Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Increase in oxidized low-density lipoprotein level according to hyperglycemia in patients with cardiovascular disease: A study by structure equation modeling

Takayuki Yamada, Kazuo Ogawa, Toshikazu D. Tanaka, Tomohisa Nagoshi, Kosuke Minai, Takayuki Ogawa, Makoto Kawai, Michihiro Yoshimura*

Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, Minato-ku, Tokyo, Japan

ARTICLE INFO

Article history:
Received 9 November 2019
Received in revised form
13 January 2020
Accepted 27 January 2020
Available online 29 January 2020

Keywords:
Dyslipidemia
Hyperglycemia
MDA-LDL

ABSTRACT

Aims: Malondialdehyde-modified low-density lipoprotein (MDA-LDL) level has been reported to be strongly associated with the pathogenesis of cardiovascular diseases. We focused on diabetic status and investigated its possible contribution to MDA-LDL level.

Methods: The study sample consisted of 2705 patients who were admitted to our hospital and underwent cardiac catheterization. Blood samples were obtained to measure the levels of fasting blood sugar (FBS), hemoglobin A1c (HbA1c), insulin, LDL, MDA-LDL and others. Body mass index (BMI) was also used in constructing structural equation modeling and Bayesian estimation.

Results: To explore the factors theoretically associated with MDA-LDL level, we performed structural equation modeling. We generated a path model that revealed that BMI, LDL level and FBS were significantly associated with MDA-LDL level ($P < 0.001$ for each factor), whereas insulin level and HbA1c level were not significantly associated (P = NS for both factors). Noted above was clearly demonstrated on the image of 2-D contour line by Bayesian structure equation modeling.

Conclusions: This study clearly showed that hyperglycemia affects MDA-LDL level. An interaction between diabetes and dyslipidemia was shown in terms of activation of lipid oxidation.

1. Introduction

Dyslipidemia and diabetes play important roles in the pathogenesis of cardiovascular diseases. Many patients with diabetes have dyslipidemia, and this dyslipidemia is believed to be important in mediating cardiovascular risk in diabetes. Therefore, diabetic dyslipidemia has been the main focus of discussions regarding the interaction between glucose and lipid metabolism. However, the real linkage between glucose and lipid metabolism is much more complex.

Malondialdehyde-modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA,

* Corresponding author.
E-mail address: oga-n@jikei.ac.jp (K. Ogawa).
https://doi.org/10.1016/j.diabres.2020.108036
0168-8227/

leading to the production of a large amount of aldehyde when LDL degenerates and becomes oxidized [1]. MDA-LDL levels are known to be elevated in subjects with dyslipidemia [2]. In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to be increased even when there are no other differences in the other lipid profiles [3]. In addition, MDA-LDL level has been shown to be potentially useful as a predictor of restenosis after percutaneous coronary intervention in patients with diabetes [4]. Based on these findings, the MDA-LDL level has been speculated to be an important indicator of the progression of arteriosclerosis; however, the clinical factors affecting MDA-LDL level have not been fully clarified.

We previously investigated the clinical factors affecting MDA-LDL levels in high-risk patients requiring catheter intervention [5]. As a result, we found that the MDA-LDL level was affected by multiple factors such as smoking status (as indicated by Brinkman index), LDL level and male gender. However, we could not find a significant relationship by multivariate analysis between glycated hemoglobin (HbA1c) level and MDA-LDL level in that research. This remained to be unexplainable for us in some ways.

Previous literatures find that inflammatory responses are augmented by hyperglycemia and glucose fluctuations via increased mitochondrial superoxide production and endoplasmic reticulum stress [6,7]. Importantly, the inflammatory responses induced by a transient increase in hyperglycemia would last during subsequent normoglycemia [8]. The long-lasting inflammatory response would lead to insulin resistance and much further hyperglycemia [9]. The molecular pathways that integrate hyperglycemia, oxidative stress, and vascular disturbance have been clearly described with a focus on reduced endothelial nitric oxide synthase activity [10]. It has been widely acceptable that endothelial dysfunction serves as the initial trigger for excessive contraction and atherosclerosis in the coronary arteries [11–14].

We hypothesized that hyperglycemia might increase the oxidization of LDL, although we could not clarify an interaction between HbA1c level and MDA-LDL level [5]. Therefore, we planned to study this interaction with the use of more precise components of examination. We searched for the factors affecting MDA-LDL level using fasting blood sugar (FBS) and insulin levels together with HbA1c and LDL levels.

Unsurprisingly, FBS, insulin and HbA1c levels are mutually related and confounding because they are increased in the same manner in patients with diabetes. It is difficult to use these confounding factors to make one equation for multivariate analysis; therefore, another statistical procedure that is well-grounded in theory should be used. Structural equation modeling or covariance structural analysis is one of the appropriate methods. This method plays an important role in understanding how the relationship among observed variables might be generated by other observed variables and/or hypothesized latent variables. Once a model is established as relevant to a given data set, it is important to evaluate the significance of specific parameters within the model, such as coefficients of regression among latent variables. Structure equation modeling has often used in the psychiatric field, but its use is now widely spreading to the rest of the medical field. To reassess the current data from another perspective of statistical view, Bayesian structure equation modeling was also applied. Bayesian estimation has been used in a variety of recent big data analyses [15] and can be used effectively in structural equation modeling. Bayesian analysis in structure equation modeling is based on the Markov Chain Monte Carlo calculation method. Estimating accuracy can be improved by specifying the prior distribution. In general, it may be possible to avoid inadequate solutions due to the small number of samples. In addition, Bayesian structure equation modeling is easy to understand because the results can be represented graphically.

2. Methods

2.1. *Study patients*

The study population consisting of 2705 patients who underwent cardiac catheterization from January 2012 to June 2017 were examined in this study. The baseline patient characteristics, including clinical parameters and biochemical data, were collected retrospectively from hospital medical records. Patients with insulin therapy and patients on emergent admission were excluded. This study was approved by the ethics committee of the Jikei University School of Medicine (Study protocol: 31-166(9665)); and we complied with the routine ethical regulation of our institution as follows. This is a retrospective study, and informed consent was unable to be obtained from each patient. Instead of informed consent from each patient, we publicly posted a notice about the study design and contact information at a public location in our institution.

2.2. *Data collection*

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine, hemoglobin A1c (HbA1c), fasting blood sugar (FBS), triglyceride (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) levels. As described in detail previously [5], MDA-LDL level was measured by an ELISA using an anti-MDA-LDL monoclonal antibody (ML25) and β-galactosidase anti-apoB monoclonal antibody (AB16) [1]. It is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL [1]. The concentration of MDA-LDL was defined at 1 mg/L of MDA-LDL produced artificially, which shows the same signal as 1 U/L of MDA-LDL in the serum. Serum levels of LDL were determined enzymatically (Sekisui Medical Co., Ltd., Tokyo, Japan).

2.3. *Blood sampling and measurement of biochemical examination*

Blood sampling was conducted for every patient with IHD during cardiac catheterization. Serum biochemical analyses were performed in a central laboratory in our hospital.

2.4. *Statistical analysis*

Continuous variables were expressed as the means ± standard deviation (SD) or the median. Statistical analyses were performed using IBM SPSS Statistics version

23.0 (SPSS Inc., Chicago, IL, USA). Single and multiple regression analyses were adopted on an as-needed basis. Path analysis based on structure equation modeling was used to investigate the relationship among clinical factors in this study population and particularly to identify the significant factors in organic stenosis or acute coronary syndrome (ACS). Path analysis was performed using IBM SPSS AMOS version 23 (Amos Development Corporation, Meadville, PA, USA). The obtained structural equation models were tested and confirmed at the significance level for P values of <0.05. The implementation procedures of structure equation modeling have been described previously [16].

In addition, we applied Bayesian structure equation modeling using the program included in IBM SPSS AMOS (version 23.0). In general, Bayesianism permits uncertainty in spite of a little information and Bayesian approaches allow us to incorporate background knowledge into our analyses. We believe that additional testing by Bayesian structure equation modeling would be rationalized and helpful to reassess our data from a different angle of statistics. In IBM SPSS AMOS, the summary table in the Bayesian structure equation modeling window becomes available. Frequency polygons were described with the marginal posterior distributions of the estimands. The selected 2-D contour line was applied in this study as it is easily visualized.

3. Results

3.1. Study patient characteristics

The clinical characteristics of the 2705 cases are shown in Table 1. The average age was 65.7 ± 11.3 years, and 82.6% were male. The mean MDA-LDL level was 111.9 ± 38.2 U/L, and the mean HbA1c level was 6.1 ± 0.8%. (Table 1).

3.2. Single and multiple regression analysis results

Single regression analysis revealed a significant correlation among the respective factors such as log FBS, log HbA1c, log insulin, log BMI, log TG, log HDL and log LDL with log MDA-LDL as shown in Table 2. Multiple regression analysis revealed a significant correlation between the respective factors log FBS, log TG, log HDL and log LDL with log MDA-LDL. However, there was no significant correlation among the respective factors log HbA1c, log BMI and log insulin with log MDA-LDL (Table 2).

3.3. Concept of the proposed path model

As a matter of logic, structure equation modeling was used to search for an independent risk affecting log MDA-LDL level (Fig. 1). The theoretical path model was proposed by positioning log BMI, log HbA1c, log FBS, log insulin, log TG, log HDL and log LDL in parallel. Paths between variables were drawn from independent to dependent variables with directional arrows, which were able to examine influencing factors. Most of the explanatory factors would be confounding with each other; and the association between any two factors was linked by the two-way arrows.

3.4. Result of the path model and Bayesian analysis

As shown in Fig. 1 and Table 3, the path model revealed that log FBS, log TG, log HDL and log LDL were associated with log MDA-LDL ($P < 0.001$ for each factor). Intriguingly, there were no significant associations between log BMI and log MDA-LDL, between log insulin and log MDA-LDL or between log HbA1c and log MDA-LDL level (P = NS for each association).

Noted above was clearly demonstrated on the image of 2-D contour line by Bayesian SEM (Fig. 2).

4. Discussion

Diabetes and dyslipidemia are very important risk factors for atherosclerosis and ischemic heart disease, respectively, along with other risk factors such as hypertension, obesity and cigarette smoking. An interaction between diabetes and dyslipidemia would be highly hazardous because their actions would be additive and probably synergistic. One of the intervention factors would be oxidization of LDL. It is conceivable that MDA-LDL levels are elevated in patients with dyslipidemia and possibly diabetes, although the precise mechanisms for the formation process of MDA-LDL are unclear under these conditions. Regardless, reducing the levels of MDA-LDL for the prevention of cardiovascular disease would be effective; thus, it is important to clarify the factors affecting MDA-LDL level. As shown in our previous study, LDL per se and smoking status were important factors that increased MDA-LDL levels; however, HbA1c did not achieve statistical significance [5]. In this study, we again tried to examine a possible contribution of diabetes to an increase in MDA-LDL levels with indicators such as FBS, insulin and HbA1c using appropriate statistical methods.

Since hyperglycemia, hyperinsulinemia, high HbA1c level and dyslipidemia are associated and mutually confounding, structure equation modeling was a good candidate for an effective solution in this study. As a result of this study, we clearly showed that high FBS was associated with high BMI, high LDL level, and high MDA-LDL level. A high TG level was associated with high MDA-LDL level by structure equation modeling, whereas there was no association between insulin level and MDA-LDL level and between HbA1c level and MDA-LDL level. The association between high glucose level and MDA-LDL level was of high importance in this study. On the other hand, the current result regarding the non-significance of HbA1c was almost same as in our previous study [5]. The current study reinforces previous reports showing the importance of hyperglycemia and glucose fluctuations on cardiovascular diseases [6–8].

In this analysis, we could not detect a harmful effect of hyperinsulinemia on MDA-LDL level. Nevertheless, hyperinsulinemia is associated with hypertension, obesity, dyslipidemia and glucose intolerance; these conditions are collectively known as metabolic syndrome [17,18]. Furthermore, hyperinsulinemia has been shown to play a role in obesity-related hypertension due to increasing renal sodium retention [17]. Additionally, hyperinsulinemia increases

Table 1 – Patient Characteristics.

	Mean ± SD, Median [interquartile range] or N (%)
Number of patients	2705
Age, years	65.7 ± 11.3
Male, gender	2233 (82.6)
BMI, kg/m^2	24.2 ± 3.9, 24.0 [21.9, 26.3]
FBS, mg/dL	111.3 ± 25.2, 24.0 [21.9, 26.3]
HbA1c, %	6.1 ± 0.8, 5.9 [5.6, 6.5]
LDL, mg/dL	98.4 ± 27.9, 95 [79, 115]
HDL, mg/dL	51.2 ± 15.0, 49 [41, 59]
TG, mg/dL	122.8 ± 68.7, 106 [77, 149]
MDA-LDL, U/L	111.9 ± 38.2, 106 [86, 131]
Insulin, μIU/mL	7.6 ± 7.3, 6.1 [4.4, 8.9]
Disease	
Diabetes mellitus	922 (34.1)
Hypertension	2042 (75.5)
Dyslipidemia	1923 (71.1)
Medicine	
Statin	1692 (62.6)
OHA	649 (24.0)
Principal reason for hospitalization	
Angina pectoris	1712 (63.3)
AMI	27 (1.0)
OMI	399 (14.8)
Cardiomyopathy	138 (5.1)
Aortic disease	16 (0.59)
Congenital heart disease	18 (0.67)
Pulmonary hypertension	26 (0.96)
Arrhythmia	56 (2.1)
Valvular disease	225 (8.3)
Congestive heart failure	75 (2.8)

BMI = body mass index; FBS = fasting blood sugar; HbA1c = hemoglobin A1c; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; MDA-LDL = malondialdehyde-modified low-density lipoprotein; OHA = oral hypoglycemic agent; AMI = acute myocardial infarction; OMI = old myocardial infarction.

Table 2 – The results of the single and multiple regression analyses.

Independent variable	Single regression analysis			Multiple regression analysis (R^2 = 0.495)	
	Standardized Regression coefficient	95% CI	P value	Standardized Regression coefficient	P value
Log FBS	0.072	0.057–0.183	<0.01	0.076	<0.01
Log HbA1c	0.054	0.043–0.242	<0.01	−0.028	0.119
Log Insulin	0.137	0.059–0.103	<0.01	−0.009	0.598
Log BMI	0.128	0.192–0.351	<0.01	0.027	0.096
Log TG	0.405	0.254–0.301	<0.01	0.264	<0.01
Log HDL	−0.12	−0.186 to −0.098	<0.01	−0.089	<0.01
Log LDL	0.621	0.681–0.750	<0.01	0.592	<0.01

Dependent variable: log MDA-LDL. FBS = fasting blood sugar; HbA1c = hemoglobin A1c; BMI = body mass index; TG = triglyceride; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; MDA-LDL = malondialdehyde-modified low-density lipoprotein.

cardiac hypertrophy [19]. Therefore, the lack of an association between hyperinsulinemia and MDA-LDL level shown in this study does not diminish the importance of hyperinsulinemia in cardiovascular disease.

Hyperglycemia is an important factor in cardiovascular diseases, working through different mechanisms such as the activation of protein kinase C, polyol and hexosamine pathways, and advanced glycation end products production [20]. All of these pathways promote reactive oxygen species (ROS) accumulation. ROS can directly damage lipids. There is much evidence from experimental studies that polyunsaturated fatty acids (PUFAs) in the plasma membrane, because of their multiple double bonds, are extremely susceptible to attack by free radicals [21]. Hydroxyl radicals initiate a free radical chain reaction and remove a hydrogen atom from one of the carbon atoms in PUFAs and lipoproteins, causing lipid peroxidation that is characterized by membrane protein damage through subsequent ROS attacks [22]. This relationship would be a possible mechanism for the formation of oxidized LDL from hyperglycemia.

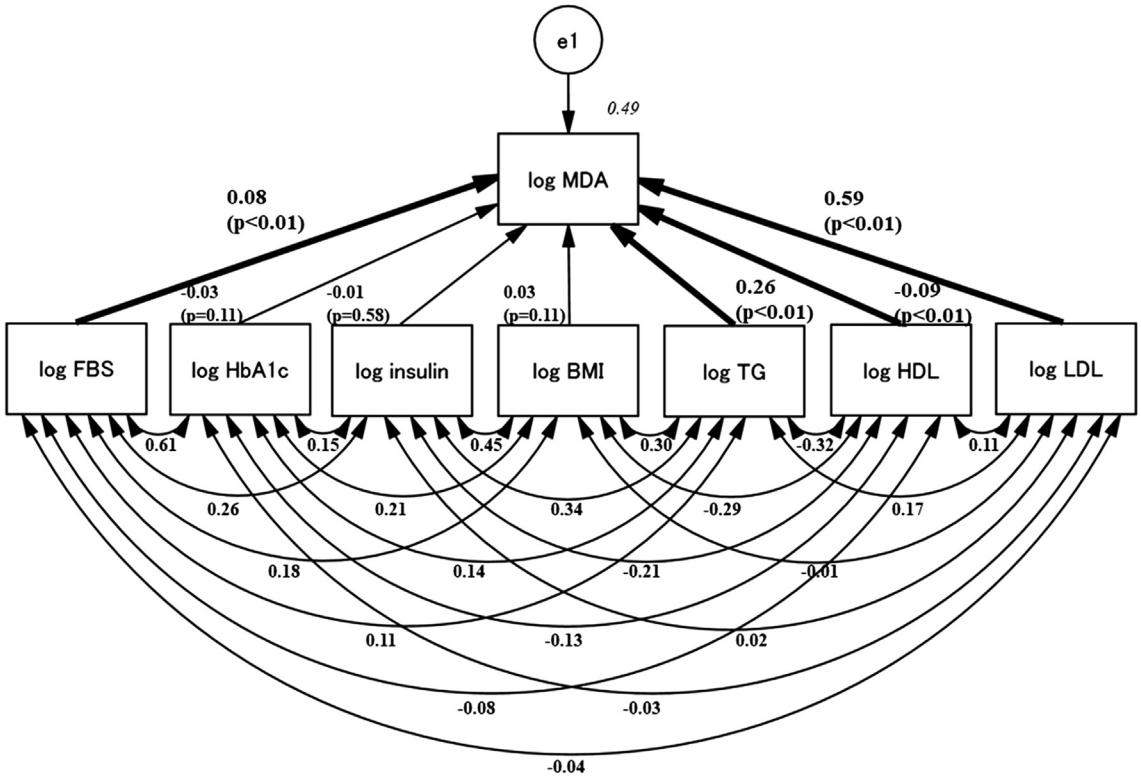

Fig. 1 – Path diagram devised by structure equation modeling. This path diagram examines the effect of each factor on log MDA. Each factor may be confounded, so the path diagram takes this into account. As a result, for example, it is found that log FBS, log HbA1c, and log insulin are related (conjugated), but only log FBS affects log MDA. Log MDA: logarithmic value of malondialdehyde-modified low-density lipoprotein cholesterol, log FBS: logarithmic value of fasting blood sugar, log HbA1c: logarithmic value of hemoglobin A1c, log BMI: logarithmic value of body mass index, log TG: logarithmic value of triglyceride, log HDL: logarithmic value of high-density lipoprotein cholesterol, log LDL: logarithmic value of low-density lipoprotein cholesterol.

Table 3 – Estimates of regression weight and standard regression weight.

	Estimate	Standard error	Test statistic	P-value	Standard regression coefficient
log MDA-LDL<–log FBS	0.126	0.030	4.237	<0.001	0.075
log MDA-LDL<–log HbA1c	−0.074	0.047	−1.559	0.112	−0.028
log MDA-LDL<–log Insulin	−0.005	0.010	−0.553	0.580	−0.009
log MDA-LDL<–log BMI	0.054	0.034	1.592	0.111	0.026
log MDA-LDL<–log TG	0.181	0.011	16.817	<0.001	0.264
log MDA-LDL<–log HDL	−0.109	0.018	−6.119	<0.001	−0.093
log MDA-LDL<–log LDL	0.678	0.016	41.396	<0.001	0.588

MDA-LDL = malondialdehyde-modified low-density lipoprotein; FBS = fasting blood sugar; HbA1c = hemoglobin A1c; BMI = body mass index; TG = triglyceride; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol.

5. Study limitation

A few comments on structure equation modeling. Structural equation modeling is a method of performing factor analysis and multiple regression analysis simultaneously. In general, structural equation modeling is useful for exploratory and descriptive factor analysis. Fortunately, this method can overcome problems if the explanatory factors are confusing. Using the advantages of structural equation modeling, we have recently proposed several path models to explain the mysterious phenomena in the field of cardiovascular disease [16–22]. As described in the methodology and above, structure equation modeling is an effective method, and the relation between cause and effect can be mentioned. However, when discussing causes and effect, note the following. The confounding variable or the third variable that affects both the cause variable and the result variable needs to be examined for its causal relationship with the target. Furthermore, to be causal, more strictly speaking, priorities must be discussed before the event occurs, in terms of the temporal

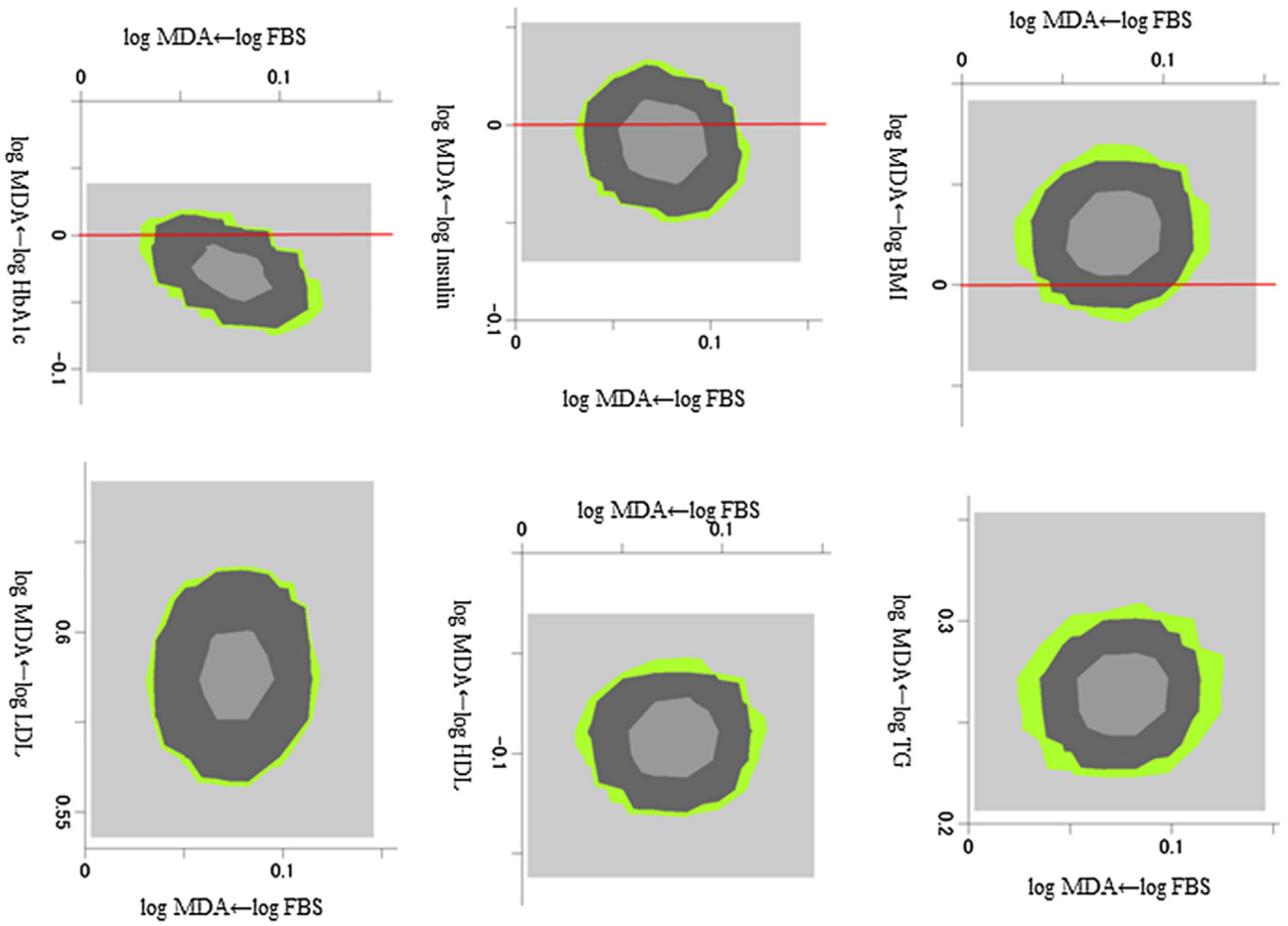

Fig. 2 – Bayesian structure equation modeling. In this study, Bayesian analysis was added after structural equation modeling. Frequency polygons were described by marginal posterior distributions of the estimates. The two-dimensional plot of the bivariate posterior density shows the relationship between the bivariate marginal posterior plots. From light to dark, the three shades of gray represent 50%, 90%, and 95% reliable regions, respectively. For example, in the upper left figure, the horizontal axis represents the effect of log FBS on log MDA, and the vertical axis represents the effect of logHbA1c on log MDA. Although the distribution slightly overlaps the zero on the vertical axis, it is quite far from the zero on the horizontal axis. Visually, it is clear that the effect of log FBS on log MDA is much stronger than the effect of log HbA1c on log MDA. Log MDA: logarithmic value of malondialdehyde-modified low-density lipoprotein cholesterol, log FBS: logarithmic value of fasting blood sugar, log HbA1c: logarithmic value of hemoglobin A1c, log BMI: logarithmic value of body mass index, log TG: logarithmic value of triglyceride, log HDL: logarithmic value of high-density lipoprotein cholesterol, log LDL: logarithmic value of low-density lipoprotein cholesterol.

priority at which the causal event occurs. Care must be taken to conclude that there was an exact causal relationship without such consideration. There will be a need to continue examining this conclusion using a variety of methodologies. Another study limitation is the timing of blood glucose measurements. This time, the analysis is performed using only the FBS level. On the other hand, the effects of postprandial blood glucose are not mentioned in this study. This is another issue to consider in the future.

6. Conclusion

In conclusion, this study clearly showed that hyperglycemia affects MDA-LDL level. An interaction between diabetes and dyslipidemia was shown in terms of activation of lipid oxidation.

Funding

No funding was received for this study.

Acknowledgments

We would like to thank Kumiko Nishiyama for data assistance.

Declaration of Competing Interest

None of the authors have any conflicts of interest to disclose.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2020.108036.

REFERENCES

[1] Kotani K, Maekawa M, Kanno T, Kondo A, Toda N, Manabe M. Distribution of immunoreactive malondialdehyde-modified low-density lipoprotein in human serum. Biochim Biophys Acta 1994;1215:121–5.

[2] Kitano S, Kanno T, Maekawa M, Sakurabayashi I, Kotani K, Hisatomi H, et al. Improved method for the immunological detection of malondialdehyde-modified low-density lipoproteins in human serum. Anal Chim Acta 2004;509:229–35.

[3] Tanaga K, Bujo H, Inoue M, Mikami K, Kotani K, Takahashi K, et al. Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. Arterioscler Thromb Vasc Biol 2002;22:662–6.

[4] Shigematsu S, Takahashi N, Hara M, Yoshimatsu H, Saikawa T. Increased incidence of coronary in-stent restenosis in type 2 diabetic patients is related to elevated serum malondialdehyde-modified low-density lipoprotein. Circ J: Off J Japanese Circ Soc 2007;71:1697–702.

[5] Ogawa K, Tanaka T, Nagoshi T, Sekiyama H, Arase S, Minai K, et al. Increase in the oxidised low-density lipoprotein level by smoking and the possible inhibitory effect of statin therapy in patients with cardiovascular disease: a retrospective study. BMJ Open 2015;5 e005455.

[6] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.

[7] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–7.

[8] El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 2008;205:2409–17.

[9] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944–8.

[10] Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Investig 2001;108:1341–8.

[11] Kugiyama K, Yasue H, Okumura K, Ogawa H, Fujimoto K, Nakao K, et al. Nitric oxide activity is deficient in spasm arteries of patients with coronary spastic angina. Circulation 1996;94:266–71.

[12] Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, et al. T-786–>C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 1999;99:2864–70.

[13] Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med 1999;340:115–26.

[14] Gimbrone Jr MA, Garcia-Cardena G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res 2016;118:620–36.

[15] Collins SP, Lindsell CJ, Pang PS, Storrow AB, Peacock WF, Levy P, et al. Bayesian adaptive trial design in acute heart failure syndromes: moving beyond the mega trial. Am Heart J 2012;164:138–45.

[16] Kinoshita K, Kawai M, Minai K, Ogawa K, Inoue Y, Yoshimura M. Potent influence of obesity on suppression of plasma B-type natriuretic peptide levels in patients with acute heart failure: an approach using covariance structure analysis. Int J Cardiol 2016;215:283–90.

[17] Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, et al. A link between hypertension obesity and glucose intolerance. J Clin Investig 1985;75:809–17.

[18] Dankner R, Chetrit A, Shanik MH, Raz I, Roth J. Basal-state hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up: a preliminary report. Diabetes Care 2009;32:1464–6.

[19] Fernandez-Twinn DS, Blackmore HL, Siggens L, Giussani DA, Cross CM, Foo R, et al. The programming of cardiac hypertrophy in the offspring by maternal obesity is associated with hyperinsulinemia, AKT, ERK, and mTOR activation. Endocrinology 2012;153:5961–71.

[20] Fiorentino TV, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Curr Pharm Des 2013;19:5695–703.

[21] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biol Med 1991;11:81–128.

[22] Halliwell B. Oxidation of low-density lipoproteins: questions of initiation, propagation, and the effect of antioxidants. Am J Clin Nutrit 1995;61:670s–7s.