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Determinants of VLDL composition and apo B-containing particles in familial combined hyperlipidemia

Ivette Cruz-Bautista [a], Roopa Mehta [a], Javier Cabiedes [b], Cristina García-Ulloa [a], Luz Elizabeth Guillen-Pineda [a], Paloma Almeda-Valdés [a], Daniel Cuevas-Ramos [a], Carlos A. Aguilar-Salinas [a,*]

[a] Endocrinology and Metabolism Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Sección XVI, Tlalpan, 14000 Mexico City, Mexico
[b] Immunology and Rheumatology Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Sección XVI, Tlalpan, 14000 Mexico City, Mexico

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ABSTRACT

Background: In familial combined hyperlipidemia (FCHL) the severity of the dyslipidemia is determined by an overproduction of VLDL (very low density lipoprotein) particles and by its abnormal lipid composition. However, few are known regarding the metabolic factors that determine these abnormalities. We investigated the impact of metabolic factors on the number of atherogenic particles (apolipoprotein B level (apoB)) and the triglyceride content of very low-density lipoproteins (VLDLs-TG).
Methods: A cross-sectional study done in FCHL subjects and gender and age-matched healthy subjects. A clinical assessment, lipid profile and plasma concentrations of insulin, apolipoprotein CIII (apo CIII), apolipoprotein AII (apo AII), high sensitive C-reactive protein (HS-CRP), adiponectin and leptin were documented in 147 FCHL patients and 147 age-matched healthy subjects. Multivariate regression models were performed to investigate the independent determinants of VLDL-TG and apo B levels adjusting for confounding factors.
Results: The variables that determined the VLDL-triglyceride content as a surrogate of VLDL composition were apo CIII ($\beta = 0.365$, $p < 0.001$), insulin ($\beta = 0.281$, $p < 0.001$), Apo AII ($\beta = 0.145$, $p < 0.035$), and adiponectin levels ($\beta = -0.255$, $p < 0.001$). This model explained 34% of VLDL composition (VLDL-TG) variability. However, none of these variables were independent contributors of apo B-containing particles.
Conclusions: In patients with FCHL apo CIII, apo AII and adiponectin are major novel factors determining the VLDL particle composition. However, such factors do not explain apo B-containing particles.

1. Introduction

Familial combined hyperlipidemia (FCHL) is a highly prevalent, complex disorder associated with increased cardiovascular mortality [1–3]. It is the most frequent primary dyslipidemia in Mexico [4]. The disease is characterized by elevated apolipoprotein B levels (apo B) (above the 90th percentile for the specific ethnic group) in combination with isolated hypertriglyceridemia, isolated hypercholesterolemia or mixed hyperlipidemia [5]. The FCHL phenotype shows remarkable variability. However, the factors which determine the phenotypic expression of the disease are only partially known [6,7]. The high levels of apolipoprotein B and a predominance of small dense low-density lipoprotein (LDL) particles are markers of atherosclerotic burden [8]. In subjects with FCHL, the development of atherosclerosis is thought to be associated with an overproduction of liver-derived apolipoprotein B (in very low-density lipoproteins (VLDLs)), an abnormal VLDL particle composition, and a reduction in lipoprotein lipase (LPL) activity. The severity of the dyslipidemia associated with FCHL can be evaluated by measuring some of these features.

Variables that may regulate the phenotypic expression of FCHL include hyperinsulinemia, the plasma concentration of several apolipoproteins, genetic factors, and inflammatory mediators [9,10].

Hyperinsulinemia is a well-known factor to regulate apo B containing lipoprotein synthesis and catabolism. However, its role as a determinant of the severity of the dyslipidemia is controversial, as only around 50% of FCHL subjects have hyperinsulinemia [11,12]. Changes in the concentrations of the apolipoproteins AII (apo AII) and CIII (apo CIII) have also been implicated in the pathogenesis of FCHL based on animal models and clinical observations. In animal models, the overexpression of apo AII results in the overproduction of VLDL, insulin resistance and low levels of HDL-C (high density lipoprotein cholesterol) [13–15]. Apo CIII inhibits LPL activity and regulates lipid and lipoprotein synthesis in the liver [16–21]. Adipose tissue secretes several adipocytokines (i.e. adiponectin [22–24], leptin [25,26], and others) that regulate appetite, immunity, inflammation, and glucose/lipid metabolism. The

* Corresponding author. Tel.: +52 55 56554523; fax: +52 55 55130002.
E-mail address: caguilarsalinas@yahoo.com (C.A. Aguilar-Salinas).

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influence of the majority of these factors on the severity of the FCHL dyslipidemia is unknown.

2. Subjects and methods

2.1. Study subjects

A total of 294 Mexican subjects were recruited for this study. The cases were selected from a cohort of families with FCHL studied in our institution, many of whom were newly identified and therefore currently not taking lipid-lowering therapy. FCHL was defined by an apo B level >90th percentile for the Mexican population (>108 and >99 mg/dl in men and women, respectively) [5] and total cholesterol and/or triglycerides levels >90th age–sex specific population percentiles. The age/sex-specific population percentiles for lipids were based on the results of a previous Mexican population-based nationwide survey [4]. All subjects had either a personal or family history of premature coronary heart disease (before 55 and 65 y in men and women, respectively) and each participant had at least one first-degree relative with a different hyperlipidemic phenotype. We excluded subjects with current treatment for dyslipidemia and the presence of xanthomas. In addition we excluded subjects with other chronic diseases such as hepatic or renal failure, alcoholism, any other primary or secondary dyslipidemia and any acute disease during the previous 6 weeks. Hypothyroid patients were included if they had a normal thyroid profile while on a thyroid hormone replacement.

FCHL cases were divided into 2 groups according to their lipid phenotype; either isolated hypercholesterolemia or hypertriglyceridemia/mixed hyperlipidemia. Isolated hypercholesterolemia was defined as a total cholesterol level >200 mg/dl and triglycerides <150 mg/dl. Hypertriglyceridemia/mixed hyperlipidemia phenotype was defined as a cholesterol level >200 mg/dl with triglycerides level >150 mg/dl [4].

The control group was composed of healthy individuals with plasma cholesterol and triglyceride levels <75th age–sex specific population percentiles [4]. These subjects were matched with cases (1:1) by age (±5 y) and gender. The Human Research Ethics Committee of our institution approved the study. All procedures were done in accordance with the Declaration of Helsinki. Each subject involved in this study provided a written informed consent.

2.2. Biochemical and anthropometric measurements

Participants were evaluated in the morning (8:00 to 10:00 AM) after an overnight fast of 8 to12 h. Subjects had to refrain from smoking and drinking alcohol for at least 72 h prior to sampling. Any lipid-lowering medication had to be withdrawn for at least 6 weeks prior to all measurements. Glucose, total cholesterol and HDL cholesterol were determined by enzymatic colorimetric commercially available reagents using the Synchron CX Delta (Beckman Coulter). Apolipoprotein B was measured by nephelometry (Beckman Coulter). VLDL lipoproteins were isolated using sequential ultracentrifugation (Optima Beckman LE80-K) of 40,000 RPM at 4 °C for 18 h. Insulin concentration was measured using microparticle enzyme-linked immunoassay (MEIA) (Axym System Abbott). Apo CIII concentrations were measured using ELISA assays (Dade Lab). HS-CRP and apo AII were measured using immunonephelometry (BN-Prospect). Adiponectin and leptin levels were assayed in duplicate using commercially available enzyme-linked immunosorbent assays (Merck Millipore). The homeostasis model assessment for insulin resistance (HOMA IR) was calculated as follows: fasting insulin (μU/ml) × fasting glucose (mg/dl) / 405 [27].

A complete medical, family history and anthropometric measurements were obtained from all subjects. Patients were weighed on calibrated scales and height was determined with a floor scale's stadiometer. Waist circumference was measured at the midpoint of a line between the rib border and the iliac crest, in the late exhalation phase while standing. Arterial hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg [28], or self-reported use of antihypertensive medications. The smoking status was categorized as current smokers (smoking at least one cigarette in the previous month) or nonsmokers. Alcoholism was defined as the consumption of at least 10 alcoholic beverages per week within the previous 2 months.

2.3. Statistical analyses

We used the Kolmogorov–Smirnov's test to explore the distribution of each variable. Log transformations were applied to approximate normality in those variables showing a non-parametric distribution. Data are presented as mean ± SD or as median and interquartile range. Categorical variables are reported as frequencies and percentages. Variables were compared between FCHL subjects and controls using the Student's t test. The frequency distribution of the categorical variables in the 2 groups was compared using chi-squared tests. One-way ANOVA was used for comparison between serum levels of VLDL-TG and apo B and apo CIII tertiles. Correlations coefficients between VLDLs-TG and biochemical features were calculated using Pearson's r test or using partial correlation analysis when adjusted for waist circumference. We did the same analysis between apo B levels and the biochemical features. Stepwise multiple lineal regression models were performed to evaluate the impact of variables in serum VLDL-TG and apo B levels for all participants. Variables were removed from the model until the best fitting model with the maximum adjusted R^2 was achieved. The variables selected to enter into regression analyses were those that correlated significantly with serum VLDL-TG and apo B levels (Table 2). Subsequently, analysis was also performed after classifying FCHL subjects according to their lipid phenotype (isolated hypercholesterolemia and mixed dyslipidemia/hypertriglyceridemia) in order to explore. p value <0.05 (2-tailed) was considered statistically significant. The SPSS 21.0 for windows software package was used.

3. Results

3.1. Clinical characteristics

We evaluated 147 patients with FCHL and 147 normolipidemic normoglycemic healthy control subjects. The clinical and biochemical characteristics of the study subjects are presented in Table 1. The median age of cases and controls was 40 (IQR 33–50 y) and 36 (IQR 29–49 y), respectively. In FCHL patients, the prevalence of hypertension was significantly higher than in the normolipemic controls ($p < 0.05$). The BMI, waist circumference and weight–height ratio (W/H) were significantly higher in FCHL subjects than the normolipemic subjects ($p < 0.001$). In addition, the fasting insulin concentration, fasting plasma glucose levels and HOMA IR were significantly higher in the FCHL group ($p < 0.001$).

3.2. Metabolic markers

FCHL participants had higher levels of apo AII, apo CIII, leptin, HS-CRP, apo B and VLDL-TG levels than the control group ($p < 0.001$). Adiponectin levels were lower in FCHL subjects [8.4 μg/l (6.4–10.5)] compared with controls [11.22 μg/l (8.5–14.9)], $p < 0.001$. These differences remained statistically significant after adjusting for waist circumference (Table 1).

3.3. Metabolic factors that determine the atherogenic particle number (measured with apo B levels) and the VLDL particle composition (measured with VLDL triglyceride concentrations as surrogate of VLDL composition) (VLDL-TG)

We investigated which factors explain the variability in VLDL-TG levels; this is a marker of VLDL particle composition. In bivariate

Table 1
Clinical and biochemical characteristics of the study subjects. Data are presented as mean ± SD, percentages or as median (interquartile range).

Variable	FCHL (n = 147)	Controls (n = 147)	p value
Gender (females)	99 (67.3%)	99 (67.3%)	NS
Age (y)[a]	40 (33–50)	36 (29–49)	NS
Smoking index[a]	0 (0–1.5)	0 (0–1.5)	NS
Systolic blood pressure (mm Hg)[a]	120 (105–130)	110 (100–120)	0.004
Diastolic blood pressure (mm Hg)[a]	80 (70–80)	70 (60–80)	<0.001
Body mass index (kg/m^2)[a]	27.1 (24.6–30)	24 (21.8–26.4)	<0.001
Waist (cm)[a]	90 (83.2–100)	83.2 (75.5–90)	<0.001
Waist-to-height ratio[a]	0.55 (0.51–0.60)	0.51 (0.47–0.55)	<0.001
Total cholesterol (mg/dl)[a,b]	234 (215–266)	174 (157–185)	<0.001
Triglycerides (mg/dl)[a,b]	238 (170–343)	83 (68–109)	<0.001
HDL cholesterol (mg/dl)[a,b]	40 (35–47)	48 (42–55)	<0.001
No HDL cholesterol (mg/dl)[a,b]	193 (172–221)	123 (110–135)	<0.001
Fasting glucose (mg/dl)[a,b]	89.1 ± 10.2	83.8 ± 8.4	<0.001
Fasting insulin (mIU/l)[a,b]	12.5 (7.8–16.5)	7 (4.7–9.2)	<0.001
HOMA IR[a,b]	2.5 (1.6–3.8)	1.5 (1–2)	<0.001
Serum apoprotein B (g/l)[a,b]	133 (121–151)	79 (70–89)	<0.001
Serum apoprotein AII (mg/dl)[a,b]	36.8 (33–40.7)	32.6 (29.5–35.5)	<0.001
Serum apoprotein CIII (mg/dl)[a,b]	12.6 (5.7–24.1)	3.3 (1.91–5.13)	<0.001
Serum hs-CRP (mg/l)[a,b]	2.2 (1.1–4.2)	1.09 (0.45–2.01)	<0.001
Serum adiponectin (μg/l)[a,b]	8.4 (6.4–10.5)	11.22 (8.5–14.9)	<0.001
Serum Leptin (ng/ml)[a,b]	16.2 (9.5–25.0)	10.4 (5.6–18.5)	<0.001
VLDL-TG (mg/dl)[a,b]	176 (105–263)	47 (31–69)	<0.001

hs-CRP, high sensitive C-reactive protein; VLDL-TG, triglyceride content in very low density lipoprotein.
[a] Log-transformed before analysis.
[b] p value <0.001 with adjustment for waist circumference.

correlation analysis adjusted for waist circumference there was a significant correlation between the VLDL-TG levels, HOMA (r = 0.26, p = 0.002), apo AII (r = 0.24, p = 0.004), apo CIII (r = 0.43, p < 0.001), adiponectin (r = −0.34, p < 0.001) and insulin levels (r = 0.264, p = 0.001) (Table 2). When we generated a multiple linear regression model including the above variables, we found that apo CIII, insulin, adiponectin, and apo AII concentrations continued to show statistical significance (r^2 = 0.35, p = 0.03, Table 2). We did not find any significant associations between the apo B levels and the study variables. In addition we performed the same bivariate analysis in control subjects and found that only apo CIII (r = 0.23, p < 0.01), and HS-PCR levels (r = 0.175, p = 0.04) were correlated with VLDL-TG (Table 2). In the multiple lineal regression model both of these variables determine the variability in VLDL-TG levels (r = 0.13, p = 0.03) (Table 3). Consistently, no correlation between apo B levels and these study variables was found in the control group as well.

Table 2
Bivariate correlations between Apo B and VLDL-triglycerides levels with biochemical and anthropometric variables adjusted by waist circumference.

Total population n = 294	Apo B		VLDL-TG	
	r	p	r	p
Cases (n = 147)				
Age[a]	0.044	0.604	−0.116	NS
Insulin[a]	−0.021	0.802	0.264	0.001
HOMA IR[a]	0.014	0.864	0.258	0.002
Glucose[a]	0.164	0.050	0.015	NS
Apo AII[a]	0.080	0.342	0.238	0.004
Apo CIII[a]	0.099	0.237	0.428	<0.001
Leptin[a]	−0.008	0.926	−0.069	NS
Adiponectin[a]	−0.079	0.344	−0.339	<0.001
CRP[a]	0.044	0.603	−0.013	NS
Controls (n = 147)				
Age[a]	0.011	0.901	0.009	NS
Insulin[a]	−0.080	0.342	0.063	NS
HOMA IR[a]	−0.068	0.430	0.071	NS
Glucose[a]	0.053	0.534	0.045	NS
Apo AII[a]	−0.070	0.418	0.103	NS
Apo CIII[a]	0.139	0.103	0.228	0.007
Leptin[a]	−0.040	0.642	−0.116	NS
Adiponectin[a]	0.029	0.740	−0.143	NS
hs-CRP[a]	0.018	0.830	0.175	0.041

VLDL TG: VLDL triglyceride content in very low-density lipoprotein; hs-CRP, high sensitive C-reactive protein.
[a] Log transformed before analysis.

3.4. Apo CIII concentrations: effect on plasma lipids in cases and controls

We evaluated the relationship between Apo CIII and the severity of dyslipidemia with other study parameters. We divided the cases and control according to tertiles of apo CIII levels. The FCHL individuals localized in the highest tertiles of Apo CIII had the highest concentrations of VLDL-TG (p < 0.001) In addition; these individuals had the lowest concentrations of HDL-C (p < 0.001). We found no association between the levels of Apo B and the tertiles of apo C III in both cases and controls (Table 4).

3.5. Biochemical characteristics by lipid phenotypes

In addition we compared the same study variables dividing the FCHL cases according to lipid phenotype (isolated hypercholesterolemia and mixed hyperlipidemia/hypertriglyceridemia). Isolated hypercholesterolemia was present in 12.9% of the study sample. This phenotype was associated with the lower levels of HOMA-IR (p = 0.012), insulin (p = 0.008), Apo CIII (p = 0.001), Apo B (<0.001) and VLDL-TG concentrations (p < 0.001). This group had the highest levels of adiponectin (p = 0.005). As expected the mixed hyperlipidemia/isolated hypertriglyceridemia phenotype (87.1%) had the highest Apo CIII (p = 0.001), apo B (p < 0.001) and insulin levels (p < 0.001) and the lowest adiponectin levels (p < 0.001). There was no difference in waist circumference, leptin, HS-CRP and glucose levels between lipid phenotypes (Table 5).

3.6. Determinants of HDL-C levels in FCHL

Finally we investigated the factors that determine the variability in HDL cholesterol levels in FCHL cases. In bivariate partial correlation analysis adjusted for waist circumference, HDL-cholesterol showed correlations with insulin (r = −0.221, p = 0.008), apo AII (r = 0.164, p = 0.049), apo CIII (r = −0.208, p = 0.013) and adiponectin levels (r = 0.189, p = 0.024). In the multiple lineal regression analysis the abovementioned variables remained significantly associated with the HDL-C concentration (r^2 = 0.448, p = 0.033). We performed the same bivariate analysis in control subjects and found that HDL-C correlated only with leptin (r = 0.204, p = 0.012), apo AII (r = 0.246, p = 0.004) and adiponectin levels (r = 0.434, p < 0.001). These factors were independent contributors to the HDL-C concentration.

Table 3
Multiple stepwise multiple regression analysis showing independent variables associated with VLDL-TG levels.

VLDL-TG	Independent variables	B	Standardized β	t	p variable
Cases[a]	Apoprotein CIII	0.164	0.365	5.2	<0.001
	Insulin	0.405	0.281	4.0	<0.001
	Adiponectin	−0.544	−0.255	−3.6	<0.001
	Apoprotein AII	0.545	0.145	2.1	0.035
Controls[b]	Apoprotein CIII	0.132	0.223	2.8	0.005
	hs-CRP	0.101	0.176	2.1	0.030

hs-CRP, high sensitive C-reactive protein. The analysis also included waist circumference, leptin and glucose which were excluded in the final model.
[a] Parameters of the model: r^2 = 0.346, F = 4.51, p = 0.035.
[b] Parameters of the model: r^2 = 0.136, F = 4.8, p = 0.047.

Table 4
Apo C III concentration is associated with VLDL-TG and HDL-C levels in cases.

Variable	FCHL subjects Apoprotein C III tertiles				Controls Apoprotein C III tertiles			
	1 (n = 20)	2 (n = 45)	3 (n = 82)	p value[a]	1 (n = 20)	2 (n = 45)	3 (n = 82)	p value[a]
VLDL-TG	92.4 ± 65.8	147.8 ± 74.8	301.3 ± 250.4	<0.001	52.6 ± 48.2	75.1 ± 107.2	60.8 ± 24.1	0.032
Apo B	137.5 ± 31.3	133.2 ± 19.3	139.5 ± 25.6	0.415	77.3 ± 13.9	79.6 ± 11.7	82.1 ± 13.2	NS
HDL-c	48.3 ± 12.2	45.1 ± 9.5	39.5 ± 10.4	<0.001	50.8 ± 11.1	49.1 ± 11.3	46.1 ± 7.7	NS

FCHL, familial combined hyperlipidemia; VLDL-TG, VLDL triglyceride content in very low density lipoprotein. Data are presented as mean ± SD.
[a] Adjusted for waist circumference.

4. Discussion

Our results show that in subjects with FCHL the factors that modulate the composition of VLDL particles are apo CIII, apo AII, insulin, and adiponectin levels. These parameters explained 34% of the variability in VLDL-TG levels. We did not find any determinants of the number of the apo B containing particles.

Our data confirm that FCHL subjects have higher insulin, HOMA, apo AII, apo CIII, HS-CRP, leptin, apo B, and VLDL-TG levels; and lower adiponectin and HDL-C levels compared with healthy controls even after adjusting for waist circumference. This finding suggests that the pathogenic pathways involved in FCHL appear to be independent of the presence of obesity.

These results are partially in agreement with the current literature. With respect to VLDL triglyceride content, our results confirm an association with insulin levels, although, this variable was not the predominant factor. Insulin resistance is not uniformly found in all FCHL cases, and only partially explains the dyslipidemia severity [12,29]. Insulin plays a critical role in the regulation of essential steps in lipoprotein metabolism. Hyperinsulinemia stimulates lipogenesis and *APO B* expression [30,31]. In contrast, hepatic insulin resistance results in decreased inhibitory actions on triglyceride synthesis and VLDL secretion [32]. Insulin represses VLDL secretion through the inhibition of the expression of *MTP (Microsomal Transfer Protein)* gene, by stimulating proteasome apolipoprotein B degradation and by inhibiting ARF (ADP-ribosylation factor) and phospholipase D1, proteins involved in the transfer of VLDL precursors through the Golgi. Thus, insulin resistance results in increased lipogenesis in combination with decreased inhibition of triglyceride synthesis and VLDL overproduction. These abnormalities explain the association between higher insulin levels and increased VLDL-TG concentrations.

On the other hand, we did not demonstrate an association between insulin levels and the total number of atherogenic particles (apo B concentration). This is in agreement with previous observations demonstrating that approximately 80% of FCH subjects had apo B levels above the 90th percentile at any level of insulin or amount of intra-abdominal adipose tissue [12]. The main determinant of plasma apolipoprotein B concentration is LDL catabolism. Although insulin has regulatory actions on the number and function of the LDL receptors, insulin resistant states are not characterized by severe defects in LDL clearance. The differential effect of insulin resistance in various processes involved in the metabolism of the apo B containing lipoproteins may explain why insulin is strongly associated with VLDL-TG, but it is not a determinant of apo B levels in FCHL patients.

Table 5
Inflammatory and biochemical markers by lipid phenotype in FCHL subjects.

Characteristic	Mixed/HTG hyperlipidemia (n = 128)	Hypercholesterolemia (n = 19)	p value
Age (y)	41 ± 12	46 ± 15	NS
Waist (cm)	91.7 ± 11	87.3 ± 10	NS
Waist-to-hip ratio	0.56 ± .06	0.55 ± .07	NS
Apoprotein AII (mg/dl)	37 (33–41)	36 (33–37)	NS
Apoprotein CIII (mg/dl)	15 (7–29)	3.4 (2.1–4.7)	0.001
Apoprotein B (mg/dl)	135 (122–154)	122 (111–130)	<0.001
VLDL-TG (mg/dl)	190 (127–286)	59 (37–87)	<0.001
Glucose (mg/dl)	89 (82–96)	90 (82–94)	NS
Insulin (μUI/ml)	12.6 (8.4–16.6)	7.7 (4.9–13.5)	0.008
HOMA IR	2.7 (1.8–3.8)	1.6 (1.0–3.0)	0.012
Adiponectin (μg/ml)	8.3 (6.1–10.2)	9.9 (7.6–12.3)	0.005
Leptin (ng/ml)	16.1 (9.7–24.7)	16.6 (8.2–26.6)	NS
hs-CRP (mg/l)	2.1 (1.0–4.2)	2.3 (1.1–4.4)	NS

HOMA, homeostasis model assessment; hs-CRP, high sensitive C-reactive protein; VLDL-TG, VLDL triglyceride content. Data are presented as mean ± SD or median and IQR.

As indicated, insulin is an important contributor to the metabolic abnormalities observed in FCHL, but other less-well identified factors should certainly be considered. In our study, apo CIII was the most important variable that influenced VLDL composition. This finding reinforces the renewed interest in the regulation and actions of apo CIII. Apo CIII is involved in both hepatic lipoprotein synthesis (i.e. increasing VLDL-1 secretion) and LPL inhibition. Apo CIII plasma levels and synthesis are positively correlated with triglyceride concentrations, and its secretion is increased in insulin resistance states characterized by free fatty acid and increased glucose levels [33,34]. Caron et al. showed that glucose increases Apo CIII gene expression via hepatic nuclear factor-4 alpha and carbohydrate-responsive element binding protein, but not the liver X receptor [35,36]. Under physiologic conditions, insulin inhibits its expression, but this regulatory action is lost in insulin resistant states. As a result, *APOC3* gene expression is remarkably augmented in insulin resistant states, including FCHL. This abnormality may stimulate the secretion of the VLDL-1 particles; these have higher triglyceride content and are characteristically increased in FCHL. However, the association between Apo CIII plasma levels and the VLDL-triglycerides remained significant after adjusting for waist circumference and insulin levels (Table 4). In several populations, including Mexicans, SNPs located in *APOC3* and in the *APOA1/C3/A4/A5* cluster regulate *APOC3* expression and are associated with the FCHL phenotype. Thus, genetic and metabolic interactions may explain the prominent role of apo CIII in the pathogenesis of FCHL. In addition, in our study when classifying FCHL subjects according to the apo CIII tertiles, we found that those in the highest tertile had the lowest adiponectin and HDL-levels and the highest apo AII and HS-CRP. As apo CIII inhibits the anti-inflammatory action of HDL particles, increased apo CIII levels may contribute to the atherogenic risk of FCHL by altering HDL number and function and play a role in the abnormal HDL metabolism found in this condition [37].

Another novel metabolic marker influencing the VLDL particles composition in the FCHL group was apo AII. This protein is the second most abundant of HDL-C and has been linked to FCHL [13]. Several studies have shown that apo AII promotes insulin resistance and has diverse effects on lipid homeostasis. It displaces paraoxonase from HDL particles altering its anti-inflammatory properties [38,39]. It also has been observed that apo AII takes apo AI off the surface of HDL particles, making them less susceptible to lecithin acyl transferase action (LCAT). This affects reverse cholesterol transport and contributes to the pro-atherogenic profile associated with apo AII [40]. Furthermore, this

alteration in function and structure of the HDL particles results in decreased lipoprotein lipase mediated lipolysis, causing an increase in apo AII rich HDL particles, typically found in FCHL. An additional interesting mechanism demonstrated by Julve et al. [41] shows that ApoAII enrichment of HDL particles displaces ApoCII, CIII and ApoE out of HDL particles decreasing its potential LPL activation in mice and humans.

Adiponectin was also found to modulate VLDL-triglyceride levels in our study. The low levels of adiponectin have been implicated in the pathogenesis of FCHL, contributing directly to the presence of an atherogenic lipid profile, regardless of the level of insulin sensitivity and obesity. The level of adiponectin in our patients was an additional determinant of the composition of the VLDL particles, regardless of the adjustment for waist circumference. As demonstrated by Kazumi T et al. the adiponectin level was negatively associated with Apo B and VLDL-TG [42].

Finally, we analyzed the FCHL population by lipid phenotype. Our data show that hypertriglyceridemia FCHL patients had a different metabolic profile compared to patients with isolated hypercholesterolemia. The former group seemed to have the most atherogenic profile associated with higher levels of apo CIII, apo B, VLDL-TG, insulin and HOMA. In addition, individuals in the isolated hypercholesterolemia group had lower insulin concentrations and higher adiponectin levels.

We acknowledge that this study has limitations; these include the cross-sectional design and the sample size. These factors limit the power of the study for confirming the differences encountered between lipid phenotypes. In addition, we cannot extrapolate our results to other populations due to genetic differences between ethnic groups. One of the principal strengths of this study is that the cohort of subjects with FCHL and their family members has been extensively studied resulting in a high degree of certainty regarding the diagnosis of this primary dyslipidemia in these subjects.

5. Conclusion

In summary, novel factors were identified that determine the VLDL-triglyceride content as surrogate of VLDL particle composition such as apo CIII, apo AII, insulin, and adiponectin. We did not find any factors that explained the number of apolipoprotein B-containing particles in FCHL patients. Our data confirms the complex nature of the metabolic derangements present in FCHL and adds novel parameters associated with the atherogenic physiopathology of this disease.

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References

[1] Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest 1973;52:1544–68.
[2] Hopkins PN, Heiss G, Ellison RC, et al. Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case–control comparison from the National Heart, Lung, and Blood Institute Family Heart Study. Circulation 2003;108:519–23.
[3] Nikkila EA, Aro A. Family study of serum lipids and lipoproteins in coronary heart-disease. Lancet 1973;1:954–9.
[4] Aguilar-Salinas CA, Olaiz G, Valles V, et al. High prevalence of low HDL cholesterol concentrations and mixed hyperlipidemia in a Mexican nationwide survey. J Lipid Res 2001;42:1298–307.
[5] Valles V, Aguilar-Salinas CA, Gomez-Perez FJ, et al. Apolipoprotein B and A-I distribution in Mexican urban adults: results of a nationwide survey. Metab Clin Exp 2002;51:560–8.
[6] Aguilar Salinas CA, Zamora M, Gomez-Diaz RA, Mehta R, Gomez Perez FJ, Rull JA. Familial combined hyperlipidemia: controversial aspects of its diagnosis and pathogenesis. Semin Vasc Med 2004;4:203–9.
[7] Ayyobi AF, Brunzell JD. Lipoprotein distribution in the metabolic syndrome, type 2 diabetes mellitus, and familial combined hyperlipidemia. Am J Cardiol 2003;92:27J–33J.
[8] Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 2001;358:2026–33.
[9] Sniderman AD, Castro Cabezas M, Ribalta J, et al. A proposal to redefine familial combined hyperlipidaemia — third workshop on FCHL held in Barcelona from 3 to 5 May 2001, during the scientific sessions of the European Society for Clinical Investigation. Eur J Clin Invest 2002;32:71–3.
[10] van der Vleuten GM, van Tits LJ, den Heijer M, Lemmers H, Stalenhoef AF, de Graaf J. Decreased adiponectin levels in familial combined hyperlipidemia patients contribute to the atherogenic lipid profile. J Lipid Res 2005;46:2398–404.
[11] Ascaso JF, Merchante A, Lorente RI, Real JT, Martinez-Valls J, Carmena R. A study of insulin resistance using the minimal model in nondiabetic familial combined hyperlipidemic patients. Metab Clin Exp 1998;47:508–13.
[12] Purnell JQ, Kahn SE, Schwartz RS, Brunzell JD. Relationship of insulin sensitivity and ApoB levels to intra-abdominal fat in subjects with familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 2001;21:567–72.
[13] Allayee H, Castellani LW, Cantor RM, de Bruin TW, Lusis AJ. Biochemical and genetic association of plasma apolipoprotein A-II levels with familial combined hyperlipidemia. Circ Res 2003;92:1262–7.
[14] Castellani LW, Goto AM, Lusis AJ. Studies with apolipoprotein A-II transgenic mice indicate a role for HDLs in adiposity and insulin resistance. Diabetes 2001;50:643–51.
[15] Escola-Gil JC, Julve J, Marzal-Casacuberta A, Ordonez-Llanos J, Gonzalez-Sastre F, Blanco-Vaca F. Expression of human apolipoprotein A-II in apolipoprotein E-deficient mice induces features of familial combined hyperlipidemia. J Lipid Res 2000;41:1328–38.
[16] Cohn JS, Patterson BW, Uffelman KD, Davignon J, Steiner G. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. J Clin Endocrinol Metab 2004;89:3949–55.
[17] Dallinga-Thie GM, Groenendijk M, Blom RN, De Bruin TW, De Kant E. Genetic heterogeneity in the apolipoprotein C-III promoter and effects of insulin. J Lipid Res 2001;42:1450–6.
[18] Olivieri O, Bassi A, Stranieri C, et al. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. J Lipid Res 2003;44:2374–81.
[19] Peacock RE, Hamsten A, Johansson J, Nilsson-Ehle P, Humphries SE. Associations of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis and high density lipoprotein subclasses. Clin Genet 1994;46:273–82.
[20] Ribalta J, La Ville AE, Vallve JC, Humphries S, Turner PR, Masana L. A variation in the apolipoprotein C-III gene is associated with an increased number of circulating VLDL and IDL particles in familial combined hyperlipidemia. J Lipid Res 1997;38:1061–9.
[21] Xu CF, Talmud P, Schuster H, Houlston R, Miller G, Humphries S. Association between genetic variation at the APO AI-CIII-AIV gene cluster and familial combined hyperlipidaemia. Clin Genet 1994;46:385–97.
[22] Baratta R, Amato S, Degano C, et al. Adiponectin relationship with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. J Clin Endocrinol Metab 2004;89:2665–71.
[23] Chan DC, Watts GF, Ng TW, et al. Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. Clin Chem 2005;51:578–85.
[24] Koenen TB, van Tits LJ, Holewijn S, et al. Adiponectin multimer distribution in patients with familial combined hyperlipidemia. Biochem Biophys Res Commun 2008;376:164–8.
[25] Haluzik M, Fiedler J, Nedvidkova J, Ceska R. Serum leptin concentrations in patients with combined hyperlipidemia: relationship to serum lipids and lipoproteins. Physiol Res 1999;48:363–8.
[26] van der Vleuten GM, Veerkamp MJ, van Tits LJ, et al. Elevated leptin levels in subjects with familial combined hyperlipidemia are associated with the increased risk for CVD. Atherosclerosis 2005;183:355–60.
[27] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[28] Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560–72.
[29] de Graaf J, Veerkamp MJ, Stalenhoef AF. Metabolic pathogenesis of familial combined hyperlipidaemia with emphasis on insulin resistance, adipose tissue metabolism and free fatty acids. J R Soc Med 2002;95(Suppl. 42):46–53.
[30] Marsh JB, Welty FK, Lichtenstein AH, Lamon-Fava S, Schaefer EJ. Apolipoprotein B metabolism in humans: studies with stable isotope-labeled amino acid precursors. Atherosclerosis 2002;162:227–44.
[31] Anant S, Davidson NO. Molecular mechanisms of apolipoprotein B mRNA editing. Curr Opin Lipidol 2001;12:159–65.

[32] Shelness GS, Sellers JA. Very-low-density lipoprotein assembly and secretion. Curr Opin Lipidol 2001;12:151–7.
[33] Zheng C, Khoo C, Furtado J, Sacks FM. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. Circulation 2010;121:1722–34.
[34] Pavlic M, Valero R, Duez H, et al. Triglyceride-rich lipoprotein-associated apolipoprotein C-III production is stimulated by plasma free fatty acids in humans. Arterioscler Thromb Vasc Biol 2008;28:1660–5.
[35] Caron S, Staels B. Apolipoprotein CIII: a link between hypertriglyceridemia and vascular dysfunction? Circ Res 2008;103:1348–50.
[36] Caron S, Verrijken A, Mertens I, et al. Transcriptional activation of apolipoprotein CIII expression by glucose may contribute to diabetic dyslipidemia. Arterioscler Thromb Vasc Biol 2011;31:513–9.
[37] Scheffer PG, Teerlink T, Dekker JM, et al. Increased plasma apolipoprotein C-III concentration independently predicts cardiovascular mortality: the Hoorn Study. Clin Chem 2008;54:1325–30.
[38] Ribas V, Palomer X, Roglans N, et al. Paradoxical exacerbation of combined hyperlipidemia in human apolipoprotein A-II transgenic mice treated with fenofibrate. Biochim Biophys Acta 2005;1737:130–7.
[39] Ribas V, Sanchez-Quesada JL, Anton R, et al. Human apolipoprotein A-II enrichment displaces paraoxonase from HDL and impairs its antioxidant properties: a new mechanism linking HDL protein composition and antiatherogenic potential. Circ Res 2004;95:789–97.
[40] Aouizerat BE, Kane JP. Apolipoprotein A-II: active or passive role in familial combined hyperlipidemia. Circ Res 2003;92:1179–81.
[41] Julve J, Escola-Gil JC, Rotllan N, et al. Human apolipoprotein A-II determines plasma triglycerides by regulating lipoprotein lipase activity and high-density lipoprotein proteome. Arterioscler Thromb Vasc Biol 2010;30:232–8.
[42] Kazumi T, Kawaguchi A, Hirano T, Yoshino G. Serum adiponectin is associated with high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein particle size in young healthy men. Metab Clin Exp 2004;53:589–93.