Rosuvastatin, Proprotein Convertase Subtilisin/Kexin Type 9 Concentrations, and LDL Cholesterol Response: the JUPITER Trial

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BACKGROUND: Although statin therapy is known to increase concentrations of PCSK9, whether this effect is related to the magnitude of LDL reduction is uncertain. This study was undertaken to understand the extent of this effect and examine the relationship between PCSK9 and LDL cholesterol (LDL-C) reduction.

METHODS: We measured plasma PCSK9 concentrations by ELISA at baseline and at 1 year in 500 men and 500 women participating in the Justification for Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial that randomly allocated participants to rosuvastatin 20 mg daily or placebo. We also evaluated rs11591147, a single nucleotide polymorphism known to have an impact on plasma PCSK9 concentrations.

RESULTS: At baseline, median (interquartile range) PCSK9 concentrations were higher in women [73 (62–90)] ng/mL than in men [69 (57–81) ng/mL] ($P <$ 0.005). During 1 year, there was no change in PCSK9 concentrations in the placebo arm, suggesting stability in time. In contrast, the rosuvastatin increased PCSK9 by 35% in women [101 (82–117) ng/mL] and 28% in men [89 (71–109) ng/mL] ($P < 0.0001$). Among those allocated to rosuvastatin, greater reductions in LDL-C were associated with greater increases in PCSK9 on both absolute and relative scales ($r = -0.15$, $P <$ 0.0005). Furthermore PCSK9 (rs11591147) did not alter the magnitude of LDL-C reduction associated with rosuvastatin use.

CONCLUSIONS: In this randomized trial, rosuvastatin increased plasma concentration of PCSK9 in proportion to the magnitude of LDL-C reduction; the LDL-C response to statin could not be inferred by PCSK9 concentrations.

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The major mechanism of action of statins is mediated through upregulation of the LDL receptor (LDLR)[5] found predominantly on hepatocytes (*1*). Recently, a critical role for the PCSK9 protein was found in the cellular processing of the LDLR (*2, 3*), and it has subsequently been reported that mutations in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene associate with wide variation in LDL cholesterol (LDL-C) concentrations. Specifically, gain-of-function mutations in *PCSK9* are associated with marked increases in LDL-C, similar to those seen in familial hypercholesterolemia due to defects in the LDLR protein (*4*), whereas loss-of-function mutations are associated with lower PCSK9 concentrations, low lifelong LDL-C concentrations, and reduced cardiovascular risk (*5, 6*). Given these interrelationships, there has been considerable interest in understanding the effect of statins on PCSK9 concentrations, particularly since agents designed to inhibit PCSK9 are likely to be used as adjuncts to statin therapy. To date, it has been established that several statins increase plasma PCSK9 concentrations, but whether this effect is related to the magnitude of LDL-C reduction associated with statin treatment or is modified by genetic status at rs11591147 remains uncertain.

We addressed these issues among 500 men and 500 women participating in the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial (*7*). These individuals underwent genotyping at rs11591147 and had baseline

Table 1. Patient demographics stratified by sex and intervention group.[a]				
	Men		Women	
	Placebo	Rosuvastatin	Placebo	Rosuvastatin
Age, years	64 (59–70)	62 (57–69)	70 (67–75)	70 (66–74)
Glucose				
mmol/L	5.3 (5.0–5.6)	5.3 (5.0–5.7)	5.2 (4.8–5.5)	5.2 (4.8–5.4)
mg/dL	95 (90–101)	96 (90–102)	93 (87–99)	93 (87–98)
Hb A_{1c}, %	5.6 (5.3–5.8)	5.6 (5.3–5.8)	5.7 (5.5–5.9)	5.6 (5.4–5.8)
Body mass index, kg/m^2	29 (26–32)	29 (26–32)	29 (25–33)	29 (25–33)
Systolic blood pressure	130 (122–140)	130 (120–140)	132 (120–142)	130 (122–140)
Diastolic blood pressure	80 (72–82)	80 (72–84)	78 (70–81)	77 (70–81)
Metabolic syndrome[b]	95 (38)	97 (39)	99 (40)	92 (37)
Smoking	41 (16)	38 (15)	13 (5)	18 (7)

[a] Data are median (25th–75th percentile) or n (%). No statistical difference exists between the 2 groups in either sex (n = 250 in each group).
[b] As defined by the consensus criteria of the American Heart Association.

and 1-year blood samples evaluated for both PCSK9 and LDL-C concentrations.

Methods

The patient population was derived from participants in the JUPITER trial, a primary prevention trial comparing rosuvastatin 20 mg daily to placebo in 17 802 apparently healthy men and women with LDL-C <3.4 mmol/L (130 mg/dL) and high-sensitivity C-reactive protein (hsCRP) >2.0 mg/L. For the purposes of the current analysis, 500 men and 500 women allocated to either rosuvastatin or placebo were selected using a stratified randomization method from compliant trial participants who provided consent for genetic and plasma biomarker studies as approved by the Review Ethics Board.

Concentrations of LDL-C were measured centrally as part of the overall JUPITER trial protocol. Genotyping was performed using the Omni 1M Quad platform (Illumina) as part of an ongoing genome-wide association study being conducted within the JUPITER trial. Using these data, genetic information on rs11591147, a previously described polymorphism at the PCSK9 gene locus that leads to an arginine-for-leucine substitution at position 46 (R46L), was available in 955 of the 1000 participants. We evaluated plasma PCSK9 concentrations using a sandwich ELISA as described (8). This assay measures total PCSK9, i.e., both mature and furin-cleaved forms (9, 10).

On the Basis of on our previous reports using PCSK9 ELISA in a healthy population, we assumed a mean value of 89 μg/L at baseline and SD 32 (8). With a likely effect of 12.5%, an α value of 0.01, and a 90% power, we expected that a minimum of 200 participants per group would be required. The estimated cohort size effect is considered a minimum and is likely to be larger. Because each subject serves as his or her own control, we anticipated achieving sufficient statistical robustness for firm conclusions. Therefore we analyzed 250 men and 250 women randomized to placebo and rosuvastatin and determined PCSK9 concentrations at baseline and at 1 year. In a sex-specific analysis, we compared baseline clinical characteristics between the placebo and rosuvastatin groups using t-tests for continuous variables and the χ^2 statistic for categorical variables. The difference in the change in PCSK9 and LDL-C over time in all groups was tested using Spearman rank correlation coefficients. We used ANOVA and Tukey–Kramer multiple comparison to determine differences in PCSK9 by quintile of LDL-C, adjusted for age, sex, body mass index, blood pressure, serum glucose, serum concentrations of lipids and lipoprotein lipids (total cholesterol, triglycerides, LDL-C, HDL-C), apolipoproteins AI and B, and hsCRP. Correlations between PCSK9 and these variables were analyzed by Spearman rank correlation coefficients.

Results

Baseline characteristics of the 500 men and 500 women participating in this study, stratified by rosuvastatin or placebo use, are shown in Table 1. As would be anticipated in a randomized trial, there were no significant differences at study entry within each sex-specific group according to treatment allocation.

Baseline and 1-year concentrations of measured lipid parameters, hsCRP, and PCSK9 are shown in Ta-

Table 2. Baseline and 12-month lipids, apolipoproteins A and B, and hsCRP.[a]

	Baseline		12 months	
	Placebo	Rosuvastatin	Placebo	Rosuvastatin
Men				
Cholesterol, mg/dL	184 (171–194)	180 (165–194)	182 (168–199)	125 (109–139)[b]
Triglycerides, mg/dL	123 (82–174)	125 (85–190)	121 (88–167)	99 (72–151)[b]
LDL-C, mg/dL	108 (95–118)	106 (88–117)	107 (94–120)	50 (40–61)[b]
HDL-C, mg/dL	46 (38–55)	44 (37–54)	46 (40–55)	49 (41–59)[b]
Apo B, mg/dL	107 (95–119)	106 (92–119)	108 (96–120)	64 (55–74)[b]
Apo A, mg/dL	153 (137–172)	152 (136–167)	157 (139–176)	159 (142–180)[b]
hsCRP, mg/L	3.8 (2.5–6.4)	3.5 (2.4–6.5)	3.2 (1.8–5.6)	2.2 (1.2–4.3)
Women				
Cholesterol, mg/dL	194 (179–206)	192 (179–207)	196 (181–214)[b]	134 (121–149)[b]
Triglycerides, mg/dL	116 (86–158)	117 (93–159)	116 (88–167)	98 (74–125)[b]
LDL-C, mg/dL	110 (95–121)	108 (96–120)	112 (97–124)[b]	49 (41–62)[b]
HDL-C, mg/dL	57 (48–70)	58 (49–68)	58 (49–67)	62 (53–73)[b]
Apo B, mg/dL	107 (94–119)	105 (93–118)	108 (94–120)	61 (54–70)[b]
Apo A, mg/dL	175 (156–201)	178 (158–198)	182 (161–203)	188 (168–204)[b]
hsCRP, mg/L	4.3 (3.0–6.9)	4.7 (3.1–7.6)	4.1 (2.5–6.7)	2.3 (1.3–4.1)[b]

[a] Data are median (25th–75th percentile).
[b] $P < 0.005$ baseline vs 12 months ($n = 250$ in each group unless stated otherwise in the text).

ble 2 and Fig. 1. Median (interquartile range) baseline concentrations of PCSK9 were higher in women [73 (62–90) ng/mL (n = 499)] than in men [69 (57–81) ng/mL (n = 500)] ($P < 0.005$). Participants randomized to placebo had no significant change in any measured variable, including PCSK9, suggesting considerable stability of PCSK9 concentrations over time. In contrast, participants randomized to rosuvastatin had
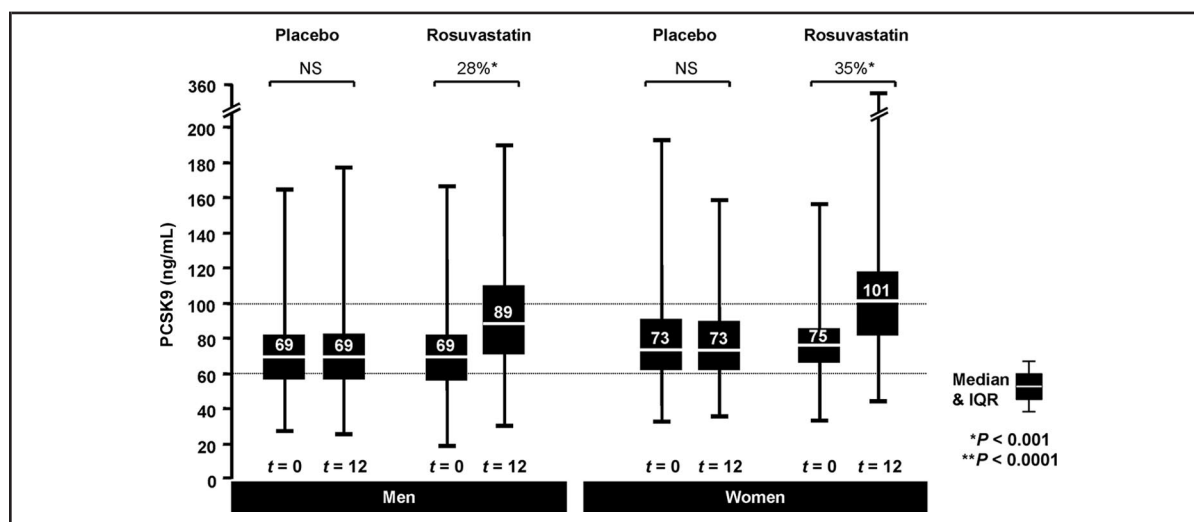
Fig. 1. Stability of PCSK9 concentrations over 1 year in the JUPITER placebo arm vs the rosuvastatin arm. PCSK9 concentrations in men and women at baseline and over 1 year.

Vertical bars, minimum and maximum values; box, interquartile range (IQR); horizontal bar, median; NS, nonsignificant; $t = 0$, baseline values; $t = 12$, 1-year values. *$P < 0.0001$.
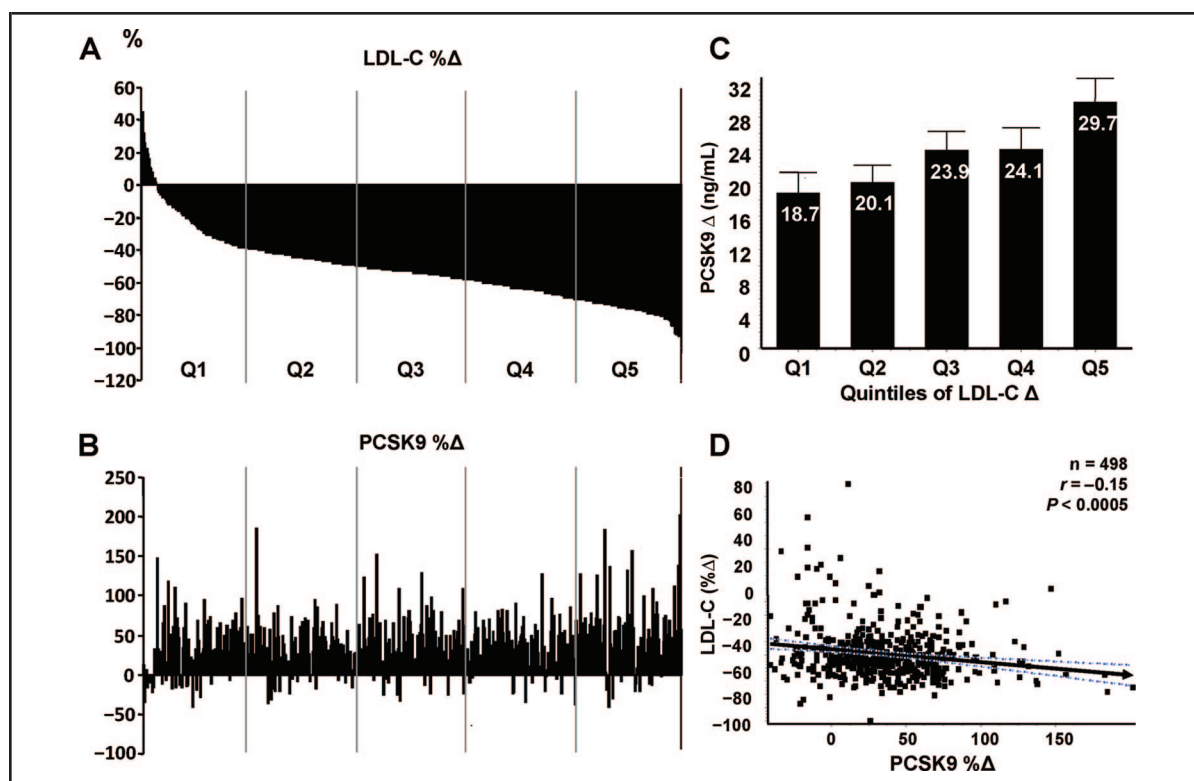
Fig. 2. Rosuvastatin increased plasma concentrations of PCSK9 in proportion to the magnitude of LDL-C reduction.

(A), Individual percentage LDL-C change in response to rosuvastatin 20 mg ranked by magnitude of effect. (B), Corresponding change in PCSK9 concentrations for each study subject ranked by the magnitude of LDL-C change. (C), Changes in PCSK9 ranked by quintiles of LDL-C change on rosuvastatin ($P = 0.0231$ by ANOVA). (D), LDL-C percentage change [(LDL-C at 12 months − LDL-C baseline)/LDL-C baseline $\times$ 100] on rosuvastatin correlates significantly with PCSK9 percentage change [(PCSK9 at 12 months − PCSK9 baseline)/(PCSK9 baseline $\times$ 100)].

significant reductions in total cholesterol, LDL-C, triglycerides, and hsCRP as well a modest increase in HDL-C. These effects were accompanied by a 35% increase in median concentrations of PCSK9 in women from 75 (66−85) ng/mL to 101 (82–117) ng/mL, $P < 0.0001$, and a 28% increase in men from 69 (57–8) ng/mL to 89 (71–109) ng/mL, $P < 0.0001$ (Fig. 1). At baseline, there was a significant correlation (n = 999, $r = 0.15$, $P < 0.0001$) between concentrations of PCSK9 and LDL-C; however, this relationship was no longer observed on rosuvastatin treatment (n = 498, $r = -0.01$, $P = 0.84$) (see Supplemental Fig. 1, A and B, which accompany the online version of this article at http://www.clinchem.org/content/vol58/issue1).

Furthermore, the individual LDL-C percent change from baseline in response to rosuvastatin treatment was plotted in a declining manner as shown in Fig. 2A and corresponding individual percent change from baseline in PCSK9 in Fig. 2B. Although individual

variation among those allocated to rosuvastatin was wide, a significant relationship was observed between the magnitude of LDL-C reduction and the increase in PCSK9 concentrations; this was seen in both an analysis of quintiles of LDL-C reduction (Fig. 2C) and when the LDL-C change was treated as a continuous variable (Fig. 2D). A similar response was observed for apolipoprotein B and non–HDL-C changes and PCSK9 changes (data not shown).

Polymorphism at rs11591147 PCSK9 (R46L) was found in 46 of 955 individuals (24 male heterozygotes, 21 female heterozygotes, and 1 male homozygote). The allele frequency was thus 47 of 1910 chromosomes or 0.0246, close to that previously reported in population-based studies (6). The sole homozygous subject was excluded from further analysis. Serum PCSK9 at baseline was lower in carriers of the R46L SNP by −19% ($P < 0.005$) (see online Supplemental Fig. 2A). And as seen in previous studies (6), the baseline LDL-C con-

centrations in R46L carriers were lower by −9% in men (n = 24, mean 94 mg/dL, vs n = 451, mean 103 mg/dL) and by −7% in women (n = 21, mean 100 mg/dL, vs n = 458, mean 107 mg/dL). In the rosuvastatin arm with available genetic status (n = 478), no significant differences in LDL-C reduction were observed as influenced by the genetic status and thus no increased response to statin therapy in the R46L carriers (see online Supplemental Fig. 2B).

Discussion

We examined 1000 patients in the JUPITER trial. Half (n = 500) were randomized to placebo and half to rosuvastatin 20 mg/day. We established that plasma PCSK9 concentrations were stable as a biomarker over time among those allocated to placebo, but increased by approximately 30% among those allocated to rosuvastatin 20 mg. Although individual responses were variable, we observed a significant relationship between the magnitude of LDL-C reduction and the increase in PCSK9 concentrations on both absolute and relative scales. Specifically, across the full study cohort, greater LDL-C reductions were associated with greater increases in plasma PCSK9 concentrations, an effect present in both sexes despite higher baseline PCSK9 concentrations in women compared with men.

PCSK9 gain-of-function mutations were identified as 1 of 4 molecular causes of familial hypercholesterolemia (4). PCSK9 is the ninth member of the mammalian proprotein convertase family of serine endoproteases (11). PCSK9 is recognized as a key regulator of serum LDL-C concentrations. The gene for PCSK9 is located on chromosome 1p34 and encodes a 692–amino acid protein that is mostly expressed in the liver and intestine (11). The protein comprises a 30–amino acid signal, peptide (SP), a prodomain (amino acids 31–152), a catalytic domain (amino acids 153–454), and a cysteine- and histidine-rich C-terminal domain (amino acids 455–692) (12, 13). The only known substrate for PCSK9 is itself. PCSK9 catalytic domain contains the main binding structure for the epidermal growth factor–like repeat A (EGF-A) domain on the LDLR (14), whereas the C-terminal domain binds cell-surface proteins, including annexin A2 (15). The major function of PCSK9 is to mediate the degradation of the LDLR protein, and evidence exists for both intracellular and extracellular sites of interaction between the LDLR EGF motif and PCSK9 (16–19). However, the predominant source of circulating PCSK9 in blood originates from the extracellular pathway in the liver, and this correlates with the concentration of plasma cholesterol (18).

PCSK9 is regulated at the transcriptional level by sterol regulatory element binding protein 2 (SREBP-2) (20, 21), possibly by SREBP-1c (22), and especially by hepatocyte nuclear factor 1α (HNF-1α) (23). PCSK9 expression is downregulated by cholesterol via SREBP-2 (24) and upregulated by statins via SREBP-2 (25). The liver specific receptor LXR and insulin also regulate PCSK9 (26, 27). Therefore, mRNA levels of LDLR and PCSK9 are regulated by SREBP-2 and statins, inducing an upregulation of both LDLR and PCSK9 (28). The results obtained in the present study are consistent with this observation. The concept that the statin-mediated increase in PCSK9 may limit the efficacy of statins in humans (13, 29), however, is not supported by the present study.

The effect of statins on PCSK9 has been inferred or documented in previous studies (11, 30–32). Here we show that rosuvastatin at a daily dose of 20 mg reduces LDL-C by approximately 50% on average and increases PCSK9 by 28% in men and 34% in women. Baseline PCSK9 concentrations correlate with LDL-C (n = 999, r = 0.15, P < 0.0001). Although a statistically significant correlation between serum concentrations of PCSK9 and LDL-C is present at baseline (r = 0.15), only approximately 2.25% of the variance in LDL-C concentrations is explained by PCSK9 concentrations. On rosuvastatin, this correlation is no longer observed (n = 498, r = −0.01, P = 0.84) (see online Supplemental Fig. 1, A and B). Similar findings with the drug atorvastatin have been reported (31). The observation that the increase in PCSK9 concentrations with rosuvastatin is not associated with a blunted LDL-C response is counterintuitive, based on the postulated mechanisms of action of PCSK9. In fact, the data presented here show a significant negative correlation between the percentage change in PCSK9 concentration on rosuvastatin and the percentage change of LDL-C (n = 498, r = −0.15, P < 0.0005) (Fig. 2D). This observation warrants further mechanistic explanation. Based on these data (Fig. 2, A and B), however, plasma PCSK9 concentrations cannot be used to predict individual response to statin therapy. It remains to be determined whether the measurement of PCSK9 concentration in blood aids in diagnosing patients who are refractory to statins. The development of PCKS9 inhibitors may require such measurement if it can be demonstrated that measuring PCSK9 concentrations influences the choice and success of treatment.

The R46L allele frequency in our study was 2.5%, similar to that of the general population, leading to approximately 8% lower LDL-C than noncarriers (6, 33). Given the ethnic heterogeneity of our population, we cannot, however, perform meaningful statistical comparisons without a much larger sample size. Nonetheless, the presence or absence of this single nucleotide polymorphism was not associated with a difference in the response to rosuvastatin (see online Supplemental Fig. 2B); therefore, knowing the R46L

variant would not likely guide therapy in our population.

We confirm the observation that statins increase PCSK9 (23, 25, 29). Whereas individual LDL-C response to rosuvastatin cannot be predicted by PCSK9 concentrations (Fig. 2, A and B); there is a significant association between the magnitude of change in LDL-C and changes in PCSK9 concentrations (see online Supplemental Fig. 1C). A possible explanation is that the transcriptional regulatory protein SREBP-2 mediates the coordinate expression of the LDLR and PCSK9 in response to cellular cholesterol deprivation (24).

As a biomarker and a therapeutic target, PCSK9 is appealing (12), but because of stoichiometric interactions between the EGF-A region of the LDLR and PCSK9, it has, to date, not been amenable to targeting by small molecules. Several approaches have thus been examined to decrease PCSK9 concentrations and are at an advanced stage. One such approach is the use of antisense mRNA that modulates the expression of PCSK9 mRNA, leading to reduced PCSK9 production (34–36). Another approach is the inhibition of PCSK9 binding to the LDLR by using antibodies against PCSK9, leading to inhibition of LDLR degradation mediated by PCSK9 (19, 37). These approaches require the manufacture of biological substrates (antisense oligonucleotides or antibodies) and are likely to entail production costs that will restrict their application to patients in whom statins at maximally tolerated doses still do not allow target levels to be reached as proposed in national guidelines (38, 39). Given this interest, several monoclonal antibodies to PSCK9 are already in phase 2 clinical trials (see www.clinicaltrials.gov), giving clinicians a potentially effective add-on treatment alternative. A strategy based on the measurement of LDL-C response and PCSK9 concentrations may help identify those statin-resistant subjects who may benefit from PCSK9 modulation for therapeutic benefits and the smaller proportion of subjects unable to tolerate statins (40).

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