Original research

Pre-heparin lipoprotein lipase mass as a potential mediator in the association between adiponectin and HDL-cholesterol in type 2 diabetes

Raelene E. Maser [a,b,*], M. James Lenhard [b,c], Ryan T. Pohlig [d], P. Babu Balagopal [e]

[a] Department of Medical Laboratory Sciences, University of Delaware, Newark, DE 19716, USA
[b] Diabetes and Metabolic Research Center, Christiana Care Health System, Newark, DE 19713, USA
[c] Diabetes and Metabolic Diseases Center, Christiana Care Health System, Wilmington, DE 19801, USA
[d] Biostatistics Core Facility, University of Delaware, Newark, DE 19716, USA
[e] Biomedical Research & Analysis Laboratory, Nemours Children's Specialty Care & Mayo Clinic College of Medicine, Jacksonville, FL 32207, USA

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ABSTRACT

Aim: Lipoprotein lipase (LPL) is a major enzyme in lipid metabolism. Dyslipidemia, characterized by decreased high-density lipoprotein cholesterol (HDL-C), is prevalent in persons with type 2 diabetes mellitus (T2DM). The aim of this study was to determine whether pre-heparin LPL mass mediates the association between adiponectin and HDL-C in individuals with T2DM.
Methods: Pre-heparin LPL mass was measured via an enzyme-linked immunosorbent assay, adiponectin by radioimmunoassay, and HDL-C was determined enzymatically. Participants' (n = 50) demographics, HbA1c, adiposity, homeostasis model assessment for insulin resistance (HOMA-IR), serum creatinine, and lipids were measured. Path analysis was utilized to test whether pre-heparin LPL mass is a mediator in the relationship between adiponectin and HDL-C.
Results: All four criteria for mediation were satisfied in the path analysis. The indirect effect of adiponectin on HDL-C through pre-heparin LPL mass was significant, p = 0.001, whereas the direct effect of adiponectin on HDL-C was not significant, p = 0.074. These results remained consistent even after adjustments for age, gender, body mass index, HOMA-IR, and serum creatinine in the model.
Conclusion: The findings in this study suggest that pre-heparin LPL mass may mediate the association between adiponectin and HDL-C in T2DM. This relationship for measures of HDL-C functionality requires future investigation.

Introduction

Lipoprotein lipase (LPL), a key multifunctional enzyme, is primarily synthesized in adipose tissue, skeletal muscle, and the heart but is also expressed in many other cells and tissues (e.g., macrophages, liver) [1]. LPL is involved in lipid metabolism with its main biological function to catalyze the hydrolysis of triglyceride-rich lipoproteins, producing remnant particles for subsequent clearance from the circulation [1]. A reduction in LPL activity can influence dyslipidemia characterized by decreased high-density lipoprotein cholesterol (HDL-C) and elevated triglycerides, often seen in type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [2]. Measurement of LPL activity, performed after the intravenous injection of heparin, is a standard procedure for evaluating the enzyme in vivo [3]. There is, however, a large amount of LPL found in pre-heparin serum with an undetectable amount of LPL activity [4]. Thus, the majority of circulating LPL is catalytically inactive but remains a ligand for receptors [4], potentially participating in lipoprotein metabolism via its ligand function rather than its lipolytic function [5]. Shirakawa et al. have recently shown that LPL activity and concentration were significantly correlated with the particle size of remnant lipoproteins in both pre- and post-heparin samples, although there were some differences depending upon ethnicity and disease state [5,6]. Thus as suggested by these investigators, the physiological relevance of pre-heparin LPL mass in lipid metabolism is important [5,6]. Pre-heparin LPL mass has been shown to be associated with lipids [7], insulin resistance

[8], and in a prospective study reduced pre-heparin LPL concentrations were shown to be related to an increased risk of future coronary artery disease [9].

Adiponectin, an adipokine with insulin-sensitizing and anti-inflammatory activity, correlates inversely with adiposity [10]. Its circulating levels are reduced in CVD [11] and in T2DM [12]. Adiponectin has a positive association with HDL-C in the general population [13] and in persons with T2DM [14]. Anti-atherogenic HDL-C has been shown to be an independent protective factor of CVD [15]. Mechanisms linking adiponectin and HDL-C, however, are not clear and some studies have reported a bidirectional relationship between adiponectin and HDL-C [16]. Nevertheless, it is known that lipases are a key factor in the regulation of HDL-C levels [17]. Previous investigators have also demonstrated an association of decreased post-heparin LPL activity and low plasma adiponectin in T2DM [18]. They speculated that increased LPL activity stimulated by increased adiponectin might result in increased HDL-C [18]. In the current study, we hypothesized that the association between adiponectin and HDL-C in T2DM would be mediated by pre-heparin LPL mass. The relationship between adiponectin, pre-heparin LPL mass, and HDL-C was examined using a statistical approach called path analysis. Path analysis is an extension of multiple regression analysis and can evaluate conditions with more than one dependent variable. In addition, path analysis examines direct and indirect effects (e.g., situations with a chain of influence where one variable influences another variable, which in turn affects another variable) [19].

Methods

Subjects The study design/protocol and the participant characteristics of this study have been described in detail previously [20,21]. This study had approval of the Institutional Review Board of Christiana Care Corporation and written informed consent was obtained from all participants before taking part in the study. Briefly, there were 50 individuals who were evaluated at the Diabetes and Metabolic Research Center, Christiana Care Health System, Newark, DE, USA. Individuals with T2DM and age ⩾18 years old were included in the study. Exclusion criteria included the following: (a) a known history of cardiac issues such as having had coronary artery bypass graft surgery, myocardial infarction, recent/ongoing atrial fibrillation, acute myocardial ischemia, etc.; (b) medication or dose changes for lipid lowering agents, antihypertensive and antidiabetes medications 2 months before taking part in the study; and (c) chronic kidney disease (i.e., stage 3b, 4, and 5).

Blood analytes Pre-heparin LPL mass was measured via an enzyme-linked immunosorbent assay (Immuno-Biological Laboratories Co., Ltd, Japan). Total cholesterol, triglycerides, and HDL-C levels were measured on the Vitros 5,1 FS chemistry system (Ortho-Clinical Diagnostics, Rochester, NY, USA). Low-density lipoprotein cholesterol was calculated using the Friedewald formula. The methods for determination of other blood analytes (i.e., insulin, C-peptide, glucose, serum creatinine, HbA1c, leptin, and total adiponectin) have been reported previously [20]. An online calculator downloaded from http://www.dtu.ox.ac.uk was used to estimate the degree of insulin resistance (i.e., homeostasis model assessment for insulin resistance [HOMA-IR]) [22].

Statistical analyses Descriptive data are presented as mean ± SD for variables that were normally distributed whereas the median ± semi-interquartile ranges are reported for variables that were non-normally distributed. Spearman rank correlation coefficients were used to evaluate potential bivariate associations between pre-heparin LPL mass, demographics (e.g., gender, body mass index (BMI)), and metabolic parameters (e.g., HbA1c, HOMA-IR, serum creatinine, lipids, leptin, adiponectin). A path analysis was performed to test if pre-heparin LPL mass was a mediator in the relationship between adiponectin and HDL-C. In brief, path analysis is a structural equation model where all variables included are non-latent (directly observed or measured). It is a powerful multivariate statistical approach that allows complex conceptual models to be evaluated. It can be viewed as a set of regression models with more than one outcome or dependent variable. Whereas multiple regression would require an individual model for each outcome, path analysis simultaneously estimates them. Another advantage of path analysis is that it can involve estimating and hypothesis testing for both direct and indirect effects. A direct effect can be thought of as a regression-like relationship between two variables involving a direct association between them (e.g., A → B). An indirect effect is an association between two variables that operates through another variable (e.g., A → B → C) or variables (e.g., A → B → C → D). All four criteria for mediation established by Baron and Kenny [23] were satisfied. All results are reported using standardized coefficients that allow for relative comparisons among the effects. Further, they can be interpreted as the magnitude of change in the outcome in standard deviations associated with an increase of one standard deviation in the predictor. Additionally R^2 values are reported.

Results

Table 1 provides participants' physical characteristics and biological factors. Physical characteristics and some of the blood analytes from this study have been reported [21]. However, for clarity of discussion of the new data that we are reporting in the present study some of the previously reported data that are pertinent to the present study have also been included in Table 1.

Significant Spearman rank bivariate correlations of adiponectin included HOMA-IR (r = −0.53, p < 0.001), HDL-C (r = 0.64, p < 0.001), triglycerides (r = −0.44, p < 0.01), leptin (r = 0.31, p < 0.05), and gender (r = 0.46, p < 0.01). Significant Spearman rank

Table 1

Descriptive statistics* for clinical and biochemical characteristics of the participants (n = 50).

Characteristics	
Age (years)	63 (10)
Duration of diabetes (years)	13 [5]
Male/female (n)	21/29
Diabetes medications[†] (n)	
Metformin	38
Thiazolidinediones	6
Sulfonylureas	14
Meglitinides	3
Incretin agonists	11
DPP-4 inhibitors	13
Insulin	20
Alpha-glucosidase inhibitors	3
Serum creatinine (mg/dL)	0.80 [0.15]
HbA1c (%)	7.0 [0.7]
Body mass index (kg/m²)	32.7 (5.3)
Homeostasis model assessment for insulin resistance	1.8 [1.0]
Leptin (ng/mL)	15.7 [11.9]
Total adiponectin (mg/L)	5.9 [2.1]
Pre-heparin lipoprotein lipase mass (ng/mL)	67.5 [14.5]
Triglycerides (mg/dL)	94.0 [49.5]
Total cholesterol (mg/dL)	163.9 (40.9)
High-density lipoprotein cholesterol (mg/dL)	50.6 (12.7)
Low-density lipoprotein cholesterol (mg/dL) (n = 49)	87.6 (34.4)

*Values presented as mean (SD) for variables that are normally distributed, median [semi-interquartile range] for variables non-normally distributed, or number of observations.

[†]Most participants were using more than one medication to control their diabetes. DPP-4 inhibitors, Dipeptidyl peptidase-4 inhibitors.
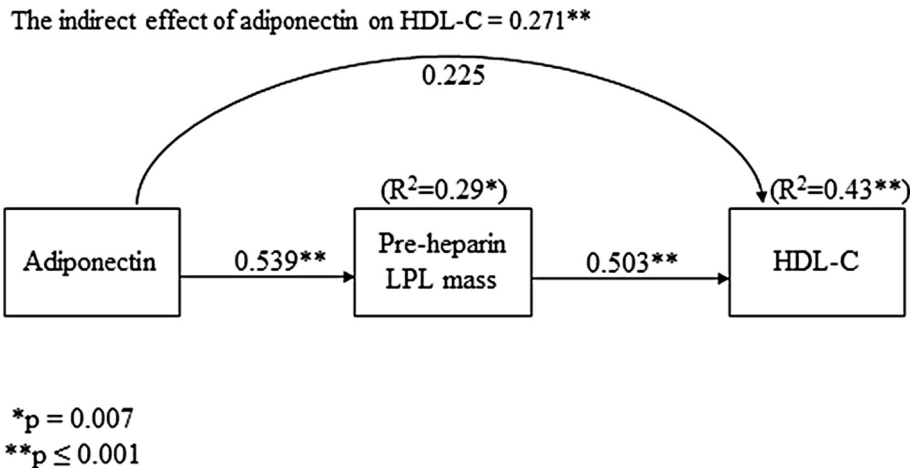
The indirect effect of adiponectin on HDL-C = 0.271**

*p = 0.007
**p ≤ 0.001

Fig. 1. Standardized path coefficients of pre-heparin lipoprotein lipase (LPL) mass mediating the relationship between adiponectin and HDL-cholesterol (HDL-C).

The specific indirect effect of adiponectin on HDL-C = 0.131†
The total indirect effect of HOMA-IR on HDL-C = -0.182†
The specific indirect effect of HOMA-IR on pre-heparin LPL mass = -0.149†

†p < 0.05
*p ≤ 0.01
**p ≤ 0.001

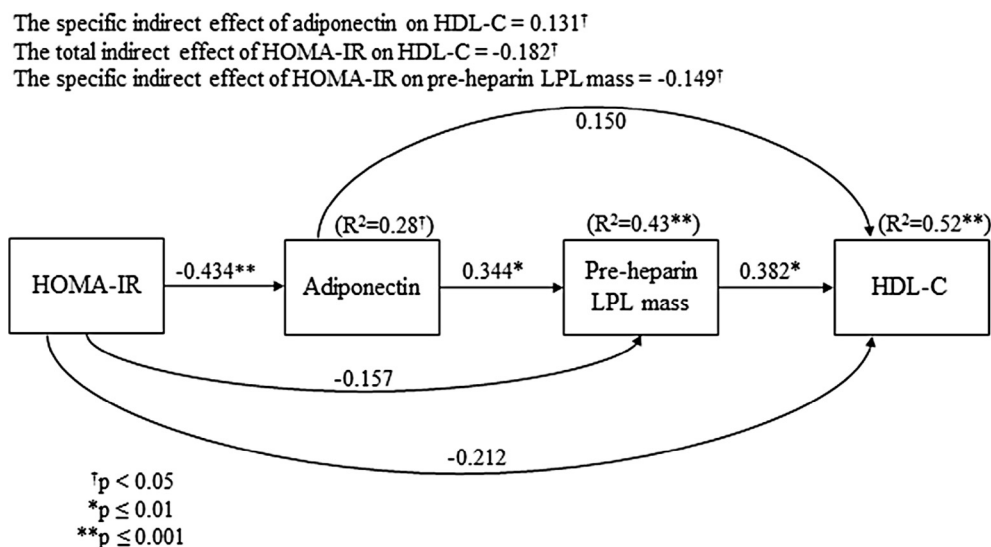**Fig. 2.** Standardized path coefficients of pre-heparin lipoprotein lipase (LPL) mass mediating the relationship between adiponectin and HDL-cholesterol (HDL-C) adjusted for gender, age, body mass index (BMI), homeostasis model assessment for insulin resistance (HOMA-IR) and serum creatinine.

bivariate correlations of pre-heparin LPL mass included adiponectin ($r = 0.67$, $p < 0.001$), HOMA-IR ($r = -0.45$, $p < 0.01$), HDL-C ($r = 0.52$, $p < 0.001$), triglycerides ($r = -0.42$, $p < 0.01$), serum creatinine ($r = -0.42$, $p < 0.01$), and gender ($r = 0.51$, $p < 0.001$).

Results of the simple mediated path model are shown in Fig. 1. The four criteria for mediation were satisfied [23]. First, adiponectin was found to be associated with pre-heparin LPL mass ($p < 0.001$). Second, adiponectin was also significantly associated with HDL-C ($p < 0.001$). Third, pre-heparin LPL mass was significantly associated with HDL-C, adjusting for adiponectin ($p < 0.001$); in this model adiponectin was not significant ($p = 0.093$). Fourth, in the mediation model, both endogenous variables (i.e., pre-heparin LPL mass and HDL-C) were significant ($R^2 = 0.29$, $p = 0.007$, and $R^2 = 0.43$, $p < 0.001$, respectively). The indirect effect of adiponectin on HDL-C was significant ($b = 0.271$, $p = 0.001$), while the direct path was not ($b = 0.225$, $p = 0.074$). Thus, the magnitude of the indirect effect of adiponectin on HDL-C through pre-heparin LPL mass is stronger than the magnitude of a direct effect of adiponectin on HDL-C and 43% of the variance of HDL-C was explained by adiponectin and pre-heparin LPL mass.

The same mediation model was analyzed again, after including age, gender, BMI, HOMA-IR, and serum creatinine as covariates. The findings remained consistent (Fig. 2) and the only additional path that was significant when including all the covariates was from HOMA-IR to adiponectin ($b = -0.434$, $p = 0.001$). The specific indirect path from HOMA-IR to HDL-C through adiponectin and pre-heparin LPL mass was not significant ($b = -0.057$, $p = 0.081$). Additionally triglycerides were examined as another potential covariate. Triglycerides had a significant direct effect on pre-heparin LPL mass but no significant indirect effects on HDL-C (data not shown). Thus, triglycerides were not reported in the model presented here.

Discussion

In this study, we investigated whether the association between adiponectin and HDL-C is mediated by pre-heparin LPL mass in T2DM. We found a significant correlation between adiponectin and HDL-C similar to previous studies. More importantly, results of the path analysis suggest a novel mediatory effect of pre-heparin LPL mass in the relationship between adiponectin and HDL-C in our study of participants with T2DM.

T2DM is associated with reduced HDL-C levels [2] and adiponectin is decreased in obesity and T2DM [12]. Zietz et al. reported a significant association between adiponectin and HDL-C in 523 T2DM individuals after controlling for age, gender, BMI, and fasting

insulin levels and suggested a potential role for adiponectin in HDL-C levels [14]. However, they did not examine LPL in this relationship [14]. Other investigators examining adiponectin and post-heparin LPL activity showed, via regression analysis, a strong relationship between adiponectin and LPL in T2DM, accounting for 26% of the variation [18]. Results of the path analysis in our study for the simple mediated model indicated that 29% of the variance of pre-heparin LPL mass was explained by adiponectin. Thus as previously speculated by von Eynatten et al., LPL stimulated by increased adiponectin may be involved in the regulation of HDL-C levels [18]. Results of the simple mediated path model in our study further support this hypothesis indicating that approximately 43% of the variance of HDL-C was explained by adiponectin and pre-heparin LPL mass.

Insulin is known to be a regulator of the LPL system [24]. Comparing pre-heparin LPL mass in our study for those on insulin therapy (n = 20) versus those who were not (n = 30) indicated no significant difference between the groups (76.1 ± 33 (mean ± SD) vs. 72.5 ± 29 ng/mL, p = 0.77). von Eynatten et al. suggested that utilizing HOMA-IR, thereby integrating insulin levels with glycemia, would be a more reliable parameter than examining insulin per se [18]. Indeed our results showed a bivariate inverse association of HOMA-IR and pre-heparin LPL mass. It should be noted also that there was a significant indirect effect between HOMA-IR and pre-heparin LPL mass in the path analysis model that adjusted for other covariates. Thus, while it is possible that hyperinsulinemia, insulin resistance, or exogenous insulin use could affect LPL, we have shown that adiponectin is strongly associated with pre-heparin LPL mass whereas neither HOMA-IR nor use/non-use of exogenous insulin (data not shown) had a significant direct effect on pre-heparin LPL mass.

Calderon et al. showed that adiponectin and post-heparin LPL activity were independently related to HDL-C, using regression analyses, in a cohort of 127 individuals with type 1 diabetes mellitus (T1DM) [25]. It should be noted that these investigators did not test for mediation and it is possible that partial mediation occurred. Thus, whether the results of this cohort of individuals with T1DM differ from our results of persons with T2DM is not clear. If mediation of adiponectin and HDL-C via LPL is not true in T1DM, a possible explanation may be with regard to obesity in T2DM. The average BMI was 33 kg/m^2 in our cohort of T2DM individuals whereas it was 26–27 kg/m^2 for the T1DM patients in the study by Calderon et al. [25]. In T2DM, lower adiponectin levels, reduced LPL, and lower HDL-C appear to be more of an issue. This suggests that the clinical management of obese patients with T2DM and hypoalphalipoproteinemia may benefit from strategies that help increase adiponectin. Such interventions may include chronic endurance training and weight loss via calorie restriction [26]. While the importance of adiponectin and HDL-C levels has been shown, adiponectin is not routinely measured in T2DM. Evidence is mounting for the measurement of adiponectin not only for research purposes but for clinical use as well.

Previous studies [18,25], described above, examined adiponectin and LPL activity following the intravenous injection of heparin whereas the current study evaluated pre-heparin LPL mass and adiponectin. Recently, Shirakawa et al. examined adiponectin and pre-heparin LPL concentration in normal healthy controls and in Japanese individuals with T2DM and reported a positive and significant correlation between them [5,6], as we did in our cohort with T2DM. Shirakawa et al. did not, however, find a positive association of adiponectin and post-heparin LPL activity in healthy controls [5]. It is likely that differences in patient cohorts may contribute to conflicting results across studies. It is also possible that post-heparin LPL activity may be less diagnostically useful than the determination of pre-heparin LPL mass, as previously suggested [5].

The landmark report of the Framingham study cohort [15] and numerous other clinical and epidemiological studies [27] have consistently shown that reduced HDL-C levels are associated with increased cardiovascular risk. Modulation of the serum levels of HDL-C had received attention as one of the targets to achieve further reduction in cardiovascular risk [28]. Nevertheless, clinical trials to test the hypothesis that raising HDL-C levels will result in reduction of cardiovascular events have not been successful [29,30]. As a result of methodological issues with previous clinical trials, it has been suggested that additional studies are needed to enhance the understanding of the effect of pharmacologic agents on raising HDL-C levels and determine if HDL particle size and its functionality contribute to cardiovascular risk and clinical outcomes [28]. To the best of our knowledge, the association of pre-heparin LPL mass and HDL particle size with its functionality in T2DM has not been examined but provides an important area for future investigation.

Findings from the current study, utilizing pre-heparin LPL mass, may be translated to clinically useful information in several ways. For instance, one area is in the evaluation and treatment of patients with heterozygous LPL deficiency, given that the diagnosis and determination of the prevalence of this disorder is difficult. Heterozygous LPL deficiency is clinically important as it can lead to an increased risk of ischemic heart disease [31]. The examination of pre-heparin LPL mass may shed light on the underlying mechanisms that link adiponectin and HDL-C. It has been speculated that adiponectin activates LPL through the peroxisome proliferator-activated receptor gamma pathway, increasing the concentration of LPL [6]. Indeed, lipases play a role in regulating HDL-C levels [17]. Taken together, these relationships could explain a potential mechanism connecting adiponectin to HDL-C levels via the mediation of pre-heparin LPL mass. Further studies are obviously required as neither path analysis nor the cross-sectional design of this study allows for the determination of cause-effect relationships.

An important strength to our study was the use of path analysis to test for mediation. Conceptually, path analysis can be thought of as an extension of multiple regression and is especially useful for the analysis of complicated models, such as in our study [19]. This study also has some potential limitations that deserve mention. The sample size was relatively small. Nonetheless, the magnitude of the standardized path coefficients would not be expected to change with a larger sample, as the estimates of the coefficients are not sample size dependent. HOMA-IR was used as a surrogate index of insulin resistance. Although HOMA-IR is not the gold standard, it is a clinically useful measure employed in many studies. Participants were using medications that could have influenced adiponectin, pre-heparin LPL mass, and/or HDL-C. For example, thiazolidinediones could have increased adiponectin levels. It should be noted, however, that there were no dose changes for antihypertensive and antidiabetes medications or lipid lowering agents 2 months prior to enrollment. Covariates and confounders that have not been measured in the current study such as physical activity and abdominal adiposity could have affected the results and their interpretation. Given that well designed studies may have flaws, future studies should be designed to account for these variables. In addition, the association between adiponectin, pre-heparin LPL mass, and HDL-C should further be examined by using pharmacological or genetic (knockout) models to provide additional information. To our knowledge these studies have not yet been performed.

In summary, we demonstrated via path analysis that pre-heparin LPL mass potentially mediates the relationship between adiponectin and HDL-C in persons with T2DM. To our knowledge the potential mediatory effect of pre-heparin LPL mass in this relationship found in the current study has not been shown before.

While it still remains unclear if HDL-C qualifies as a target for therapy in T2DM *per se*, other measures of HDL functionality and the potential effect of adiponectin mediated pre-heparin LPL mass pose important questions for future study.

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Conflict of interest

The authors declare they have no conflicts of interest.

References

[1] Li Y, He PP, Zhang DW, Zheng XL, Cayabyab FS, Yin WD, et al. Lipoprotein lipase: from gene to atherosclerosis. Atherosclerosis 2014;237:597–608.

[2] de Vries R, Borggreve SE, Dullaart RP. Role of lipases, lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in abnormal high density lipoprotein metabolism in insulin resistance and type 2 diabetes mellitus. Clin Lab 2003;49:601–13.

[3] Terazawa-Watanabe M, Tsuboi A, Fukuo K, Kazumi T. Association of adiponectin with serum preheparin lipoprotein lipase mass in women independent of fat mass and distribution, insulin resistance, and inflammation. Metab Syndrome Relat Disord 2014;12:416–21.

[4] Machida T, Miyashita K, Sone T, Tanaka S, Nakajima K, Saito M, et al. Determination of serum lipoprotein lipase using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer. Clin Chim Acta 2015;442:130–5.

[5] Shirakawa T, Nakajima K, Shimomura Y, Kobayashi J, Stanhope K, Havel P, et al. Comparison of the effect of post-heparin and pre-heparin lipoprotein lipase and hepatic triglyceride lipase on remnant lipoprotein metabolism. Clin Chim Acta 2015;440:193–200.

[6] Shirakawa T, Nakajima K, Yatsuzuka S, Shimomura Y, Kobayashi J, Machida T, et al. The role of circulating lipoprotein lipase and adiponectin on the particle size of remnant lipoproteins in patients with diabetes mellitus and metabolic syndrome. Clin Chim Acta 2015;440:123–32.

[7] Kobayashi J, Nohara A, Kawashiri MA, Inazu A, Koizumi J, Nakajima K, et al. Serum lipoprotein lipase mass: clinical significance of its measurement. Clin Chim Acta 2007;378:7–12.

[8] Hanyu O, Miida T, Obayashi K, Ikarashi T, Soda S, Kaneko S, et al. Lipoprotein lipase (LPL) mass in preheparin serum reflects insulin sensitivity. Atherosclerosis 2004;174:385–90.

[9] Rip J, Nierman MC, Wareham NJ, Luben R, Bingham SA, Day NE, et al. Serum lipoprotein lipase concentration and risk for future coronary artery disease: The EPIC-Norfolk Prospective Population Study. Arterioscler Thromb Vasc Biol 2006;26:637–42.

[10] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79–83.

[11] Goldstein BJ, Scalia RG, Ma XL. Protective vascular and myocardial effects of adiponectin. Nat Clin Pract Cardiovasc Med 2009;6:27–35.

[12] Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–9.

[13] Christou GA, Kiortsis DN. Adiponectin and lipoprotein metabolism. Obes Rev 2013;14:939–49.

[14] Zietz B, Herfarth H, Paul G, Ehling A, Muller-Ladner U, Scholmerich J, et al. Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. FEBS Lett 2003;545:103–4.

[15] Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977;62:707–14.

[16] Van Linthout S, Foryst-Ludwig A, Spillmann F, Peng J, Feng Y, Meloni M, et al. Impact of HDL on adipose tissue metabolism and adiponectin expression. Atherosclerosis 2010;210:438–44.

[17] Tall AR. Metabolic and genetic control of HDL cholesterol levels. J Intern Med 1992;231:661–8.

[18] von Eynatten M, Schneider JG, Humpert PM, Rudofsky G, Schmidt N, Barosch P, et al. Decreased plasma lipoprotein lipase in hypoadiponectinemia: an association independent of systemic inflammation and insulin resistance. Diabetes Care 2004;27:2925–9.

[19] Streiner DL. Finding our way: an introduction to path analysis. Can J Psychiatry 2005;50:115–22.

[20] Maser RE, Lenhard MJ, Pohlig RT. Vitamin D insufficiency is associated with reduced parasympathetic nerve fiber function in type 2 diabetes. Endocr Pract 2015;21:174–81.

[21] Maser RE, Lenhard MJ, Pohlig RT, Balagopal PB. Osteopontin and osteoprotegerin levels in type 2 diabetes and their association with cardiovascular autonomic function. J Diabetes Complications 2016;30:507–10.

[22] Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191–2.

[23] Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51:1173–82.

[24] Ruge T, Sukonina V, Kroupa O, Makoveichuk E, Lundgren M, Svensson MK, et al. Effects of hyperinsulinemia on lipoprotein lipase, angiopoietin-like protein 4, and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 in subjects with and without type 2 diabetes mellitus. Metabolism 2012;61:652–60.

[25] Calderon RM, Diaz S, Szeto A, Llinas JA, Hughes TA, Mendez AJ, et al. Elevated lipoprotein lipase activity does not account for the association between adiponectin and HDL in type 1 diabetes. J Clin Endocrinol Metab 2015;100:2581–8.

[26] Lee S, Kwak HB. Effects of interventions on adiponectin and adiponectin receptors. J Exercise Rehabil 2014;10:60–8.

[27] Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation 2001;104:1108–13.

[28] Tariq SM, Sidhu MS, Toth PP, Boden WE. HDL hypothesis: where do we stand now? Curr Atheroscler Rep 2014;16:398.

[29] AIM-HIGH Investigators, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med 2011;365:2255–67.

[30] HPS2-THRIVE Collaborative Group, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med 2014;371:203–12.

[31] Nordestgaard BG, Abildgaard S, Wittrup HH, Steffensen R, Jensen G, Tybjaerg-Hansen A. Heterozygous lipoprotein lipase deficiency: frequency in the general population, effect on plasma lipid levels, and risk of ischemic heart disease. Circulation 1997;96:1737–44.