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Calorically Restricted Diets Decrease PCSK9 in Overweight Adolescents

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Abstract

Background and Aims—Nutritional therapy is the first line approach to treatment of hyperlipidemia in childhood. Proprotein convertase subtilisin kexin type 9 (PCSK9) is a key regulator of plasma cholesterol levels and a target of novel lipid-lowering pharmacotherapies. We examined the effects of an intensive nutritional intervention on PCSK9 levels in overweight adolescents with cardiovascular disease (CVD) risk factors.

Methods and Results—Twenty seven obese and overweight adolescents with CVD risk factors were assigned to either a low fat or low glycemic load diet. During an 8-week “*Intensive Phase*,” assigned meals were delivered to the home, and all participants received weekly in-person home nutrition counseling and phone calls. The subjects then underwent a 4-month “*Maintenance Phase*” without food provision and with no in-person contact. Anthropometric measurements, laboratory data, and serum PCSK9 protein levels were measured at baseline, 8 weeks, and 6 months. PCSK9 decreased by 16.5% at 8 weeks (201.2 ± 56.3 vs 165.6 ± 58.4 ng/mL; $p < 0.001$); PCSK9 levels returned to baseline levels at 6 months, after the *Maintenance Phase*. Change in PCSK9 was associated with change in fasting insulin, HOMA-IR, and AUC insulin, independent of weight loss.

Conclusions—PCSK9 decreased in youth participating in an intensive dietary intervention. Change in HOMA-IR was associated with change in PCSK9, independent of weight loss, suggesting an important relationship with insulin sensitivity.

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CONFLICT OF INTEREST

Dr. Ludwig reported receiving royalties for books about nutrition and obesity. Dr. de Ferranti has received royalties for UpToDate topics on treatment of pediatric lipid disorders.

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death in adulthood (1), and evidence supports that the atherosclerotic process begins in childhood (2,3). Longitudinal epidemiological studies have shown that non-high density lipoprotein cholesterol (non-HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels track into adulthood and predict severity of atherosclerosis and adult CVD (4). Abnormal lipid levels are common in adolescents (5), and can improve with lifestyle modifications, which have been shown to significantly reduce LDL-C levels (6).

Although nutritional therapy is a mainstay of dyslipidemia treatment in children and adolescents, its effect on proprotein convertase subtilisin-kexin type 9 (PCSK9), a key regulator of LDL-C clearance (7–9), is unknown. Gain-of-function mutations in PCSK9 result in elevated levels of LDL-C and increased risk for CVD; whereas loss-of-function PCSK9 mutations are associated with hypocholesterolemia and reduction in the incidence of coronary heart disease (10–12). Thus, PCSK9 has become a novel target for lipid lowering therapy, and newly approved PCSK9 inhibitors have shown to lower LDL-C levels by over 50% (13).

Despite the importance of PCSK9 in the regulation of LDL-C, little is known about its role in childhood hypercholesterolemia. Furthermore, no studies have examined the effect of nutritional therapy on PCSK9, the mainstay of CVD risk reduction in childhood (14). With the application of universal screening guidelines, more children with abnormal cholesterol levels are being identified (15,16), making it important to understand the impact of dietary modifications on PCSK9, and its relationship with glucose homeostasis, lipid parameters, and changes in adiposity, in this young population.

The primary objective of this study was to assess the effects of an intensive nutritional intervention, coupled with weight loss, on serum PCSK9 levels in overweight and obese adolescents with CVD risk factors. We also sought to determine the relationship between change in PCSK9 concentration and change in lipid parameters, insulin sensitivity, and body fat.

MATERIALS AND METHODS

Study Participants

Participants were recruited from clinical programs treating pediatric obesity and complications, Craigslist, and community practices. Overweight or obese (BMI ≥ 85th percentile) children and adolescents, ages 8–21 years, were eligible if they had at least three CVD risk factors, including fasting TG level >100 mg/dL, HDL-C <45 mg/dL for boys ages 15–19 years and <50 mg/dL for all other participants, systolic blood pressure >90th percentile for gender, age, and height, and fasting glucose ≥ 100 mg/dL or fasting insulin level > 15 uU/mL. Exclusion criteria were current or anticipated pregnancy, weight >275 lbs (125 kg), major medical illness, medications that might significantly affect CVD risk factors or weight, or abnormalities at the screening assessment that required pharmacotherapy. Written informed consent and assent were obtained from parents and participants. The study

was approved by the Institutional Review Board of Boston Children's Hospital and was registered at clinicaltrials.gov (NCT01080339).

Study Design

The parent study has previously been described in detail (17). Briefly, it was designed to compare the effects of a low fat (LF) and a low glycemic load (LGL) diet on CVD risk factors in overweight and obese adolescents, in the setting of modest weight loss, in a randomized, 6-month, parallel-group study conducted at Boston Children's Hospital (BCH) between May 2007 and March 2012. The study began with an 8-week *Intensive Phase*, during which participants received meals and snacks delivered directly to their homes according to their assigned diet (LF or LGL) in sufficient quantity to supply a caloric deficit of ~25% to induce modest weight loss over the course of 8 weeks. The diets differed mainly in macronutrient composition and glycemic load (GL) (percent fat/carbohydrate/protein; LGL: 40/40/20, LF: 20/60/20). The glycemic index (GI) was calculated as product of the glycemic index of a food and the amount of carbohydrate contained in that food, using glucose as the reference (18,19). The LGL diet aimed for a 50% glycemic index for each meal. The LF diet was based on contemporary dietary guidelines (20% total fat of which 7% were saturated fat) (20) and targeted a GI representative of prevailing dietary trends (21). Aside from differences in GL, total fat and saturated fat content, the study diets provided similar amounts of protein (20%), fiber (~30 g/day), and dietary cholesterol (200 mg/day).

The *Intensive Phase* was followed by a 4-month *Maintenance Phase* that involved only phone support, but no delivered meals or in-person contact.

Anthropometrics and Blood Pressure

Anthropometrics and blood pressure were measured at all study visits three times and averaged for use in the analysis. Height was measured to the nearest 0.1cm, weight to the nearest 0.1kg, and waist circumference to the nearest 0.1cm. Percent body fat was measured using bioelectrical impedance. Systolic and diastolic blood pressures were measured by auscultation according to standard methods (22).

Laboratory Testing

Fasting blood samples were obtained at baseline, 8 weeks, and 6 months. Serum PCSK9 protein levels were measured by ELISA (CycLex, Nagano, Japan). Additional measurements, including blood glucose, insulin, HbA1c, and a lipid panel, were measured in the fasting state according to standard methods. HOMA-IR was calculated using averaged fasting glucose and insulin values from the -20, -10, and 0 time points. The area under the curve (AUC) of glucose and insulin were calculated from a 2-hour oral glucose tolerance test (OGTT) performed at baseline and 8 weeks; samples were collected after a 75 gram oral dose of Trutol.

Statistical Analyses

Descriptive statistics are reported as mean (SD) or frequency (%). Differences in baseline demographic and clinical factors between diet groups were assessed using two-sample t-tests for continuous variables and Fisher's exact test for categorical variables. One-sample t-tests

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were used to assess pre-post changes from baseline to 8 weeks or 6 months within diet. Differences between diets in pre-post changes from baseline at each visit were assessed using two-sample t-tests. Non-normally distributed variables were logarithmically transformed, and non-parametric tests were used if normality was not attained. Associations between change in PCSK9 and continuous variables were assessed using Pearson correlation. An exploratory linear regression model was used to examine the relationship between clinical factors and the change in PCSK9, adjusting for selected covariates. All tests were performed at two-sided alpha-level of 0.05. SAS (version 9.3, Cary, NC) software was used for all analyses. The last observation carried forward method was used to impute information for any subjects with missed visits.

RESULTS

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The patient characteristics for the 27 individuals randomized into the study are shown in Table 1. Of these participants, 25 completed the study; one subject withdrew after the baseline visit, and another individual did not complete the *Maintenance Phase*. The data shown in Table 1 are from 27 participants, except for percent body fat and HbA1c, where $n=26$. By study design, the participants had a high prevalence of CVD risk factors, including a mean BMI z-score of 2.1 ± 0.3 and mean percent body fat of $35.3 \pm 3.1\%$. Participants were comparable between diet groups without differences at baseline with regard to age, sex, race/ethnicity, socio-demographic factors, anthropometrics, and CVD risk factors (17).

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In the group overall, mean PCSK9 levels declined significantly (-35.5 ± 49.5 ng/mL, $p<0.001$) during the 8-week *Intensive Phase*, in parallel with a decrease in LDL-C (Figure 1A, 1B and Table 2). This decline returned to baseline during the 4-month *Maintenance Phase*; the mean PCSK9 level at 6 months did not differ compared to baseline (-5 ± 66.7 ng/mL, $p=0.91$). Mean PCSK9 levels did not differ significantly between the two diet groups at baseline ($p=0.36$), 8 weeks ($p=0.72$), and 6 months ($p=0.76$) (Appendix Figure 1). Given these findings, all further analyses included all participants, without consideration for group assignment.

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Significant decreases were observed in BMI z-score, waist circumference, percent body fat, and systolic blood pressure after the *Intensive Phase* (Table 2), as has previously been reported (17). Change in BMI z-score was modestly correlated with change in mean PCSK9 at the 8-week time point (correlation coefficient $r=0.39$, $p<0.001$). Measurements of glucose homeostasis improved over the 8 weeks including significant declines in HbA1c, fasting insulin, AUC Insulin, and HOMA-IR. These changes were moderately correlated with change in PCSK9 ($r=0.49-0.56$, $p<0.05$). Lipid parameters also improved during the dietary intervention, with decreases in TC, LDL-C, TG, and Non-HDL-C; however, none of these lipid changes were significantly correlated with mean change in PCSK9. During the *Maintenance Phase*, many of the study outcomes trended back towards baseline (Table 2). In Figure 2, the change in PCSK9 from baseline to 8 weeks ("*Intensive Phase*", X axis) is plotted against the change in PCSK9 from 8 weeks to 6 months ("*Maintenance Phase*", Y axis), for each individual study subject, and linear crosshairs indicate where zero change occurred. This graph shows that there were several subjects who experienced minimal change in PCSK9 during the *Intensive Phase* and little change after the *Maintenance Phase*.

However, it appears that the majority of the subjects who showed moderate change or a decrease in PCSK9 during the intervention, also had a relative increase in PCSK9 during the maintenance phase. This finding is further supported by non-parametric correlative analysis that shows a significant negative association between the change in PCSK9 during the *Intensive Phase* with the change in PCSK9 during the *Maintenance Phase* (Spearman correlation coefficient -0.47 ; $P=0.01$), therefore a decrease in PCSK9 during the first 8 weeks was correlated with an increase in PCSK9 at 6 months.

We also found that improvements in measures of adiposity during the intervention persisted and were correlated with change in PCSK9 (BMI and BMI z score, $r=0.39$ – 0.41 , $p<0.05$). Change in PCSK9 was also associated with change in fasting insulin and HOMA-IR at the 8-week (Figure 3A and 3B; $p<0.05$) and 6-month visits (Figure 3C and 3D; $p<0.0001$). To further examine the relationship between clinical factors and change in mean PCSK9, an exploratory linear regression model was used. At 8 weeks, in an unadjusted model predicting log transformed change in PCSK9 from baseline, HOMA-IR was a significant predictor ($\beta=0.058$, $p=0.011$). Adjusting for change in BMI z-score did not attenuate the relationship between change in HOMA-IR and natural-log transformed change in PCSK9. HOMA-IR remained a significant predictor ($\beta=0.055$, $p=0.012$). Adjusting separately for change in waist circumference ($\beta=0.057$, $p=0.014$), percent body fat ($\beta=0.057$, $p=0.010$), or LDL ($\beta=0.056$, $p=0.016$) demonstrated similar results.

At 6 months, in an unadjusted model predicting log transformed change in PCSK9 between baseline, HOMA-IR was a significant predictor ($\beta=0.098$, $p<0.001$). Adjusting for change in BMI z-score did not attenuate the relationship between change in HOMA-IR and natural-log transformed change in PCSK9; HOMA-IR remained a significant predictor ($\beta=0.090$, $p=0.008$). Adjusting separately for change in waist circumference ($\beta=0.096$, $p<0.001$), percent body fat ($\beta=0.10$, $p<0.001$), or LDL ($\beta=0.098$, $p<0.001$) demonstrated similar results.

DISCUSSION

To our knowledge, this is the first study to assess the effect of a dietary intervention on PCSK9 levels in adolescents with CVD risk factors. We found that after an intensive dietary modification, using either a low glycemic index or a low fat approach with accompanying weight loss, mean PCSK9 levels were decreased, but returned to baseline during the maintenance phase of the study. Furthermore, we showed that, while weight loss was correlated with decreases in PCSK9, measures of insulin resistance, including fasting insulin and HOMA-IR, were the strongest predictors of change in PCSK9 that we examined, independent of weight loss. Change in PCSK9 was not correlated with change in lipid parameters in this small study.

Despite the demonstrated effects of an intensive dietary intervention on PCSK9 levels, dietary assignment did not differentially affect PCSK9. By design, the diets delivered in our study differed in fat (particularly saturated fat) and glycemic load. A previous study on the effects of diets high in fat, protein and fructose on PCSK9 showed that a high-load fructose diet can dramatically increase PCSK9 levels by 93% in healthy adult participants (23).

Rodent studies have suggested that this effect may be mediated through hepatic transcriptional regulators of PCSK9 (24). Interestingly, the Mediterranean diet, which is enriched with monounsaturated fatty acids and has been proposed to prevent metabolic syndrome, resulted in decreased PCSK9 levels in healthy men after 5 weeks (25), and other studies have shown similar results with polyunsaturated fatty acids (26,27). Although the low-fat diet in our study was low in saturated fats, it was not increased in mono- or polyunsaturated fatty acids.

The effects of the *Intensive Phase* on PCSK9 may have been related to the caloric restriction of the dietary intervention. The meals and snacks during the *Intensive Phase* of this study provided a deficit of ~25% in total calories in order to induce weight loss (17). Previously, it has been shown that starvation reduces PCSK9 levels by 70 to 80% in human subjects (28), and that fasting may affect PCSK9 expression at the transcriptional level in rodents (29). Therefore, given that the BMI in our study subjects increased back to near baseline levels at the end of the *Maintenance Phase*, it is likely that many of the subjects resumed unrestricted diets and baseline lifestyle behaviors by the end of the study, and thus many of the positive changes were not sustained. This is consistent with findings from a study of obese, dyslipidemic men, who received personalized healthy eating and physical activity counseling, as well as a hypocaloric diet for one year, in which no significant effect of such a lifestyle intervention on PCSK9 levels (30). These data suggest that an intensive approach to dietary change can elicit an effect on PCSK9 levels, over a relatively short period of time.

Our results suggest that variations in insulin resistance may be related to changes in PCSK9. As insulin resistance (HOMA-IR) improved, PCSK9 levels decreased. This finding is supported by other dietary studies showing positive associations between changes in PCSK9 and measurements of peripheral and hepatic insulin resistance (23,30). Furthermore, this association between PCSK9 and glucose metabolism has also been observed in several large population studies (31), including one with children (32). Additionally, animal experiments have demonstrated a possible mechanistic role for insulin in the regulation of PCSK9 (29,33,34).

The association between insulin resistance and PCSK9 appeared to be independent of change in BMI. During the *Intensive Phase*, all of the study subjects experienced modest weight loss, and change in BMI was correlated with change in PCSK9. However, when insulin resistance was included in the analysis, BMI was not an independent predictor of PCSK9, and neither changes in waist circumference nor percent body fat were associated with PCSK9 in this study. Previous studies have shown the lack of association between PCSK9 and adipose tissue accumulation, body fat distribution, or adipokines (30). Interestingly, other studies have also shown dietary effects on PCSK9 with and without weight loss (25), suggesting that change in weight or BMI may not have a significant effect on PCSK9. Therefore, the effect of insulin resistance on PCSK9 may occur via mechanisms that are independent of change in body mass or fat distribution.

Despite the role of PCSK9 in regulating plasma LDL-C levels, we found no relationship between change in PCSK9 and lipid parameters over the course of the intervention. This finding has been observed in another lifestyle intervention study (30), and may be due to the

small variance in circulating LDL-C, potentially as low as 7%, which can be explained by PCSK9 (31). Additionally, elevated LDL-C was not an inclusion criterion for this study, therefore the ranges for baseline and change in LDL-C levels were relatively narrow, thus correlation analyses may not be particularly informative in this case. It is also possible that the effect of the dietary intervention on PCSK9 in this study may not be large enough to make a measurable impact on LDL-C, and a larger study may be needed to see a significant correlation.

Our study was limited by small sample size and therefore should be considered exploratory. Small size may have restricted the capacity to detect moderate correlations between PCSK9 with small changes in various parameters, such as LDL-C. Furthermore, the results of the statistical models depend upon measurement accuracy and precision of the covariates. We did observe a significant decrease in PCSK9 after the dietary intervention; however, it is difficult to assess the clinical importance of the magnitude of reduction in PCSK9, limiting our ability to determine the clinical impact of our findings. Additionally, the change in PCSK9 returned to baseline by the end of the *Maintenance Phase*, suggesting that interventions may need to remain intensive in order to see beneficial effects. Nevertheless, this is a novel study in regards to dietary intervention in a young cohort at risk for CVD, and provides an opportunity to study PCSK9 in a well-phenotyped pediatric population, relatively free of other medications or other medical problems.

In summary, our findings suggest that improvement in insulin resistance via dietary intervention may be related to changes in circulating PCSK9 concentrations, apparently independent of change in BMI. We propose that, in addition to the overall positive effect of nutritional treatment on CVD risk factors, intensive dietary intervention, with either a low fat or low glycemic diet, may also effectively decrease PCSK9, and could serve as adjunctive therapy to PCSK9 inhibitors, which are very effective in reducing LDL-C (30). Larger, long-term studies are needed to better understand changes in PCSK9 due to dietary modification, and potential lasting effects on the early development of atherosclerosis and CVD in adulthood.

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Appendix

Appendix Figure 1.

Change in PCSK9 by dietary group at baseline (A), 8 weeks (B), and 6 months (C).

References

1. Murphy SL, Kochanek KD, Xu J, Heron M. Deaths: Final Data for 2012. *Natl Vital Stat Rep*. 2015; 63(9):1–117.
2. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med*. 1998; 338(23):1650–6. [PubMed: 9614255]
3. McGill HC, McMahan CA, Zieske AW, Sloop GD, Walcott JV, Troxclair DA, Malcom GT, Tracy RE, Oalmann MC, Strong JP. Associations of coronary heart disease risk factors with the intermediate lesion of atherosclerosis in youth. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Arterioscler Thromb Vasc Biol*. 2000; 20(8): 1998–2004. [PubMed: 10938023]
4. Frontini MG, Srinivasan SR, Xu J, Tang R, Bond MG, Berenson GS. Usefulness of childhood non-high density lipoprotein cholesterol levels versus other lipoprotein measures in predicting adult subclinical atherosclerosis: the Bogalusa Heart Study. *Pediatrics*. 2008; 121(5):924–9. [PubMed: 18450895]
5. Prevalence of abnormal lipid levels among youths — United States, 1999–2006. *MMWR Morb Mortal Wkly Rep*. 2010; 59(2):29–33. [PubMed: 20094024]
6. Ho M, Garnett SP, Baur L, Burrows T, Stewart L, Neve M, Collins C. Effectiveness of lifestyle interventions in child obesity: systematic review with meta-analysis. *Pediatrics*. 2012; 130(6):e1647–71. [PubMed: 23166346]
7. Abifadel M, Varret M, Rabès J-P, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derré A, Villéger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf J-M, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003; 34(2):154–6. [PubMed: 12730697]
8. Benjannet S, Rhoads D, Essalmani R, Mayne J, Wickham L, Jin W, Asselin M-C, Hamelin J, Varret M, Allard D, Trillard M, Abifadel M, Tebon A, Attie AD, Rader DJ, Boileau C, Brissette L, Chrétien M, Prat A, Seidah NG. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. *J Biol Chem*. 2004; 279(47):48865–75. [PubMed: 15358785]

9. Kotowski IK, Pertsemlidis A, Luke A, Cooper RS, Vega GL, Cohen JC, Hobbs HH. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am J Hum Genet.* 2006; 78(3):410–22. [PubMed: 16465619]
10. Berge KE, Ose L, Leren TP. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. *Arterioscler Thromb Vasc Biol.* 2006; 26(5):1094–100. [PubMed: 16424354]
11. Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med.* 2006; 354(12):1264–72. [PubMed: 16554528]
12. Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, Horton JD, Cohen JC, Hobbs HH. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. *Am J Hum Genet.* 2006; 79(3):514–23. [PubMed: 16909389]
13. Chapman MJ, Stock JK, Ginsberg HN. PCSK9 inhibitors and cardiovascular disease: heralding a new therapeutic era. *Curr Opin Lipidol.* 2015; 26(6):511–520. [PubMed: 26780005]
14. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics.* 2011; 128(Suppl):S213–56. [PubMed: 22084329]
15. Ritchie SK, Murphy EC-S, Ice C, Cottrell LA, Minor V, Elliott E, Neal W. Universal versus targeted blood cholesterol screening among youth: The CARDIAC project. *Pediatrics.* 2010; 126(2):260–5. [PubMed: 20624798]
16. Zachariah JP, McNeal CJ, Copeland LA, Fang-Hollingsworth Y, Stock EM, Sun F, Song JJ, Gregory ST, Tom JO, Wright EA, VanWormer JJ, Cassidy-Bushrow AE. Temporal trends in lipid screening and therapy among youth from 2002 to 2012. *J Clin Lipidol.* 9(5 Suppl):S77–87.
17. De Ferranti SD, Milliren CE, Denhoff ER, Quinn N, Osganian SK, Feldman HA, Ebbeling CB, Ludwig DS. Providing food to treat adolescents at risk for cardiovascular disease. *Obesity (Silver Spring).* 2015; 23(10):2109–17. [PubMed: 26337820]
18. Foster-Powell K, Miller JB. International tables of glycemic index. *Am J Clin Nutr.* 1995; 62(4): 871S–890S. [PubMed: 7572722]
19. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *Am J Clin Nutr.* 1991; 54(5):846–54. [PubMed: 1951155]
20. Daniels SR, Greer FR, Committee on Nutrition. Lipid screening and cardiovascular health in childhood. *Pediatrics.* 2008; 122(1):198–208. [PubMed: 18596007]
21. Lin C-S, Kimokoti RW, Brown LS, Kaye EA, Nunn ME, Millen BE. Methodology for adding glycemic index to the National Health and Nutrition Examination Survey nutrient database. *J Acad Nutr Diet.* 2012; 112(11):1843–51. [PubMed: 23102184]
22. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics.* 2004; 114(2 Suppl):555–76. 4th Report. [PubMed: 15286277]
23. Cariou B, Langhi C, Le Bras M, Bortolotti M, Lê K-A, Theytaz F, Le May C, Guyomarc'h-Delasalle B, Zaïr Y, Kreis R, Boesch C, Krempf M, Tappy L, Costet P. Plasma PCSK9 concentrations during an oral fat load and after short term high-fat, high-fat high-protein and high-fructose diets. *Nutr Metab (Lond).* 2013; 10(1):4. [PubMed: 23298392]
24. Dong B, Singh AB, Azhar S, Seidah NG, Liu J. High-fructose feeding promotes accelerated degradation of hepatic LDL receptor and hypercholesterolemia in hamsters via elevated circulating PCSK9 levels. *Atherosclerosis.* 2015; 239(2):364–74. [PubMed: 25682035]
25. Richard C, Couture P, Desroches S, Benjannet S, Seidah NG, Lichtenstein AH, Lamarche B. Effect of the Mediterranean diet with and without weight loss on surrogate markers of cholesterol homeostasis in men with the metabolic syndrome. *Br J Nutr.* 2012; 107(5):705–11. [PubMed: 21787450]
26. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, Pulkki K, Basu S, Uusitupa M, Rudling M, Arner P, Cederholm T, Ahlström H, Risérus U. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr.* 2012; 95(5):1003–12. [PubMed: 22492369]
27. Graversen CB, Lundbye-Christensen S, Thomsen B, Christensen JH, Schmidt EB. Marine n-3 polyunsaturated fatty acids lower plasma proprotein convertase subtilisin kexin type 9 levels in

- pre- and postmenopausal women: A randomised study. *Vascul Pharmacol.* 2016; 76:37–41. [PubMed: 26143741]
28. Persson L, Cao G, Ståhle L, Sjöberg BG, Troutt JS, Konrad RJ, Gälman C, Wallén H, Eriksson M, Hafström I, Lind S, Dahlin M, Amark P, Angelin B, Rudling M. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. *Arterioscler Thromb Vasc Biol.* 2010; 30(12):2666–72. [PubMed: 20884874]
 29. Costet P, Cariou B, Lambert G, Lalanne F, Lardeux B, Jarnoux A-L, Grefhorst A, Staels B, Krempf M. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. *J Biol Chem.* 2006; 281(10):6211–8. [PubMed: 16407292]
 30. Arsenault BJ, Pelletier-Beaumont E, Alméras N, Tremblay A, Poirier P, Bergeron J, Després J-P. PCSK9 levels in abdominally obese men: association with cardiometabolic risk profile and effects of a one-year lifestyle modification program. *Atherosclerosis.* 2014; 236(2):321–6. [PubMed: 25128757]
 31. Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. *J Clin Endocrinol Metab.* 2009; 94(7):2537–43. [PubMed: 19351729]
 32. Baass A, Dubuc G, Tremblay M, Delvin EE, O'Loughlin J, Levy E, Davignon J, Lambert M. Plasma PCSK9 is associated with age, sex, and multiple metabolic markers in a population-based sample of children and adolescents. *Clin Chem.* 2009; 55(9):1637–45. [PubMed: 19628659]
 33. Miao J, Manthena PV, Haas ME, Ling AV, Shin D-J, Graham MJ, Crooke RM, Liu J, Biddinger SB. Role of Insulin in the Regulation of Proprotein Convertase Subtilisin/Kexin Type 9. *Arterioscler Thromb Vasc Biol.* 2015; doi: 10.1161/ATVBAHA.115.305688
 34. Ai D, Chen C, Han S, Ganda A, Murphy AJ, Haeusler R, Thorp E, Accili D, Horton JD, Tall AR. Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice. *J Clin Invest.* 2012; 122(4):1262–70. [PubMed: 22426206]
 35. Koren MJ, Giugliano RP, Raal FJ, Sullivan D, Bolognese M, Langslet G, Civeira F, Somaratne R, Nelson P, Liu T, Scott R, Wasserman SM, Sabatine MS. Efficacy and safety of longer-term administration of evolocumab (AMG 145) in patients with hypercholesterolemia: 52-week results from the Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) randomized trial. *Circulation.* 2014; 129(2):234–43. [PubMed: 24255061]
 36. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JJP. Efficacy and Safety of Alirocumab in Reducing Lipids and Cardiovascular Events. *N Engl J Med.* 2015; 372(16):150315080052000.
 37. Zhang X-L, Zhu Q-Q, Zhu L, Chen J-Z, Chen Q-H, Li G-N, Xie J, Kang L-N, Xu B. Safety and efficacy of anti-PCSK9 antibodies: a meta-analysis of 25 randomized, controlled trials. *BMC Med.* 2015; 13:123. [PubMed: 26099511]

HIGHLIGHTS

- The first line treatment for dyslipidemia in childhood is nutritional therapy
- Effect of diet on PCSK9, a regulator of LDL-C, is unknown in children
- An intensive nutritional intervention reduces PCSK9 in overweight adolescents
- Change in PCSK9 with diet is correlated with change in insulin resistance
- Improvements in insulin sensitivity with diet may modify PCSK9 in adolescents

Figure 1.

Change in PCSK9 (A) and LDL-cholesterol (B) levels across three study visits. Boxes display mean (+), median (center line), interquartile range (top and bottom boundaries), and minimum and maximum (vertical lines). Gray lines depict time course for each of 27 individual subjects. *Paired comparison showed a significant mean decline between baseline and 8 wk ($p < 0.05$ by t-test).

Figure 2.

Change in PCSK9 from baseline to 8 weeks (“*Intensive Phase*”, X axis) vs change in PCSK9 from 8 weeks to 6 months (“*Maintenance Phase*”, Y axis). Black circles represent each individual study subject. Linear crosshairs indicate where zero change occurred.

Figure 3.

Correlation between change in (ln)PCSK9 and change in HOMA-IR and fasting insulin at 8 weeks (A, B) and 6 months (C, D).

Table 1

Characteristics of the study population at baseline (n=27)

Patient Characteristic ^a	n (%) or mean (SD)
Age (years)	13.2 (2.5)
Sex (n, % female)	18 (67)
Race (n, % non-Caucasian)	44 (52)
Ethnicity (n, % Hispanic)	7 (26)
BMI (kg/m ²)	29.8 (3.9)
BMI z-score	2.1 (0.3)
Waist Circumference (cm)	99.8 (9.4)
Body Fat (% , n=26)	35.3 (3.1)
Systolic BP (mmHg)	105.4 (7.7)
Diastolic BP (mmHg)	68.9 (7.1)
HbA1c (% , n=26)	5.6 (0.3)
Fasting Glucose (mg/dL)	88.5 (8.3)
Fasting Insulin (mU/mL)	21.4 (9.2)
HOMA-IR (mg/dL* μ U/mL)	4.7 (2.2)
Total Cholesterol (mg/dL)	158.0 (35.7)
LDL-C (mg/dL)	96.4 (32.1)
HDL-C (mg/dL)	36.6 (5.9)
Non-HDL-C (mg/dL)	121.0 (35.7)
PCSK9 (ng/mL)	201.1 (56.3)

^a All data are reported as n (%) or mean (SD) when appropriate.

Table 2

Changes in clinical parameters and correlation with change in PCSK9 at 8 weeks and 6 months (n=27).

Clinical Parameter	Change BL to 8 Weeks		Change BL to 6 Months	
	Mean (SD) [§]	Correlation with ln(PCSK9) ^{§§}	Mean (SD) [§]	Correlation with ln(PCSK9) ^{§§}
<i>Anthropometrics and Blood Pressure</i>				
BMI (kg/m ²)	−1.37 (1.1) *	0.39 *	−0.82 (1.3) *	0.39 *
BMI z-score	−0.16 (0.1) *		−0.13 (0.1) **	0.41 *
Waist Circumference (cm)	−3.1 (4.2) *		−2.6 (4.4) *	
Percent Body Fat	−1.13 (1.8) *		−1.83 (2.4) **	
Systolic Blood Pressure (mm Hg)	−4.94 (7.1) *		−3.20 (7.3) *	
Diastolic Blood Pressure (mm Hg)	−2.01 (6.9)		−2.17 (9.3)	
<i>Insulin & Glucose Parameters</i>				
Fasting Insulin (uU/mL)	−7.61 (9.7) *	0.56 *	−3.53 (10.9)	0.82 **
Fasting Glucose (mg/dL)	−2.77 (10.0)		0.24 (7.9)	
HOMA-IR (mg/dL * uU/mL)	−1.68 (2.2) *	0.50 *	−0.69 (2.7)	0.79 **
HbA1c (%)	−0.12 (0.3) *		0.03 (0.3)	
AUC Insulin (uU/mL * 120 mins)	−58.63 (92.9) *	0.49 *	—	—
AUC Glucose (mg/dL * 120 mins)	−11.8 (48.3)		—	—
<i>Lipid Parameters</i>				
Total Cholesterol (mg/dL)	−9.85 (18.8) *		−2.48 (17.4)	
LDL-C (mg/dL)	−6.22 (15.6) *		−3.93 (15.4)	
HDL-C (mg/dL)	−0.8 (5.0)		2.12 (3.6) *	
Triglycerides (mg/dL)	−22.15 (51.5) *		−11.44 (48.6)	
Non-HDL-CI (mg/dL)	−9.06 (16.8) *		−4.6 (15.7)	
PCSK9 (ng/mL)	−35.5 (49.5) *		−5 (66.7)	

[§]From one-sample t-test testing for change from baseline to 8 weeks or baseline to 6 months;^{§§}Pearson correlation coefficient

*p<0.05,

**p<0.0001.