allele carriers had lower BMI and WC as well as improved insulin sensitivity, with the rare allele rs7849 exerting the strongest effect on WC and insulin sensitivity (Warensjö et al., 2007). A large population-based study in Costa Rica proved association of an *SCD1* polymorphism (rs1502593) and prevalence of MetS (Gong et al., 2011). Canadian authors found a positive association between CRP levels, concentrations of palmitic acid (16:0), and the SCD1 index for stearic acid (18 : 1/ 18 : 0) in European and Asian females. Of the nine *SCD1* polymorphisms analysed, only SNP rs2060792 was associated with concentrations of 16:0 and 18:0 in females of European descent. Additionally, the same SNP was associated with CRP levels in both ethnic groups of females (Stryjecki et al., 2012). In the EPIC-Potsdam Study, seven *SCD1* polymorphisms (rs1502593, rs522951, rs11190480, rs3071, rs3793767, rs10883463, and rs508384) were investigated in relation to BMI, WC, fetuin-A, triglycerides (TG), glycated haemoglobin (HBA1c), high-sensitivity C-reactive protein (hs-CRP), γ-glutamyltransferase (GGT), and alanine aminotransferase (ALT). Although rs10883463 was initially weakly associated with TG, GGT, HBA1c, and rs11190480,

these relationships became statistically non-significant after multiple testing (Arregui et al., 2012).

To our knowledge, associations between *SCD1* polymorphisms and apoB-48 concentrations have yet to be studied. The aim of this pilot study was to analyse basic clinical and laboratory measurements in relation to selected SNPs of the *SCD1* gene in patients at elevated cardiometabolic risk with different plasma apoB-48 concentrations.

Material and Methods

Study design and participants

This cross-sectional pilot study was carried out at the 4th Department of Medicine of the General University Hospital, Prague. The study protocol was approved by the Ethics Committee of the General University Hospital, Prague. Written informed consent was obtained from all participants prior to inclusion. The study was performed in accordance with the Declaration of Helsinki.

We examined a group of 220 Caucasian subjects at increased cardiometabolic risk from April 2014 to October 2015 at the Lipid and Diabetology Clinic of the 4th Department of Medicine. The group consisted of 104 men and 116 women, of whom 79 (68 %) were postmenopausal. Each subject had at least one MetS-related trait, with 164 persons (74.5 %) categorized as overweight or obese (BMI ≥ 25.0 or 30 kg/m^2, respectively), with the same prevalence of abdominal fat distribution (waist circumference ≥ 94 cm and ≥ 80 cm in men and women, respectively). Increased blood pressure (BP) (or treatment of previously diagnosed arterial hypertension) was observed in 157 persons (71 %), and hyperuricaemia (uric acid ≥ 420 μmol/l) in 26 persons (11.8 %). Impaired fasting glycaemia was observed in 57 patients (57.9 %), hypertriglyceridaemia (TG ≥ 1.70 mmol/l) in 101 patients (46 %), and decreased HDL-C (< 1.03 and < 1.30 mmol/l for men and women, respectively) in 42 patients (19 %). All subjects had maintained a stable body weight in the preceding three months.

As our examination group did not contain healthy controls, we used the median apoB-48 concentration of all subjects examined as the cut-off level. The median value for apoB-48 was estimated at 7.9 mg/l (see Fig. 1).

MetS was diagnosed according to the International Diabetes Federation criteria (IDF, 2005) (Alberti et al., 2006). The number of MetS components and related risk factors was determined based on current definitional criteria: WC, concentrations of TG and HDL-cholesterol (HDL-C), increased BP (or treatment of previously diagnosed arterial hypertension), impaired fasting glycaemia (or previously diagnosed type 2 diabetes mellitus), hyperuricaemia (> 420 μmol/l), and predominance of small-dense LDL (pattern B of the LDL phenotype) (Das, 2010).

Basic clinical data and anthropometric parameters (body weight, height, waist circumference, thickness of four skin folds) were examined in all individuals using

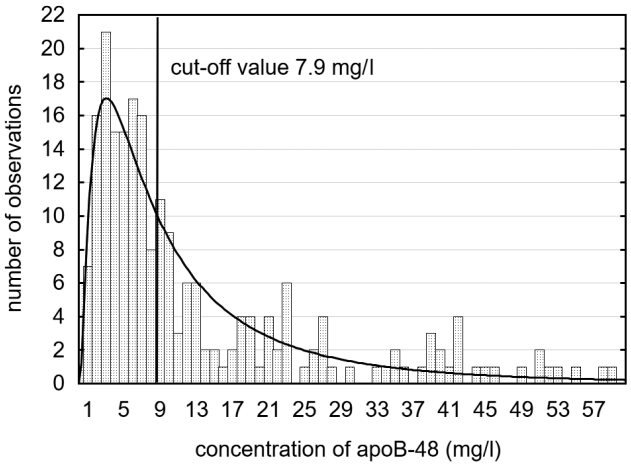

Fig. 1. Distribution of apoB-48 concentrations in the group studied

methods recommended by the Airlie Conference (Lohman et al., 1988). Body fat percentages were determined according to the Durnin and Womersley formula (Durnin and Womersley, 1974). Fat mass was calculated by multiplying body mass by the percentage ratio of fat mass.

Exclusion criteria were as follows: age < 18 years, unstable clinical condition (including body weight), current antioxidant therapy (e.g., vitamin C, vitamin E, allopurinol, N-acetylcysteine), immunosuppressive and/or anti-inflammatory therapy, supplementation with n-3 and/or n-6 polyunsaturated fatty acids, chronic kidney disease (creatinine > 150 μmol/l; urinary protein > 500 mg/l), liver cirrhosis, decompensated DM, endocrine disease, and concomitant malignancies (or their treatment). Further criteria for exclusion included: acute pancreatitis or acute relapse of chronic pancreatitis, unstable angina pectoris, acute myocardial infarction occurring less than one year prior to the study, coronary aortic bypass grafting, percutaneous coronary intervention, and stroke. Persons operated on in the gastrointestinal tract (within the previous year) and subjects having experienced systemic inflammation (within the previous six months) were also excluded as previously described (Zak et al., 2014; Zeman et al., 2017).

Laboratory measurements

Blood samples from all participants were obtained after overnight fasting (for at least 12 h). Plasma was immediately cooled to 4–6 °C and separated within 30 min at the same temperature at 1,000 *g* for 10 min. In line with routine biochemical and haematological analysis, samples were measured immediately according to standardized enzymatic-colorimetric methods, with samples requiring further analysis stored at –80 °C.

Concentrations of LDL-C were calculated according to Friedewald's equation based on the recommended criteria. Given that 35 patients had concentrations of TG above 2.5 mmol/l, LDL-C concentrations are not presented in Table 2.

Concentrations of insulin were measured using diagnostic sets and a modular analyser (Roche Diagnostic, Indianapolis, IN) by electrochemiluminescence (ECLIA). Levels of hs-CRP were measured using the hs-CRP Human ELISA kit (BioVendor, Brno, Czech Republic). Concentrations of apoB-48 were determined using a diagnostic set Human Apo B-48 ELISA Kit (Shibayagi Co., Ltd., Shibukawa, Gunma, Japan).

Subfractions of LDL were analysed by high-performance discontinued gel electrophoresis using polyacrylamide gel tubes (Lipoprint® LDL System, Quantimetrix, Redondo Beach, CA). LDL particles were separated into seven subfractions (LDL1 to LDL7). LDL1 and LDL2 subfractions represented large (buoyant) particles, while LDL3–7 represented small-dense LDL (sd-LDL). Concentrations of cholesterol in sd-LDL over 6 mg/dl or a peak LDL particle diameter ≤ 26.8 nm denoted phenotype pattern B with a predominance of sd-LDL (Gazi et al., 2006). Profiles of fatty acids (FA) were measured by chromatographic methods (Tvrzicka et al., 2002).

To measure conjugated diene concentrations in precipitated LDL, a modified spectrophotometric method was used (Ahotupa et al., 1996). Concentrations of circulating, oxidized LDL (ox-LDL) were measured by the ELISA kit "Oxidized LDL" from Mercodia (Uppsala, Sweden). Concentrations of hs-CRP were measured using the hs-CRP Human ELISA reagent kit (BioVendor).

Single-nucleotide polymorphism selection and genotyping

Single-nucleotide polymorphisms (SNPs) of two genes – apoE (*APOE*) and stearoyl-CoA desaturase-1 (*SCD1*) – were analysed in all individuals. We selected a total of eight *SCD1* SNPs for genotyping based on the following parameters in the case of European Caucasian population: frequency [HapMap Rel 24-phaseII, NCBI B36 assembly, dbSNP b126; chr10:102089500-1022114800], known and implicated functional effect (Warensjö et al., 2007; Merino et al., 2010; Gong et al., 2011; Stryjecki et al., 2012; Martin-Núñez et al., 2013; Tosi et al., 2014), and positioning and spacing along the *SCD1* gene. Nearly all of the SNPs selected (except for rs641996) exhibited a minor allele frequency > 0.05.

DNA samples were isolated from peripheral blood leukocytes according to standard desalting procedures (Miller et al., 1988). As essential parameters, DNA concentration and purity were assessed (Nanodrop ND 1000, Thermo Fisher Scientific, Wilmington, DE) in advance of subsequent methods.

Polymorphisms of *apoE* and *SCD1* were performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. In cases where the respective endonuclease or restrictive enzyme site was not found, direct DNA sequencing was employed (Sanger and Coulson, 1975; Sanger et al., 1977) using a genetic analyser (Applied Biosystems® 3500 Series Genetic Analyzer, Foster City, CA).

Isoforms of apolipoprotein E (*apoE*) were determined using the RFLP method based on a modified analytical procedure as previously described (Jáchymová et al., 2001; Zak et al., 2007). Finally, fragments were detected by electrophoresis in 3% agarose gel with the addition of ethidium bromide, and visualized under a UV lamp. Each sample was verified independently by two team members. Reaction conditions for genetic analysis of the *SCD1* gene are given in Table 1.

The homeostasis model assessment method (HOMA-IR) was used as the index of insulin resistance (Matthews et al., 1985).

Desaturase activity was calculated as the product/substrate ratio in respect of two indices of Δ 9 desaturase

Table 1. Analytic conditions for detection of polymorphisms in the SCD1 gene

Polymorphism	**Forward primer 5′→3′** **Reverse primer 3′→5′**	**Annealing (°C)**	**Method:** **RFLP, ds**
rs2167444 T>A	5 – gggagtttcttttggctgtg 3 – caagttgccagctggtgtta	59.8	AflII, SmlI
rs508384 C>A	5 – gggagtttcttttggctgtg 3 – caagttgccagctggtgtta	59.8	HgaI, BmsFI
rs7849 T>C	5 – agggtcactgaaccactgct 3 – ccagagagaggggactgaaa	59.5	ds
rs641996 T>C	5 – ggtggccatgagttcaaagt 3 – gattagggtggcaggaaaca	59.9	ds
rs55710213 C>T	5 – ggtggccatgagttcaaagt 3 – gattagggtggcaggaaaca	59.9	ds
rs56334587 C>T	5 – ggtggccatgagttcaaagt 3 – gattagggtggcaggaaaca	59.9	ds
rs10883463 T>C	5 – ggtggccatgagttcaaagt 3 – gattagggtggcaggaaaca	59.9	ds
CTTC deletion	5 – ggtggccatgagttcaaagt 3 – tttggcagagaagatgacca	59.8	ds

Legend: Gene polymorphisms and allelic variants are denominated according to the data obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).
Abbreviations: ds – direct sequencing

(D9D), e.g., D9D16 as the ratio 16:1n-7/16:0 and D9D18 as the ratio 18:1n-9/18:0. D6D activity was calculated as the ratio 18:3n-6/18:2n-6 and D5D as the ratio 20:4n6/20:3n-6 (Zak et al., 2007; Tosi et al., 2014).

Statistical methods

STATISTICA® software for Windows was used for statistical evaluation of the results. Categorical values are given in absolute values and as percentages, continuous values are expressed as mean and standard deviations (median, 25th and 75th percentiles, respectively).

Differences between continuous variables were analysed using Student's t-test or Mann-Whitney U test. Distribution of the categorical values was assessed using the χ^2-test. Frequencies of all genotypes and alleles were calculated, and analysed according to the Hardy-Weinberg principle using the χ^2-test. Pearson's χ^2-test was used to analyse the frequency differences of individual genotypes and allelic variants (with Yates' correction for small numbers). Statistical significance was set at $P < 0.05$ using the Benjamini-Hochberg procedure for multiple comparisons.

Results

Table 2 shows the clinical and biochemical characteristics of the patients divided into two groups, with plasma concentrations of apoB-48 above and below the median value (7.9 mg/l). Neither of the groups differed with regard to the age, male/female ratio, proportion of

Table 2. Basic clinical and biochemical parameters of the groups

	ApoB-48 < 7.9 (mg/l)	**ApoB-48 ≥ 7.9 (mg/l)**
Number of subjects	108	112
Males N (%)	44 (40.7)	60 (53.6) NS
Age (years)	49.9 ± 14.7[a]	52.9 ± 14.1
Probands with MSC[e] ≥ 3 N (%)	35 (32.4)	67 (59.8)[c***]
LDL subclass phenotype B N (%)	22 (20.4)	52 (46.4)[c***]
BMI (kg/m^2)	28.6 ± 5.7	29.4 ± 5.8
Waist circumference > 94/80 cm	78 (72 %)	86 (77 %)[c] NS
Waist circumference (cm)	94.6 ± 15.2	98.9 ± 14.8[b] NS†
Waist circumference men (cm)	100.0 ± 12.5	103.4 ± 13.8[b] NS†
Waist circumference women (cm)	90.5 ± 16.0	93.7 ± 14.5[b] NS†
SBP (mmHg)	131 ± 15	135 ± 18[b*]
DBP (mmHg)	87 ± 10	86 ± 11
Fat mass (kg)	32.5 ± 10.9	33.2 ± 10.8
TC (mmol/l)	5.08 ± 1.04	5.78 ± 1.35[b***]
TG (mmol/l)	0.99 [0.74–1.33]	1.95 [1.40–2.70][d***]
HDL-C (mmol/l)	1.59 ± 0.47	1.36 ± 0.36[b***]
Non-HDL-C (mmol/l)	3.49 ± 0.96	4.42 ± 1.22[b***]
ApoA1 (g/l)	1.52 ± 0.33	1.40 ± 0.28[b**]
ApoB-100 (g/l)	1.06 ± 0.31	1.28 ± 0.34[b***]
ApoB-48 (mg/l)	4.4 [2.9–6.1]	18.3 [10.6–29.2][d***]
NEFA (mmol/l)	0.54 [0.35–0.71]	0.53 [0.39–0.71]
sd-LDL-C (mg/dl)	2.0 [0.0–5.0]	5.0 [2.0–17.0][d***]
Glucose (mmol/l)	5.08 ± 0.64	5.20 ± 0.66
HOMA-IR (index)	1.654 [1.249–2.824]	2.554 [1.692–3.830][d***]
Insulin (µU/ml)	7.48 [6.05–12.55]	10.55 [7.29–15.64][d**]
CD (µmol/l)	60.7 ± 15.4	67. 8 ± 16.8[b***]
ox-LDL (U/l)	39.3 [18.7–58.6]	54.4 [29.2–71.3][d**]
hs-CRP (mg/l)	2.17 [1.03–4.20]	2.32 [1.13–4.10]
Uric acid (µmol/l)	297 ± 83	335 ± 91[b***]

Legends and abbreviations: [a] values are presented as means ± SD or medians [25th–75th percentiles]; [b] Student's t-test; [c] χ^2 test; [d] Mann-Whitney U test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; [e] MSC – metabolic syndrome components: waist circumference > 94/80 cm (M/F); TG > 1.70 mmol/l; HDL-C < 1.00/1.30 mmol/l (M/F); BP > 130/85 mmHg (or specific antihypertensive treatment); glucose > 5.60 mmol/l (or DM type 2 treatment); hyperuricaemia > 420 µmol/l; small-dense LDL (sdLDL-C > 6 mg/dl);
† using multilinear regression with gender x group interaction;
CD – conjugated dienes in LDL; DBP – diastolic BP; F – female; hs-CRP – high-sensitivity C-reactive protein; LDL-C – low-density lipoprotein cholesterol; M – male; N – number; SBP – systolic BP.

postmenopausal women (33 vs. 45 %, NS), BMI, fat mass, or concentrations of glucose, NEFA or hs-CRP. The two groups exhibited a similar prevalence of hypertension (73.2 vs. 69.4 %; P = 0.537), obesity/overweight (76.8 vs. 72.2 %; P = 0.437), and atherosclerotic cardiovascular complications [coronary heart disease /stroke/ peripheral vascular disease] (13.4 vs. 6.5 %; P = 0.088). We found no differences in apoB-48 concentrations between men and women [9.34 (4.70–22.80) vs. 7.10 (4.14–12.60), median (25^{th} – 75^{th} percentile), NS] or between pre- and post-menopausal women [6.94 (5.17–9.26) vs. 7.14 (3.82–18.51), median (25^{th}–75^{th} percentile), NS].

The group with apoB-48 ≥ 7.9 mg/l was characterized by increased systolic blood pressure, higher frequency of patients with three or more components of MetS (MSC ≥ 3), higher proportions of smokers (36.6 vs. 22.2 %; P < 0.05) and MetS patients, impaired fasting glucose (32.1 vs. 19.4 %; P < 0.05), hyperuricaemia (> 420 μmol/l) (9.4 vs. 5.6 %, P < 0.05), hypertriglyceridaemia (67.9 vs. 23.1 %; P < 0.001), and lower HDL-C (25 % vs. 13 %, P < 0.05). Laboratory parameters for the group with apoB-48 ≥ 7.9 mg/l were characterized by higher concentrations of TC, TG, and non-HDL-C. We also observed significantly higher concentrations of apoB-100 and apoB-48 and lower concentrations of apoA1. In this group, hyperinsulinaemia was associated with increased insulin resistance index values (HOMA-IR). Higher concentrations of apoB-48 were associated with increased concentrations of cholesterol in sd-LDL and more frequent instances of LDL subclass phenotype B (46.4 vs. 20.4 %, P < 0.001). Based on the surrogate parameters of oxidative stress analysed, the group with higher concentrations of apoB-48 exhibited increased concentrations of conjugated dienes in LDL (CD-LDL) and oxidatively modified LDL particles (ox-LDL).

Concentrations of apoB-48 across the entire group showed significant correlations with TG (r = 0.440, Spearman's rank correlation coefficient; P < 0.001), sd-LDL (r = 0.621; P < 0.001), total cholesterol (r = 0.466; P < 0.001), non-HDL-C (r = 0.419; P < 0.001), and conjugated dienes in LDL (r = 0.243; P < 0.001). Moreover, apoB-48 levels correlated negatively with HDL-C (r = –0.176; P < 0.01).

In the group with apoB-48 ≥ 7.9 mg/l (in comparison with those with low apoB-48), analysis of fatty acid profiles in plasma phosphatidylcholines revealed an increased D9D activity index for palmitic acid (0.0197 ± 0.006 vs. 0.0178 ± 0.0050; 16:1n-7/16:0 ratio; P < 0.001), an increased D6D activity index (0.0051 ± 0.0032 vs. 0.0043 ± 0.0017; 18:2n-6/18:3n-6 ratio; P < 0.05), and a decreased D5D activity index (3.2610 ± 0.8857 vs. 3.6498 ± 1.0060; 20:4n-6/20:3n-6 ratio; P < 0.05). However, the D9D activity index for stearic acid was reduced (0.7198 ± 0.1430 vs. 0.7871 ± 0.1280, 18:1n-9/18:0 ratio, P < 0.001) (Table 3).

All genotypes were in Hardy-Weinberg equilibrium. Genotype analysis of stearoyl-CoA desaturase-1 (*SCD1*) polymorphisms revealed significant differences in both allele and genotype frequencies for SCD1 in patients with different concentrations of apoB-48. The group with higher concentrations of apoB-48 exhibited lower prevalence of minor alleles and respective genotypes of the rs2167444 and rs508384 polymorphisms (Table 4). We found no associations between polymorphisms or alleles and *apoE* (*ε2, ε3, ε4*) in either group (data not presented).

Table 3. Fatty acid composition of plasma phosphatidylcholines

FA[a]	ApoB-48 < 7.9 (mg/l)	ApoB-48 ≥ 7.9 (mg/l)
14:0	0.19 ± 0.11	0.23 ± 0.10[h*]
16:0	31.91 ± 4.73	30.72 ± 3.51[*]
16:1n-9	0.12 ± 0.03	0.12 ± 0.03
16:1n-7	0.57 ± 0.20	0.61 ± 0.20
18:0	14.13 ± 1.66	14.89 ± 1.45[***]
18:1n-9	10.99 ± 1.43	10.65 ± 2.00
18:1n-7	1.53 ± 0.20	1.51 ± 0.22
18:2n-6	23.60 ± 3.03	23.30 ± 3.56
18:3n-6	0.10 ± 0.03	0.11 ± 0.04[*]
18:3n-3	0.24 ± 0.09	0.23 ± 0.10
20:0	0.04 ± 0.02	0.04 ± 0.02
20:1n-9	0.12 ± 0.04	0.12 ± 0.04
20:2n-6	0.41 ± 0.13	0.43 ± 0.18
20:3n-6	2.76 ± 0.79	3.06 ± 0.75[**]
20:4n-6	9.58 ± 2.41	9.64 ± 2.34
20:5n-3	0.94 ± 0.57	0.84 ± 0.37
22:4n-6	0.20 ± 0.10	0.24 ± 0.11[**]
22:5n-6	0.14 ± 0.07	0.16 ± 0.08[*]
22:5n-3	0.50 [0.28–0.73]	0.67 [0.40–0.87][**]
22:6n-3	1.71 [1.01–2.85]	2.15 [1.28–3.30]
∑SFA	46.29 ± 3.81	45.92 ± 3.48
∑MUFA	13.35 ± 1.55	13.00 ± 2.19
∑PUFAn-6	36.6 5 ± 3.43	37.06 ± 3.56
∑PUFAn-3	3.71 ± 1.79	4.03 ± 1.69
D5Dn6[c]	3.6498 ± 1.0060	3.2610 ± 0.8857[**]
D6Dn6[d]	0.0039 [0.0029–0.0051]	0.0045 [0.0035–0.0059][*]
D9D16[e]	0.0178 ± 0.0050	0.0197 ± 0.0059[**]
D9D18[f]	0.7871 ± 0.1280	0.7198 ± 0.1430[***]

Legend and abbreviations: Only relevant fatty acids are shown (proportions of fatty acids 12:0 and 14:1n-5 are not given); ΣSFA – total content (sum) of saturated fatty acids; ΣMUFA – sum of monounsaturated fatty acids; ΣPUFAn-6 – sum of polyunsaturated fatty acids (n-6 family); ΣPUFAn-3 – sum of PUFA (n-3 family); [a] shorthand notation for fatty acids: number of carbon atoms/number of double bonds; n – number of carbon atoms from the methyl end to the nearest double bond; [b] values are means ± SD or medians [25^{th}–75^{th} percentiles] (mol %); [c] D5D – Δ 5 desaturase (20:4n-6/20:3n-6); [d] D6D – Δ 6 desaturase (18:3n-6/18:2n-6); [e] D9D16 – Δ 9 desaturase for palmitic acid (16:1n-7/16:0); [f] D9D18 – Δ 9 desaturase for stearic acid (18:1n-9/18:0); [g] Mann-Whitney U test; [h] Student's *t*-test; * P < 0.05; ** P < 0.01; *** P < 0.001.

Table 4. Genotype and allele distribution of SCD1 polymorphisms in subjects with different apoB-48 concentrations

Polymorphisms ApoB-48 group		**Genotype**		**Allele frequency**		**HWE**	**Genotype/allele analysis (χ^2 test)**
rs2167444	TT	TA	AA	T	A		
< 7.9 mg/l	65 (60.2)	38 (35.2)	5 (4.6)	168 (77.8)	48 (22.2)	$\chi^2 = 0.034$	7.038 (P = 0.030) genotype*
≥ 7.9 mg/l	85 (75.9)	27 (24.1)	-	197 (87.9)	27 (12.1)	$\chi^2 = 2.104$	8.041 (P = 0.005) allele
rs508384	CC	CA	AA	C	A		
< 7.9 mg/l	64 (59.3)	39 (36.1)	5 (4.6)	167 (77.3)	49 (22.7)	$\chi^2 = 0.094$	7.025 (P = 0.030) genotype*
≥ 7.9 mg/l	84 (75.0)	28 (25.0)	-	196 (87.5)	28(12.5)	$\chi^2 = 2.286$	7.901 (P = 0.005) allele
rs7849	TT	TC	CC	T	C		
< 7.9 mg/l	66 (61.1)	41 (38.0)	1 (0.9)	173 (80.1)	43 (19.9)	$\chi^2 = 3.919^x$	3.906 (P = 0.142) genotype*
≥ 7.9 mg/l	84 (75.0)	28 (25.0)	-	196 (87.5)	28 (12.5)	$\chi^2 = 2.286^x$	4.459 (P = 0.035) allele
rs55710213	CC	CT	TT	C	T		
< 7.9 mg/l	45 (41.7)	48 (44.4)	15 (13.9)	138 (63.9)	78 (36.1)	$\chi^2 = 0.146$	2.664 (P = 0.264) genotype
≥ 7.9 mg/l	35 (31.2)	57 (50.9)	20 (17.9)	127 (56.7)	97 (43.3)	$\chi^2 = 0.149$	2.375 (P = 0.123) allele
rs56334587	CC	CT	TT	C	T		
< 7.9 mg/l	45 (41.7)	48 (44.4)	15 (13.9)	138 (63.9)	78 (36.1)	$\chi^2 = 0.146$	2.664 (P = 0.264) genotype
≥ 7.9 mg/l	35 (31.2)	57 (50.9)	20 (17.9)	127 (56.7)	97 (43.3)	$\chi^2 = 0.149$	2.375 (P = 0.123) allele

Values represent the number of subjects with specific genotypes (alleles) in individual groups; numbers in parentheses represent the percentage of subjects with specific genotypes (alleles); ^{x} genotype distribution of *SCD1 rs7849* did not correspond with HWE (P > 0.05); * Yates' correction

Abbreviations: HWE – Hardy-Weinberg equilibrium

Discussion

Increased concentrations of apoB-48, which reflect the concentrations of intestinally derived CM and their remnant particles (CMR), are caused by increased production and secretion of CM and/or by their insufficient catabolism. TRL originating in the liver, such as VLDL and their remnants (IDL), compete with lipoproteins of intestinal origin (CM and CMR) for lipoprotein lipase in skeletal muscle and adipose tissue, and for elimination by receptor-mediated uptake (LDLR and LRP) of IDL and CMR in the liver (Fazio and Linton, 2015; Nakajima and Tanaka, 2018).

Insulin resistance increases expression of microsomal triglyceride transfer protein and Niemann-Pick C1-like 1 protein and reduces activity of the ABCG5/G8 transporter. Consequently, the content of cholesterol and TG in CM along with the secretion of CM by enterocytes increase (Nakajima and Tanaka, 2018). It has been suggested that CMR particles, which are rich in cholesterol and other lipids, are also highly atherogenic. Increased concentrations of apoB-48 are understood to be associated with prolonged residence time of TRL and with increased concentrations of sd-LDL and ox-LDL. *In vitro* studies have proved the pro-atherogenic and pro-inflammatory properties of both CMR and ox-LDL (Nakajima et al., 2006). Increased concentrations of apoB-48 are recognized as a risk factor for atherothrombotic cardiovascular events (especially coronary heart disease and non-embolic ischemic stroke) and are understood to be independent of levels of total plasma, LDL- and HDL-cholesterol, and triglycerides in children, adults, diabetics and non-diabetics (Alipour et al., 2012; Mori et al., 2013; Wang et al., 2013). Elevated plasma concentrations of apoB-48 are recognized as risk factors for clinically manifest and subclinical atherosclerosis in both diabetics and non-diabetics (Tanimura et al., 2008; Valdivielso et al., 2010; Mori et al., 2013; Nakajima and Tanaka, 2018). Fasting apoB-48 has been independently associated with asymptomatic peripheral artery disease in patients with DM2T. Of the several risk factors involved (LDL-cholesterol, HDL-cholesterol, HBA_{1c}, DM duration, smoking, and systolic BP), only apoB-48 has been identified as an independent predictor of PAD (Lapice et al., 2012; Mancera-Romero et al., 2013).

High levels of apoB-48 have been reported in patients with diabetic nephropathy, chronic kidney disease, as well as end-stage renal disease (Hayashi et al., 2008), obesity and MetS (Kinoshita et al., 2009; Masuda et al., 2014; Okubo et al., 2014).

In our study, probands with increased concentrations of apoB-48 (≥ 7.9 mg/l) exhibited unfavourable lipid and lipoprotein profiles (increased TG, non-HDL-C and apoB-100; decreased HDL-C and apoA-I), elevated markers of oxidative stress (CD-LDL and ox-LDL), insulin resistance (higher insulinaemia and HOMA-IR), and higher systolic blood pressure.

The most important result of our study was that probands with lower plasma concentrations of apoB-48 had higher proportions of minor alleles and genotypes of rs2167444 and rs508384 *SCD1* polymorphisms. Our set of probands included persons with at least one component of MetS; however, no control group was used. As the normal range of apoB-48 concentrations could not be determined, we used the median for all 220 probands

as the cut-off value. In the literature, data on the reference ranges of apoB-48 in healthy persons are scarce (Nakajima and Tanaka, 2018) and available only for Japanese normolipidaemic population (Masuda et al., 2014).

The finding of a significant association between the SCD1 genotype (allele) polymorphisms rs2167444 and rs508384 (rs7849) and apoB-48 concentrations indicates higher proportion of minor, protective genotypes (alleles) in probands with lower concentrations of apoB-48. A Swedish study reported a significant association between minor alleles of four *SCD1* polymorphisms (rs10883463, rs7849, rs2167444, and rs508384) and lower BMI, lower WC, and improved insulin sensitivity. The expected differences in D9D activities between minor and common allele carriers did not reach statistical significance (Warensjö et al., 2007). In our study, we found no significant association between *SCD1* polymorphisms and D9D activity (data not shown). A Spanish study reported associations between several *SCD1* polymorphisms and obesity. However, although it revealed a lower risk of obesity among carriers of the minor alleles rs7849 and rs508384, the opposite effect was observed for the minor allele rs150259. Moreover, the same study found a statistically significant association between D9D for palmitic and stearic acid and polymorphisms rs508384, rs1267444, and rs7849 (Martín-Núñez et al., 2013). A Canadian study found an association between some cardiometabolic risk factors (TG, CRP, IL-6, and glycaemia) and three polymorphisms of *SCD1* (rs508384, rs3071, and rs3829160), and a significant association between D9D activity and one *SCD1* polymorphism (rs2234970). The same study also noted a significant interaction between rs508384, dietary PUFA n-3 intake, and glycaemic change (Rudkowska et al., 2014). The EPIC-Potsdam study, which employed univariate analysis, found a weak association between *SCD1*, rs10883463, TG, glycated haemoglobin, and liver function tests (GGT, ALT) and rs11190480. However, in multiple testing, this association was non-significant (Arregui et al., 2012). Warensjö et al. (2007) found associations between four *SCD1* polymorphisms (rs10883463, rs7849, rs2167444, and rs508384) and BMI, waist circumference, and insulin resistance. However, in our study, the group with higher concentrations (≥ 7.9 mg/l) of apoB-48 differed from the group with lower concentrations in respect of insulin resistance, but not of BMI or waist circumference.

Our results corresponded with the findings of higher apoB-48 concentrations in persons with dyslipidaemia, obesity (overweight), MetS, and insulin resistance (Sakai et al., 2003; Kinoshita et al., 2009; Otokozawa et al., 2009; Alipour et al., 2012; Masuda et al., 2014). Higher apoB-48 concentrations have also been found in elderly men and post-menopausal women (Kinoshita et al., 2009; Alipour et al., 2012; Masuda et al., 2014). In our set of probands, the group with concentrations of apoB-48≥ 7.9 mg/l did not significantly differ from the group with lower apoB-48 concentrations in respect of age, BMI, or fat mass.

Patients with higher concentrations of apoB-48 also exhibited higher concentrations of sd-LDL particles, indicating the predominance of LDL subclass phenotype B. Characterized by a preponderance of small-dense LDL particles, phenotype B is a dyslipidaemic component of MetS. Liver lipase activity and TG concentrations are metabolic predictors of sd-LDL particle levels (Ng, 2013; Diffenderfer and Schaefer, 2014). Triglyceride-rich lipoprotein particles from the liver (VLDL and IDL) and gut (CM and CMR) compete for the same lipolytic system, and for receptors that bind IDL and CMR As a result, triglyceride concentrations increase in tandem with prolonged TRL residence time. We found a positive correlation between the concentrations of apoB-48 and concentrations of TG and sd-LDL particles and a negative correlation between the concentrations of apoB-48 and HDL-C, as also previously reported by Sato et al. (2009).

Concentrations of both CD/LDL and ox-LDL reflect systemic oxidative stress. While CD/LDL is an indicator of minimally oxidized LDL (containing only oxidatively modified lipids), both lipid and protein components are oxidatively modified in ox-LDL particles (Esterbauer et al., 1992; Ahotupa et al., 1996). When evaluating the spectra of plasma phosphatidylcholine fatty acids, we observed higher activity of Δ 9 desaturase for palmitic acid (D9D16) in the group with higher concentrations of apoB-48, higher D6D activity, and lower D5D activity. D9D activity for stearic acid (D9D18) was lower in the group with higher concentrations of apoB-48. The ratio of 16:1n-7/16:0 (D9D16) was a surrogate marker of SCD1 activity, the ratio of 20:4n-6/20:3n-6 (Δ 5 desaturase, D5D) a marker of FADS1 activity, and the ratio of 18:3n-6/18:2n-6 (D6D) a marker of FADS2 activity.

The increase in D9D and D6D activity and the opposite changes in D5D activity have been described as a consequence of the characteristic alteration of FA transformation in MetS (Zak et al., 2007, 2014; Tosi et al., 2014). SCD1 (or D9D) activity is influenced by genetic and environmental factors and diseases (Merino et al., 2011; Mauvoisin and Mounier, 2011). Increased D9D activity, which is associated with dyslipidaemia and ischemic heart disease, predicts hyperglycaemia and DM2T manifestation (Warensjö et al., 2006; Mahendran et al., 2014). Elevated D9D activity is linked with tumour development (Byberg et al., 2014), obesity, adipose tissue distribution, insulin resistance (Warensjö et al., 2007), and MetS prevalence (Gong et al., 2011). Increased D6D activity is associated with hyperinsulinaemia and higher BMI. However, low D5D activity specific to MetS is independent of BMI and physical activity (Warensjö et al., 2006), and is understood to be a predictor of development of abdominal obesity (Kawashima et al., 2009).

High D6D and low D5D activities are both characteristic of conditions associated with insulin resistance (Kröger and Schulze, 2012) and are known predictors for development of DM and MetS (Saito et al., 2013). One study reported a negative correlation in the case of

both sexes between LDL-C and TG concentrations and D5D, and a positive correlation between LDL-C, TG, LDL-C, and non-HDL-C and D6D activity (Jacobs et al., 2015). A surprising finding in our study was the detection of higher D9D18 activity in the group with lower apoB-48 concentrations. Oleic acid (OA, 18:1n-9), unlike palmitoleic acid (POA, 16:1n-7), is abundantly supplied in the diet. Therefore, its concentration does not correspond with SCD1 activity. However, it does reflect OA intake. Unfortunately, we could not confirm decreased OA dietary intake because no dietary questionnaire was given to the probands in this study. Stearoyl-CoA desaturase-1 minor alleles are understood to be associated with lower D9D activity. Contrary to Martin-Núñez et al. (2013), neither we nor other authors (Warensjö et al., 2007; Stryjecki et al., 2012) have been able to detect differences in the 16:1n-7/16:0 (18:1n-9/18:0) indexes in minor allele homozygotes compared with common allele homozygotes and compound heterozygotes.

In agreement with Warensjö et al. (2007), we found an association between two *SCD1* polymorphisms (rs20167444 and rs508384) and insulin sensitivity, which might partly explain the increased concentration of apoB-48. However, other factors can also modify apoB-48 levels, such as the amount of dietary cholesterol, diverse composition of dietary fatty acids, and factors influencing cholesterol homeostasis (such as activities of Niemann-Pick C1-like 1 protein and of the ABCG5/G8 transporter), CM synthesis, and catabolism.

The limitations of the study are: (1) the small number of subjects, (2) the cross-sectional study design, (3) the lack of a dietary questionnaire, and (4) the absence of a healthy control group.

The major strength of the study is its well-defined group of high-risk individuals, none of whom were treated with lipid-lowering drugs or n-3/n-6 polyunsaturated FA supplements. Additionally, at least according to our knowledge, this is the first study to describe associations between different apoB-48 concentrations and *SCD1* polymorphisms. Our study supports the role of the *SCD1* variant in the aetiology and pathogenesis of increased apoB-48 levels. However, further studies are undoubtedly warranted, especially in relation to factors influencing homeostasis of chylomicrons and their remnants.

Conclusions

(1) We found an association between increased apoB-48 concentrations and an unfavourable lipid profile characterized by higher concentrations of TC, non-HDL-C, TG, and apoB-100, lower HDL-C, higher systolic blood pressure, insulin resistance, and oxidative stress. (2) These changes were independent of age, sex, BMI, adipose tissue mass, and adipose tissue distribution. (3) The lower prevalence of minor alleles and genotypes for two *SCD1* polymorphisms, rs2167444 and rs508384, in the subgroup with higher apoB-48 concentrations implicates the contribution of genetic factors in the pathogenesis of elevated apoB-48 concentrations, a trend associated with higher cardiometabolic risk.

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