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Lack of a relationship between plasma PCSK9 concentrations and hepatic lipoprotein kinetics in obese people

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Abstract

Obesity is associated with unfavorable alterations in plasma lipid concentrations. Data obtained from studies in cultured cells and rodent models show that Protein Convertase Subtilisin/Kexin 9 (PCSK9), a secreted protein that leads to degradation of LDL receptors in the liver is an important regulator of plasma LDL cholesterol concentrations. Recent evidence suggests that PCSK9 may also regulate the very low density lipoprotein (VLDL) receptor expression and VLDL-triglyceride (TG) metabolism. The purpose of this study was to determine whether circulating PCSK9 concentrations are correlated with VLDL-triglyceride kinetics in obese people. Plasma PCSK9 concentration and VLDL-TG kinetics were evaluated in 39 non-diabetic, obese subjects (body mass index 36.9 ± 4.3 kg/m²). Body composition was assessed by using dual energy x-ray absorptiometry and VLDL-TG kinetics were assessed by using stable isotopically labeled tracer infusion. We found that plasma PCSK9 concentrations correlated significantly with percent body fat ($r = 0.322$, $P = 0.046$) and serum LDL-cholesterol concentrations ($r = 0.333$, $P = 0.036$), but not with VLDL-TG secretion rate ($r = 0.083$, $p = 0.614$) or clearance rate ($r = 0.032$, $p = 0.845$). These data suggest that PCSK9 is likely involved in LDL-cholesterol metabolism, but is not a clinically important regulator of VLDL kinetics in obese individuals.

Introduction

Obesity is associated with alterations in plasma lipids, including increases in plasma low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-triglyceride (VLDL-TG) concentrations,(1–4) which are important risk factors for coronary heart

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disease. These lipid abnormalities are interrelated because LDL-C is derived as the end product of VLDL metabolism (5).

PCSK9 (Proprotein Convertase Subtilisin/Kexin 9), a secreted protease made primarily in the liver, is a potent regulator of serum LDL-C metabolism (6) through its ability to bind and degrade LDL receptors (LDLRs) in liver. The most important determinant of serum LDL-C concentrations is uptake of LDL particles by the liver, which is mediated by the LDL receptor (7, 8). The LDL-C/LDLR complex is delivered to endosomes, where the LDL particle and the LDLR dissociate allowing the LDLR to return to the surface of the cell for another cycle of binding to LDL particles. PCSK9 binds directly to the LDLR on the surface of hepatocytes (9). The PCSK9/LDLR complex is internalized into the hepatocyte and routed to lysosomes, where the LDLR is degraded (10–13). The clinical relevance of PCSK9 has been demonstrated by data from studies that found haploinsufficiency of PCSK9 with loss-of-function is associated with marked reductions in both plasma LDL-C concentration and cardiovascular events (14). In addition, people who do not have detectable circulating PCSK9 have very low plasma levels of LDL-C (15) due to increased levels of the LDLR at the cell surface resulting from reduced PCSK9-mediated lysosomal degradation of LDLR.

Data obtained from a series of studies conducted in cell systems, rodent models, and people suggest that PCSK9 could be an important regulator of VLDL-TG metabolism in obese people (16–18). PCSK9 increases hepatic VLDL-TG secretion (16) and binds to VLDL-TG receptors leading to their degradation in animal models (17, 18), which could decrease VLDL-TG clearance. In addition, body mass index (BMI) and intrahepatic triglyceride (IHTG) content, which are both associated with increased VLDL-TG secretion and increased plasma TG concentrations, are directly associated with plasma PCSK9 concentrations (19). However, the relationships between PCSK9 and obesity related alterations in hepatic lipoprotein metabolism have not been studied.

The primary purpose of the present study was to evaluate the relationship between VLDL-TG kinetics and plasma PCSK9 concentrations in obese men and women with a wide range of IHTG. We hypothesized that PCSK9 is involved in the pathogenesis of dyslipidemia associated with obesity, particularly in obese subjects who have increased IHTG. Hepatic VLDL-TG kinetics was determined by using stable isotope tracer infusion in conjunction with mathematical modeling.

Methods

Thirty-nine obese subjects (11 men and 28 women, 41.4 ± 10.8 years old) participated in this study. VLDL-TG kinetics were previously reported in 28 of these 39 subjects (20). All participants completed a medical evaluation which included a history and physical exam, blood tests, the Michigan Alcohol Screening Test (MAST) (21), and a 2 hour oral glucose tolerance test to test for the presence of diabetes. Subjects were excluded from the study if they had chronic liver disease other than nonalcoholic fatty liver disease (NAFLD), diabetes, a MAST score >4 , or medications known to affect metabolism. All subjects were weight stable ($<2\%$ change in weight for at least 3 months before the study) and participated in less than 1 hour of aerobic exercise per week. This study was carried out according to the principles of the Declaration of Helsinki, and was approved by the Institutional Review Board and the Center for Applied Research Sciences Advisory Committee at Washington University School of Medicine. All participants provided written informed consent before participating in the study.

Experimental Protocol

Body composition—Total body fat mass was determined by using dual-energy-X-ray absorptiometry (Hologic QDR 4500, Waltham, MA) and IHTG content was determined by using magnetic resonance spectroscopy (MRS) (Siemens, Erlanger, Germany), as previously described (22).

Hepatic Lipoprotein Kinetics: Subjects were admitted to the Washington University Clinical Research Unit in the evening and fed a standard meal. Subjects then fasted (except for water) until the completion of the hepatic lipoprotein kinetic study the next day. At 0500 h the next day, one catheter was inserted into a forearm vein for tracer infusions and a second catheter was inserted into the opposite hand, which was heated by using a thermostatically controlled box, to obtain arterialized blood samples. At 0600 h a bolus of [1,1,2,3,3-²H₅]glycerol (75 μmol/kg) was administered through the catheter in the forearm vein. Blood samples were collected before, and at 5, 15, 30, 60, and 90 min, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h after glycerol tracer injection as previously described (20).

Sample analyses—Plasma lipid concentrations were measured enzymatically by using a Hitachi 917 autoanalyzer (Hitachi, Tokyo, Japan). Plasma PCSK9 concentrations were measured by using ELISA as described previously (19). Plasma VLDL-TG concentration was determined by using a colorimetric enzymatic kit (Sigma Chemicals, St. Louis, MO). The tracer-to-tracee ratio (TTR) of plasma and VLDL-TG glycerol was measured by using electron impact ionization gas chromatography/ mass spectroscopy (MSD 5973 system with capillary column; Hewlett-Packard), as previously described (23).

Calculations—The fractional turnover rate of VLDL-TG was determined by fitting the TTR time-courses of free glycerol in plasma and glycerol in VLDL-TG to a compartmental model (24). The total rate of VLDL-TG secretion (in μmol/l-min), which represents the amount of VLDL-TG secreted by the liver per unit of plasma, was calculated by multiplying the fractional turnover rate of VLDL-TG (in pools/min) by the steady-state plasma VLDL-TG concentration (in μmol/l). The plasma clearance rate of VLDL-TG (ml/min) was calculated as the production rate (μmol/min) divided by the plasma concentration (μmol/ml).

Statistical analysis

All data sets were normally distributed according to Kolmogorov-Smirnov. Student's t-test for independent samples was used to compare BMI, IHTG content, and plasma PCSK9 concentrations between male and female subjects. Pearson's product moment correlation coefficient was used to test the statistical significance of relationships between variables. Multiple stepwise linear regression analysis (with age, sex, BMI, percent body fat, and PCSK9 as independent variables) was performed to identify significant independent predictors of plasma LDL concentrations. All results are expressed as mean ± standard deviation, and all reported P-values are two sided. A P-value of <0.05 was considered statistically significant. Statistical analyses were performed by using SPSS (Windows) v17.0.

Results

Characteristics of the study subjects are shown in Table 1. Men and women were matched on BMI (37.5 ± 3.2 and 36.7 ± 4.6 kg/m², respectively) and IHTG content (17.1 ± 11.1 and 16.4 ± 11.9 percent, respectively). Mean serum PCSK9 concentrations was higher in women (447 ± 166 ng/ml) than in men (340 ± 68 ng/ml) ($p = 0.007$). Subjects had a wide range in the metabolic variables, VLDL kinetics, and plasma PCSK9 concentrations.

Plasma PCSK9 concentration was significantly positively correlated with plasma LDL-C concentrations ($r = 0.333$, $P = 0.036$, Figure 1) and percent body fat ($r = 0.322$, $p = 0.046$, Figure 2). However, no significant correlation was detected between serum PCSK9 concentration and VLDL-TG secretion rate ($r = 0.083$, $p = 0.614$), VLDL-TG clearance rate ($r = 0.032$, $p = 0.845$), or plasma VLDL-TG concentration ($r = 0.024$, $p = 0.885$). In a stepwise linear regression analysis to identify predictors of plasma LDL-C concentration, only plasma PCSK9 concentration was a predictive (adjusted R^2 0.087, $p = 0.038$)

Discussion

In this study, we evaluated the relationship between plasma PCSK9 concentrations and VLDL-TG kinetics in obese subjects. Plasma PCSK9 concentrations correlated directly with percent body fat but was not correlated with VLDL-TG secretion or clearance rates, or plasma VLDL-TG concentrations. These data suggest that although PCSK9 might affect hepatic VLDL secretion (16) and binds to VLDL receptors (17, 18) in rodent models and isolated cell systems, circulating PCSK9 is not an important determinant of VLDL metabolism or increased plasma TG concentrations associated with obesity in people.

Plasma PCSK9 correlated directly with plasma LDL-C concentrations, which is consistent with data reported previously (19, 25–27). Moreover, we found that plasma PCSK9 only explained 8.7% of the variation in LDL-C concentrations, which is similar to that reported in most previous studies, which were conducted among different ethnic and racial groups. It is not clear why PCSK9 concentrations do not account for more of the variability in plasma LDL-C concentrations in non-diabetic subjects, given the marked effect of gain-of-function or loss-of-function mutations on serum LDL-C concentrations (28–30). It is possible that circulating levels of PCSK9 do not provide a full picture of PCSK9 activity, or that direct PCSK9 availability within the liver, rather than PCSK9 circulating in plasma is more important in affecting LDLR activity and LDL-C levels. It is also possible that the relationship between plasma PCSK9 and LDL-C is confounded by clearance of PCSK9 from plasma after it binds to the LDLR. Mice deficient in LDLR have an increased plasma half-life of PCSK9 (31), indicating that LDLR affects PCSK9 clearance.

Data from previous studies found that plasma PCSK9 concentrations were greater in women than in men (19, 26, 27). Our data extend these observations by demonstrating differences plasma PCSK9 concentrations between men and women are also present in an obese cohort. Moreover, we found that PCSK9 correlated directly with percent body fat, so it is possible that greater adiposity in women than men is responsible for the sexual dimorphism in PCSK9 concentrations. This data is in contrast to animal data, which demonstrate increased adipose tissue in visceral depots in *Pcsk9*^{-/-} mice (32). The potential mechanistic links between adipose tissue mass and hepatic PCSK9 production or clearance in humans are not known, but are likely to differ from murine models, and require further study.

Our study is limited by the small number of subjects, which could have missed a correlation between plasma PCSK9 concentrations and VLDL-TG kinetics because of inadequate power. However, we studied participants who had a large range in serum PCSK9 concentrations and VLDL-TG kinetics to increase our ability to detect any correlation between these two outcome variables. Furthermore, our VLDL kinetics protocol has an intra-subject variability of VLDL production and clearance rates of approximately 15% in NAFLD subjects (33), so the range of variability in VLDL-TG metabolic parameters in the present study largely reflects biologic variability rather than measurement precision. Thus, if PCSK9 is involved in regulating VLDL-TG kinetics, it is likely to be a very small effect.

Speculations

Our study did not demonstrate a correlation between plasma PCSK9 concentrations and VLDL-TG secretion or clearance rates, or plasma VLDL-TG concentrations in obese subjects. However, plasma PCSK9 concentration did correlate with plasma LDL-C concentration. These data suggest that PCSK9 is likely involved in LDL-C metabolism, but is not a clinically important regulator of VLDL kinetics in obese people.

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Abbreviations

BMI	body mass index
LDL-C	low density lipoprotein-cholesterol
HDL-C	high density lipoprotein-cholesterol
IHTG	intrahepatic triglyceride
MAST	Michigan Alcohol Screening Test
NAFLD	nonalcoholic fatty liver disease
PCSK9	Proprotein Convertase Subtilisin/Kexin 9
TTR	tracer to trace ratio
VLDL-TG	very low density lipoprotein triglyceride

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Figure 1.
Unadjusted correlation between plasma LDL-cholesterol and plasma PCSK9 concentrations

Figure 2.
Unadjusted correlation between Percent Body Fat and plasma PCSK9 concentrations.

Table 1

Study subject characteristics.

	Mean $\pm$ SD	Range
BMI (kg/m ²)	36.9 $\pm$ 4.3	31–45
Body Fat (%)	40.0 $\pm$ 5.5	28–49
Intrahepatic Triglyceride content (%)	16.6 $\pm$ 11.6	0.8–43.2
Total Cholesterol (mg/dl)	172.3 $\pm$ 34.8	63–238
LDL-C (mg/dl)	95.5 $\pm$ 27.0	18–151
HDL-C (mg/dl)	47.5 $\pm$ 13.9	28–83
Triglycerides (mg/dl)	156.2 $\pm$ 81.8	45–355
VLDL-TG concentration (mmol/l)	0.79 $\pm$ 0.58	0.07–2.57
VLDL-TG secretion rate (μ mol/min)	20.5 $\pm$ 10.3	6.1–58.0
VLDL-TG clearance rate (L/min)	38.3 $\pm$ 31.4	8.0–155.0
PCSK9 concentration (ng/ml)	417 $\pm$ 153	105–800

BMI: Body Mass Index. C: cholesterol. LDL: Low Density Lipoprotein. HDL: High Density Lipoprotein. VLDL: Very Low Density Lipoprotein. TG: Triglyceride. PCSK9: Protein Convertase Subtilisin/Kexin 9