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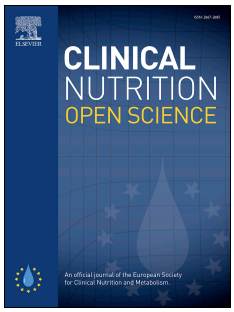

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Original Article

Early Effects of Insulin Therapy on Cholesterol Synthesis and Absorption Markers in Patients with Type 2 Diabetes

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Abstract

Background & Aim:

Metabolic abnormalities in type 2 diabetes affect the production and the clearance of plasma lipoproteins. Although the improvement of low-density lipoprotein cholesterol, triglyceride, and high-density lipoprotein cholesterol levels are important targets in diabetes, it is not established how changes occur in the production and clearance of plasma lipoproteins in the treatment of diabetes. Serum non-cholesterol sterols are introduced as practical markers to assess endogenous cholesterol synthesis and intestinal cholesterol absorption. This study aimed to investigate the effects of insulin therapy on cholesterol synthesis and absorption markers in patients with type 2 diabetes.

Methods: This was a single-center, prospective, 2-week, longitudinal pilot study. Patients with type 2 diabetes who were admitted to start insulin therapy without using lipid-lowering agents were recruited. On the day of hospitalization, the patients discontinued all oral hypoglycemic agents and started with basal-bolus insulin therapy. Cholesterol synthesis (lathosterol) and absorption (campesterol, sitosterol, and cholestanol) markers were assessed at baseline and after 2 weeks of insulin treatment.

Results: In eighteen subjects, the mean age of the patients was 56 ± 10 years (mean ± SD). At baseline, body mass index was 24.3 ± 5.0 kg/m^2, and HbA1c was 11.6 ± 1.7%.

After 2 weeks of insulin therapy, total cholesterol (from 205 ± 48 to 184 ± 43 mg/dL, p = 0.004), lathosterol (from 2.6 ± 1.3 to 2.0 ± 0.7 μg/mL, p = 0.001), campesterol (from 4.3 ± 2.7 to 3.0 ± 2.1 μg/mL, p < 0.0001), and sitosterol (from 2.4 ± 1.6 to 1.7 ± 1.4 μg/mL, p < 0.0001) were significantly decreased, and cholestanol (from 2.5 ± 1.0 to 2.3 ± 0.8 μg/mL, p = 0.05) tended to decrease.

Conclusion: This study showed that insulin therapy reduces cholesterol synthesis and absorption markers in patients with type 2 diabetes hospitalized within 2 weeks. The decrease in cholesterol synthesis and absorption seems to be useful for improving lipid metabolism and reducing the risk of atherosclerosis. Further randomized controlled studies are required to confirm the efficacy of insulin therapy for cholesterol synthesis and absorption markers.

Keywords

Basal-bolus insulin therapy, cholesterol synthesis markers, cholesterol absorption markers, type 2 diabetes, poor glycemic control

Abbreviation:

Niemann-Pick C1-Like 1 (NPC1L1), estimated glomerular filtration rate (eGFR),

55 standard deviation (SD), interquartile range (IQR), waist circumference (WC), blood
56 pressure (BP), Fasting plasma glucose (FPG), Glycated hemoglobin (HbA1c), Glycated
57 albumin (GA), Serum C-peptide immunoreactivity (S-CPR), urinary C-peptide
58 immunoreactivity (U-CPR), Immunoreactive insulin (IRI), Urinary albumin (U-Alb),
59 Lipoprotein lipase (LPL), Apolipoprotein (Apo), body mass index (BMI), sterol
60 regulatory element binding protein-2 (SREBP-2), hydroxymethylglutaryl-CoA (HMG-
61 CoA), liver X receptor (LXR), ATP-binding cassette sub-family G member 5/8
62 (ABCG5/G8)
63

64 **Introduction**

65 Type 2 diabetes is a risk factor for cardiovascular disease. The incidence of coronary
66 artery disease is two to four times higher in patients with diabetes than in those without
67 diabetes [1, 2]. Metabolic abnormalities in type 2 diabetes affect the production and
68 disturb the clearance of plasma lipoproteins. Increased triglyceride (TG), reduced high-
69 density lipoprotein cholesterol (HDL-C) and increased small dense low-density
70 lipoprotein cholesterol are characteristic patterns of diabetes, all of which are known to
71 be atherogenic [3]. However, it is not established how the production and clearance of
72 plasma lipoproteins are altered in patients with type 2 diabetes and how cholesterol

metabolism changes with diabetes treatment.

Plasma cholesterol concentrations are regulated by the synthesis of endogenous cholesterol, absorption from food, and cholesterol catabolism. [4-8]. In the early 1990s, serum non-cholesterol sterols were introduced as practical markers to assess endogenous cholesterol synthesis and intestinal cholesterol absorption [9, 10]. There are four types of serum non-cholesterol sterols: lathosterol, campesterol, sitosterol, and cholestanol. Lathosterol is a precursor of cholesterol that is synthesized in the body. Serum lathosterol levels indicate whole-body cholesterol synthesis and are used as markers of cholesterol synthesis [11].

Campesterol and sitosterol, which are plant sterols, cannot be synthesized in the body. Niemann-Pick C1-Like 1 (NPC1L1) is a transmembrane protein localized at the apical membrane of enterocytes and the canalicular membrane of hepatocytes. It functions as a sterol transporter that mediates intestinal cholesterol absorption and counterbalances the excretion of hepatobiliary cholesterol [12]. In enterocytes, NPC1L1 transports cholesterol and plant sterols, such as campesterol and sitosterol [13]. Campesterol and sitosterol are considered markers of cholesterol absorption because the serum levels of campesterol and sitosterol correlated with cholesterol absorption, even though the intestinal absorption of campesterol and sitosterol was less than 5% [9].

Cholestanol is excreted into bile and partially reabsorbed from the intestinal tract. Cholestanol is a metabolite of cholesterol and is used as a cholesterol absorption marker because the serum ratio of cholestanol to cholesterol is associated with intestinal sterol absorption [14, 15].

Sitosterolemia elevates the plasma levels of sitosterol, due to mutations in the ATP-binding cassette sub-family G member 5/8 (ABCG5/G8) genes, and accelerates atherosclerosis [16, 17]. The increasing campesterol levels were associated with the risk of coronary heart disease event reoccurrence in the Scandinavian Simvastatin Survival Study [18]. It is possible that the lowering sitosterol and campesterol reduce the risk of atherosclerosis. Residual cardiovascular disease (CVD) risk is a dilemma in clinical practice, and novel lipoprotein biomarkers are possible as targets for preventing the risk for CVD [19].

Cholesterol synthesis and absorption markers vary depending on the metabolic abnormalities [20]. Several reports showed an increase in cholesterol synthesis markers and a decrease in absorption markers in patients with type 2 diabetes. However, some results are inconsistent [20-22]. In addition, the effect of diabetes treatment on cholesterol synthesis and absorption markers is unclear. The type 2 diabetes is one of the highest risk factor for coronary artery disease. We hypothesized that there is a metabolic abnormality

in cholesterol absorption and synthesized markers in type 2 diabetes, and the treatment of diabetes normalizes the lipid metabolism abnormality and cholesterol synthesis and absorption markers. Therefore, we examined the levels of cholesterol synthesis and absorption markers in type 2 diabetic patients and how insulin treatment changes cholesterol synthesis and absorption markers.

Aim

We aimed to investigate the effects of insulin therapy on cholesterol synthesis (lathosterol) and absorption (campesterol, sitosterol, and cholestanol) markers in patients with type 2 diabetes.

Methods

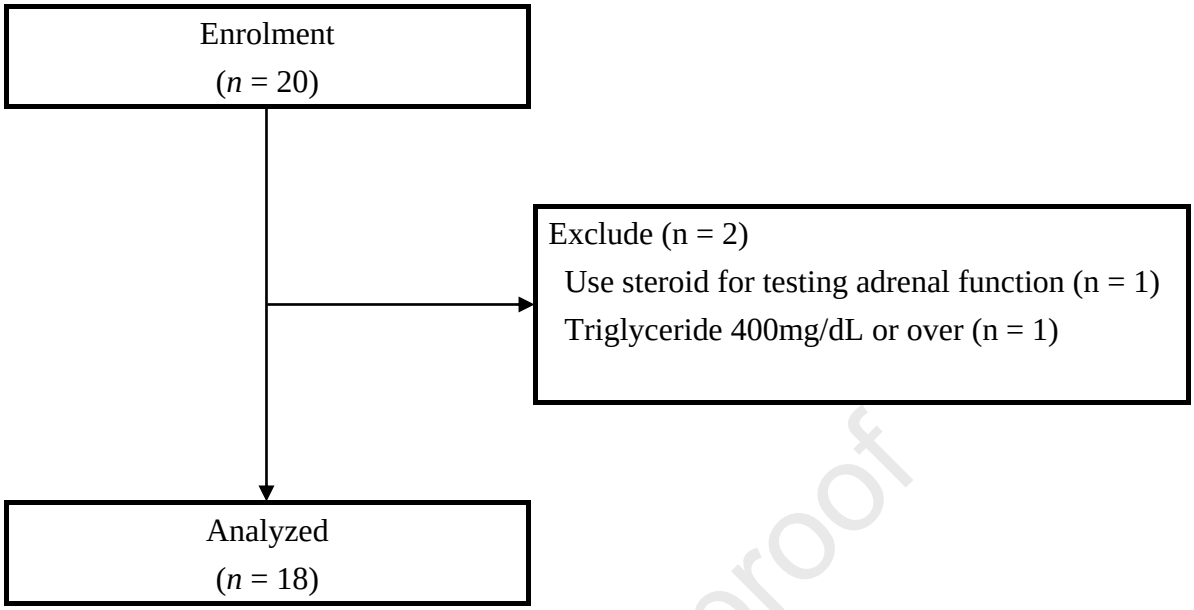
Participants

Patients with type 2 diabetes who had poor glycemic control even after diet therapy, lifestyle improvement, or oral hypoglycemic agents and who required insulin therapy were selected. In addition, the patients who needed insulin therapy immediately due to hyperglycemia were also selected. Enrolled criteria included age 20–75 years, consent to participate in the present study, and hospitalization at Nippon Medical School Hospital

(Tokyo, Japan) for glycemic control. The exclusion criteria were as follows: steroid treatment, perioperative period, history of gastrointestinal resection, severe ketosis, malignant disease, infectious disease, difficulty in ingestion, severe anemia, severe liver dysfunction, chronic kidney disease stage 3b or higher (estimated glomerular filtration rate [eGFR] <45 mL/min/1.73m^2; Japanese Society of Nephrology) [23], severe heart disease, pregnancy, planning pregnancy, breastfeeding, patients taking lipid-lowering agents (statins, cholesterol absorption inhibitor, anion exchange resin, probucol, proprotein convertase subtilisin/kexin type 9 inhibitors, microsomal triglyceride transfer protein inhibitor, fibrates, selective peroxisome proliferator-activated receptor-α modulator, nicotinic acids, and n-3 polyunsaturated fatty acids).

Twenty patients (14 men and 6 women) were assessed for eligibility between June 2017 and November 2018, and 18 patients (13 men, 5 women) were included in the study (Figure 1). Two patients were excluded because they were using steroids (n = 1) and had triglyceride levels of >400 mg/dL (n = 1). The mean age was 56 ± 10 years old (mean ± standard deviation [SD]), and the median diabetes duration was 3.5 (0.0–7.8) (interquartile range [IQR]) years. The baseline characteristics are shown in Table 1.

Figure 1 Flowchart of patient enrolment



Clinical Measurements

All participants underwent physical examination on the first morning of hospitalization, including height, body weight, waist circumference (WC), and blood pressure (BP). Blood samples were collected after overnight fasting on the second day of admission. Fasting plasma glucose (FPG) levels were measured by the glucose oxidase method (ADAMS Glucose GA-1171; Arkray Inc., Kyoto, Japan). Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (ADAMS A1c HA-8181; Arkray Inc., Kyoto, Japan) and was expressed as the percentage value of the National Glycohemoglobin Standardization Program according to the guidelines of the Japan Diabetes Society [24]. Glycated albumin (GA) was measured using an enzymatic method (JCM-BM6070; Japan Electron Optics Laboratory Co., Tokyo, Japan or

LABOSPECT008α; Hitachi High-Tech Co., Tokyo, Japan) and expressed as a percentage of total serum albumin. Serum C-peptide immunoreactivity (S-CPR) levels was measured using chemiluminescent enzyme immunoassay (LUMIPULSE G1200; Fujirebio Co., Tokyo, Japan) and urinary C-peptide immunoreactivity (U-CPR) levels was measured using solid phase radioimmunoassay (ARC-8010; Hitachi Ltd., Tokyo, Japan). Immunoreactive insulin (IRI) was measured by Fluorescence Enzyme Immunoassay (AIA-2000; Tosoh Co., Tokyo, Japan). Urinary albumin (U-Alb) levels were measured using an immune turbidimetric method (JCM-BM6070; Japan Electron Optics Laboratory Co., Tokyo, Japan or LABOSPECT008α; Hitachi High-Tech Co., Tokyo, Japan). Serum total cholesterol (TC), HDL-C, and TG levels were measured enzymatically (JCM-BM6070; Japan Electron Optics Laboratory Co., Tokyo, Japan or LABOSPECT008α; Hitachi High-Tech Co., Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula: LDL-C (mg/dL) = TC (mg/dL) – HDL-C (mg/dL) – TG (mg/dL)/5 [25]. Non-HDL-C was calculated using the following formula: non-HDL-C (mg/dL) = TC (mg/dL) – HDL-C (mg/dL). The eGFR was calculated using the following formula: Cr is serum creatinine, eGFR (mL/min/1.73 m^2) = $194 \times Cr^{-1.094} \times Age^{-0.287}$ (×0.739 if female) [23]. Lipoprotein lipase (LPL) was measured using an enzyme-linked immunosorbent assay (Microplate Reader Emax;

179 Molecular Devices, Tokyo, Japan). Apolipoprotein (Apo) A-I, Apo A-II, Apo B, Apo C-
180 II, Apo C-III, and Apo E were measured using a turbidimetric immunoassay (JCM-25050;
181 Japan Electron Optics Laboratory Co., Tokyo, Japan). Apo B-48 was measured using
182 chemiluminescent enzyme immunoassay (LUMIPULSE G1200; Fujirebio Co., Tokyo,
183 Japan). Lathosterol, campesterol, sitosterol, and cholestanol were assayed using gas
184 chromatography (GC-2010; Shimazu Co., Kyoto, Japan).
185
186 **Study Protocol and Treatment**
187 The study protocol was approved by the Nippon Medical School Institutional Review
188 Board (226038) and conformed to the provisions of the Declaration of Helsinki of 1995
189 (as revised in Edinburgh 2000). All participants provided informed consent prior to
190 enrolment.
191 On admission, all subjects discontinued using oral hypoglycemic agents and started with
192 basal-bolus insulin therapy. During the hospitalization period, dietary energy intake
193 (kcal/day) (protein 11%–18%, fat 19%–27%, and carbohydrate 58%–61%) was restricted
194 to 27.5 kcal/kg for height (m) × height(m) × 22 (ideal body weight based on the
195 recommendation of the Japan Diabetes Society). If the body mass index (BMI) is over 25
196 kg/m^2, energy intake was restricted to 25 kcal/kg for height (m) × height(m) × 22. The

197 ideal body weight was calculated using the following formula: [height (m)]^2 × 22 (kg/m^2)
198 [26]. We started insulin therapy by determining the total daily insulin dose using the
199 following formula: total daily insulin dose (U/day) = FPG (mg/dL) × 0.08. Then, we
200 divided the total daily insulin dose so that the ratio of the basal insulin dose before meals
201 to the bolus insulin dose was in the range of 1:1 to 1:2.
202 Subsequently, the daily insulin dose was appropriately modified until discharge. None of
203 the patients used lipid-lowering agents from admission until their discharge. In addition,
204 each factor (BMI, WC, BP, FPG, HbA1c, GA, S-CPR, U-CPR, IRI, S-Cr, eGFR, U-Alb,
205 TC, HDL-C, TG, LDL-C, non-HDL-C, Apo A-I, Apo A-II, Apo B, Apo C-II, Apo C-III,
206 Apo E, Apo B-48, LPL, lathosterol, campesterol, sitosterol, and cholestanol) was
207 measured at the time of admission and 2 weeks later at discharge, and the values of both
208 were compared. We evaluated the correlations between changes in cholesterol synthesis
209 marker (lathosterol) and absorption markers (campesterol, sitosterol, and cholestanol) and
210 changes in other clinical parameters during hospitalization. The cholesterol synthesis
211 marker (lathosterol) and absorption markers (campesterol, sitosterol, and cholestanol)
212 were based on the reference values for healthy Japanese individuals [27].
213

214 **Statistical Analysis**

215 Categorical variables are expressed as numbers, and continuous variables are described
216 as mean ± SD or median and IQR. The Wilcoxon signed-rank test was used to analyze
217 within-group differences between pre-treatment and post-treatment. Spearman's rank
218 correlation coefficient was used to analyze the correlation between the changes in each
219 clinical measurement during hospitalization. Statistical significance was set at $p < 0.05$.
220 All analyses were performed using JMP software (version 13.0; SAS Institute Inc., Cary,
221 North Carolina, USA).
222
223 **Result**
224 None of the patients were using insulin, four patients (22%) were using oral
225 hypoglycemic agents, and 14 patients (78%) did not use antidiabetic drugs before
226 hospitalization (Table 1).
227 The duration of hospitalization was 12–14 days (12 ± 1) (mean ± SD), and the clinical
228 characteristics of the patients on admission and after 2 weeks of treatment are shown in
229 Table 2. After 2 weeks of treatment, BMI, WC, FPG, HbA1c, GA, S-CPR, U-CPR were
230 significantly lower than those at baseline. TC, HDL-C, TG, LDL-C, non-HDL-C, Apo A-
231 I, Apo A-II, Apo B, Apo C-II, Apo C-III, and Apo E levels were also significantly lower
232 than those at baseline. However, Apo B-48 and LPL levels did not change significantly

233 during hospitalization. Lathosterol (from 2.6 ± 1.3 to 2.0 ± 0.7 μg/mL, p = 0.001),
234 campesterol (from 4.3 ± 2.7 to 3.0 ± 2.1 μg/mL, p < 0.0001), sitosterol (from 2.4 ± 1.6 to
235 1.7 ± 1.4 μg/mL, p < 0.0001) were significantly decreased and cholestanol (from 2.5 ±
236 1.0 to 2.3 ± 0.8 μg/mL, p = 0.05) tended to decrease after 2 weeks of treatment.
237 Table 3 shows the correlations between changes in cholesterol synthesis and absorption
238 markers and changes in other clinical parameters between admission and discharge. Delta
239 (Δ) was defined as the change in values between admission and discharge. There were
240 positive correlations between ΔBMI and Δcampesterol (ρ = 0.51, p = 0.03), ΔBMI and
241 Δsitosterol (ρ = 0.59, p = 0.01), and ΔBMI and Δcholestanol (ρ = 0.60, p = 0.009). A
242 negative correlation was found between ΔHbA1c and Δsitosterol (ρ = -0.50, p = 0.04),
243 but no correlation was found between other cholesterol synthesis and absorption markers
244 and glycemic parameters such as HbA1c or GA.
245

246 **Discussion**

247 Basal levels of lathosterol, sitosterol, and campesterol were higher in this study than in
248 healthy Japanese subjects' reference values of non-cholesterol sterols [27]. These results
249 indicate that both cholesterol synthesis and absorption markers are increased in our study.
250 The previous study showed the increase in cholesterol synthesis and the decrease of

absorption markers in type 2 diabetes. The discrepancy between our study and the previous study might occur due to the variation in severity of diabetes control. The mean level of HbA1c was 11% in our study but 6-7% in the previous study [28, 29]. The poor controlled state might affect the baseline of cholesterol synthesis and absorption markers. Insulin deficiency was not the primary pathophysiology in this study, as no patients were insulin deficient (Table 2). In addition, the pre-treatment S-CPR and IRI levels in this study did not suggest a state of insulin resistance, but the 24h U-CPR was indicative of insulin resistance. These results suggest that the patients in this study were in a state of insulin resistance and relative insulin deficiency associated with hyperglycemia at the time of admission. The insulin therapy in this study might contribute to insulin replacement and glycotoxicity improvement.

The effect of glucose-lowering therapy on cholesterol synthesis and absorption markers in type 2 diabetes has not been established. The superior characteristics of this study were that none of the patients used lipid-lowering agents and that insulin therapy was the only treatment to improve glycemic control. Statin administration in diabetic patients with hypercholesterolemia is the recommended therapy, and many patients take statins in clinical practice. This study is designed to examine lipid metabolism in statin-naive subjects, which can help investigate lipid metabolism by excluding the effect of statin.

269 It is difficult to determine the factors that caused decreasing in lathosterol, campesterol,
270 and sitosterol levels. Changes in the quantity and quality of the diet before and after
271 hospitalization may have contributed to changes in cholesterol synthesis and absorption
272 markers. A dietitian provides nutritional guidance during hospitalization, and the diet of
273 the patient prior to admission was interviewed. Although the date of the accurate amount
274 of food intake nor pictures of food are recorded, the dietitian asked and recorded the habits
275 of diets, the trend of the food intake, alchol comsumption, snack intake, and soft drink.
276 the trend of food intake, The four diabetologists have reviewed the nutritional guidance
277 records and evaluated each patient's pre-admission diet; the excessive in carbohydrates,
278 snack foods, and alcohol/soft drink intake, and total caloric intake through luxury foods
279 was high compared to the inpatient diet. On the other hand, the total caloric intake during
280 admission was higher in some patients but comparable in others than during pre-
281 admission. These results suggest that if diet affected sterol metabolism during
282 hospitalization, it is possible to relate food quality more than calories to sterol
283 metabolisms. To determine whether dietary influences are related to sterol metabolism, it
284 is necessary to check the change of calories, carbohydrates, protein, and fat and set control
285 as diet therapy only.
286 The decrease in BMI during 2 week hospitarization might influence the decrease in

287 cholesterol absorption markers, because the changes in BMI were positively correlated
288 with changes in cholesterol absorption markers (sitosterol, campesterol, and cholestanol)
289 but not with the cholesterol synthesis marker (lathosterol). The WC were also decreased,
290 but no correlation was observed between changes in WC and cholesterol synthesis and
291 absorption markers (Table 3). The previous study reported that weight reduction for two
292 years significantly increased the ratios of serum campesterol to cholesterol and serum
293 sitosterol to cholesterol; however, it did not affect the ratio of serum cholestanol to
294 cholesterol and serum lathosterol to cholesterol [30]. In addition, the three-month weight
295 reduction significantly decreased the serum levels of campesterol and lathosterol, but not
296 sitosterol and cholestanol [31]. Based on these previous results, the change in body weight
297 affects the levels of cholesterol synthesis and absorption markers. However, the changing
298 pattern of these markers is not consistent. The association between weight change,
299 cholesterol synthesis, and absorption markers in this study differed from the previous
300 studies, which could be due to the short duration of the study or treatment with insulin
301 therapy.
302 Different treatments for glycemic control may influence the outcome. First, the duration
303 of diabetes treatment may affect changes in cholesterol synthesis and absorption markers.
304 Twelve weeks of treatment with anagliptin did not change cholesterol synthesis and

absorption markers [32], but one month of treatment with anagliptin reduced latosterol levels in patients with type 2 diabetes [33]. It is not clear how the duration of diabetes treatment affects cholesterol metabolism makers and further investigation is needed. Second, the class of drugs may influence changes in cholesterol metabolic markers. Treatment with miglitol reduced levels of campesterol and sitosterol in patients with type 2 diabetes [32]. However, treatment with sitagliptin did not alter cholesterol synthesis and absorption markers [34]. These differences in drug effects may be related to changes in markers of cholesterol metabolism. Third, the state of glycemic control may affect cholesterol metabolic markers. In the present study, patients were treated with insulin for 2 weeks and glycemic parameters improved significantly. However, there was no correlation between changes in blood glucose parameters and markers of cholesterol synthesis and absorption during hospitalization, except for a negative correlation between HbA1c and changes in sitosterol levels. Therefore, the decrease in cholesterol synthesis and absorption markers with insulin treatment might have been caused by additional factors other than improvement in blood glucose levels. These results suggest that the cholesterol synthesis and absorption markers may differ depending on the type and duration of treatment, and that insulin dose and insulin function may affect cholesterol synthesis and absorption markers.

Sterol regulatory element-binding proteins (SREBPs) and liver X receptor (LXR) regultate cellular lipogenesis and lipid homeostasis [32, 33]. SREBP-1c activates fatty acid synthesis and SREBP-2 activate cholesterol synthesis and uptake [34] and control the production of key enzyme in cholesterol biosynthesis [35]. LXR regulate the expression of genes involved in cholesterol efflux, storage, catabolism, and elimination [36]. LXR is a nuclear receptor whose ligands include oxysterols produced when cholesterol is converted to steroid hormones and bile acids.

Insulin increases the mRNAs encoding lipogenic enzyme, such as glucokinase, acetyl CoA carboxylase, and fatty acid synthetase. Insulin treatment increase the amount of SREBP-1c mRNA, which plays a major role in the upregulation of fatty acid synthesis in response to insulin [37]. Insulin enhance LXR transcriptional regulation [38]. Activation of LXR upregulates ABCG5/G8 expression and increases the excretion of cholesterol and plant sterols into the lumen of the small intestine [32]. Thus, insulin plays an essential role in cholesterol synthesis and absorption at a crucial point. In addition, SREBPs can regulate insulin signaling. Insulin receptor substrate 2 (IRS-2) is a major mediator of insulin signaling and control insulin sensitivity. SREBPs can decrease IRS-2 expression [39], and high SREBP-1c activity resulting from hyperinsulinemia negatively correlated with IRS-2 expression in ob/ob mouse [34].

SREBP-2 activates the mevalonate pathway and promotes the metabolism of hydroxymethylglutaryl-CoA (HMG-CoA) to mevalonate. The increased circulating levels of the immediate product of HMG-CoA reductase activity, which is an index of whole-body cholesterol synthesis, is normalized after three weeks of insulin therapy in nonobese, normolipidemic, type 2 diabetic patients [40].

Our results showed that the levels of lathosterol, campesterol, and sitosterol at admission were higher than the reference values in healthy subjects [27]. However, it is speculated that the insulin treatment improved cholesterol metabolism and decreased both cholesterol synthesis and absorption markers in poorly controlled type 2 diabetes. This study is the first to report changes in cholesterol synthesis and absorption markers before and after insulin treatment for as short a period as two weeks. It was also suggested that insulin therapy might be an essential means of treatment.

Patients with diabetes are at high risk factor for atherosclerosis and are often introduced to statins to prevent ischemic heart disease. The target LDL-C control level is 120 mg/dL for diabetic patients in Japan. In the present study, the mean LDL-C level at admission was 124 mg/dL, and the condition did not immediately require lipid-lowering agents. In the present study, the cholesterol synthesis and absorption markers were high, suggesting that the synthesis and absorption markers might reflect residual risk. The results of this

study suggest that cholesterol synthesis and absorption markers can be positioned as residual risk markers of atherosclerosis beyond LDL-C levels alone. The cholesterol synthesis and absorption markers were decreased by insulin treatment, and the risk of atherosclerosis could be reduced.

Insulin regulate LPL mass and LPL activity and reduce TG levels. The levels of LPL and Apo B-48 remained unchanged in this study. This may be due to the short evaluation treatment period of 2 weeks. Also, the fact that Apo B-48 remained unchanged suggests that the number of chylomicron particles did not change, but the quality of cholesterol contained in the chylomicrons may have changed.

The present study has several limitations. Firstly, the sample size was small, which might have affected the results. In addition, cholesterol synthesis and absorption markers have different reference values for men and women [27]. Due to the small sample size, we could not consider a separate evaluation for men and women. It is necessary to verify in large-scale clinical trials. Secondly, it is possible that the single-center study caused a selection bias. It should be conducted to verify whether multicenter studies can obtain similar results. Thirdly, because the observation period was as short as 2 weeks, it was unclear how it changed in the long run. Finally, since the patients were hospitalized, they received not only insulin therapy but also dietary therapy. Differences in sterol intake

before and after hospitalization may contribute to changes in cholesterol synthesis and absorption markers. In this study, we did not investigate plant sterol intake and the change in the total amount of calories and lipid intake. Despite the nutritional guidance provided by a dietitian, we did not estimate plant sterol intake and the change in calories and lipid intake before and after hospitalization. Therefore, counting the change of calories, lipids, and plant sterol intake is advisable to investigate sterol metabolism.

Conclusion

This study demonstrated that insulin therapy reduces cholesterol synthesis and absorption markers in patients with type 2 diabetes. However, the change in plant sterol intake and energy intake could not be excluded. Further investigation is required to determine the effect of insulin treatment on cholesterol synthesis and absorption markers.

Acknowledgments

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Compliance with Ethical Standards

All procedures followed were in accordance with the ethical standards of the responsible

committee on human experimentation (Nippon Medical School Institutional Review Board / February 26, 2015 / 226038) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

Disclosure of potential conflicts of interest

All authors declare that they have no conflict of interest.

Author contributions

Yuji Yamaguchi: formal analysis; investigation; methodology; writing - original draft.

Kyoko Tanimura-Inagaki: conceptualization; investigation; methodology; project administration; writing - review and editing.

Izumi Fukuda: investigation; methodology; writing - review and editing.

Hitoshi Sugihara: investigation; methodology; writing - review and editing.

Shinichi Oikawa: conceptualization; investigation; methodology; project administration; writing - review and editing.

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530

Table 1 Baseline characteristics

Variable	*n* = 18
Age (years old)	56 ± 10
Sex (male / female)	13 / 5
Diabetes duration (years)	3.5 [0.0, 7.8]
Family history of diabetes, *n* (%)	10 (56)
Smoking history, *n* (%)	12 (67)
Diabetic microangiopathy, *n* (%)	11 (61)
Diabetic retinopathy, *n* (%)	6 (33)
Diabetic nephropathy, *n* (%)	5 (28)
Diabetic neuropathy, *n* (%)	8 (44)
Diabetic macroangiopathy, *n* (%)	2 (11)
Prehospital medication (antidiabetic agents), *n* (%)	4 (22)
Insulin, *n* (%)	0 (0)
Biguanide, *n* (%)	2 (11)
Thiazolidinediones, *n* (%)	0 (0)
Alpha-glucosidase inhibitor, *n* (%)	0 (0)
Sodium glucose co-transporter 2 inhibitor, *n* (%)	2 (11)
Dipeptidyl peptidase-4 inhibitor, *n* (%)	1 (6)
Glucagon-like peptide-1 receptor agonist, *n* (%)	0 (0)
Sulfonylurea, *n* (%)	0 (0)
Glinide, *n* (%)	0 (0)

Data are expressed as mean ± standard deviation, median [interquartile range] or number.

534 **Table 2** Comparison of parameters between pre-treatment and after-treatment

Variable	pre-treatment	after-treatment	*p*-value
BMI (kg/m^2)	24.3 ± 5.0	24.1 ± 4.9	0.02*
WC (cm)	86.8 ± 10.9	85.4 ± 11.0	0.02*
SBP (mmHg)	115 ± 11	112 ± 11	0.23
DBP (mmHg)	65 ± 8	66 ± 8	0.63
FPG (mg/dL)	225 ± 41	106 ± 19	<0.0001*
HbA1c (%)	11.6 ± 1.7	10.7 ± 1.5	<0.0001*
GA (%)	32.3 ± 5.6	26.9 ± 4.4	<0.0001*
S-CPR (ng/mL)	1.44 ± 0.54	0.83 ± 0.40	<0.0001*
U-CPR (μg/day)	118 ± 66	47 ± 26	<0.0001*
IRI (μU/mL)	4.8 ± 2.7	6.9 ± 3.9	0.03*
S-Cr (mg/dL)	0.63 ± 0.18	0.73 ± 0.20	<0.0001*
eGFR (ml/min)	100.2 ± 25.2	84.6 ± 18.7	<0.0001*
U-Alb (mg/day)	103.5 ± 216.6	45.8 ± 95.4	0.01*
TC (mg/dL)	205 ± 48	184 ± 43	0.004*
HDL-C (mg/dL)	51 ± 16	45 ± 9	0.007*
TG (mg/dL)	151 ± 86	106 ± 51	0.0004*
LDL-C (mg/dL)	124 ± 42	117 ± 39	0.04*
non-HDL-C (mg/dL)	154 ± 47	138 ± 45	0.006*
Apo A-I (mg/dL)	136.2 ± 28.9	117.3 ± 15.1	0.0009*
Apo A-II (mg/dL)	27.7 ± 6.4	23.7 ± 3.1	0.001*
Apo B (mg/dL)	107.1 ± 30.8	95.8 ± 30.2	0.003*
Apo C-II (mg/dL)	5.3 ± 2.2	4.2 ± 1.9	0.004*
Apo C-III (mg/dL)	11.4 ± 5.2	7.0 ± 2.2	<0.0001*
Apo E (mg/dL)	4.7 ± 1.6	3.4 ± 1.0	<0.0001*
Apo B-48 (μg/mL)	5.9 ± 4.7	5.3 ± 2.3	0.65
LPL (ng/mL)	43.1 ± 16.0	45.1 ± 11.6	0.15
lathosterol (μg/mL)	2.6 ± 1.3	2.0 ± 0.7	0.001*
campesterol (μg/mL)	4.3 ± 2.7	3.0 ± 2.1	<0.0001*
sitosterol (μg/mL)	2.4 ± 1.6	1.7 ± 1.4	<0.0001*
cholestanol (μg/mL)	2.5 ± 1.0	2.3 ± 0.8	0.05

535 BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP:

536 diastolic blood pressure, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, GA:

537 glycated albumin, S-CPR: serum C-peptide immunoreactivity, U-CPR: urinary C-peptide
538 immunoreactivity, IRI: immunoreactive insulin, S-Cr: serum creatinine, eGFR: estimated
539 glomerular filtration rate, TC: total cholesterol, HDL-C: high-density lipoprotein
540 cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, non-HDL-C:
541 non high-density lipoprotein cholesterol, Apo: apolipoprotein, LPL: lipoprotein lipase.
542 Data are expressed as mean ± standard deviation. *p*-value for Wilcoxon signed-rank test
543 comparing changes between groups. *$p<0.05$
544

Table 3 Spearman's rank correlation coefficients between changes in cholesterol synthesis and absorption markers and changes in other clinical measurements during hospitalization

Variable	Δ lathosterol		Δ campesterol		Δ sitosterol		Δ cholestanol	
	ρ	*p*-value	*ρ*	*p*-value	*ρ*	*p*-value	*ρ*	*p*-value
Δ BMI	0.29	0.25	0.51	0.03	0.59	0.01	0.60	0.009
ΔWC	0.22	0.39	-0.31	0.21	-0.26	0.30	0.13	0.61
Δ HbA1c	-0.24	0.34	-0.39	0.11	-0.50	0.04	-0.14	0.58
Δ GA	-0.40	0.10	-0.28	0.26	-0.40	0.10	-0.32	0.20
Δ TC	0.28	0.26	0.67	0.002	0.61	0.007	0.37	0.13
Δ HDL-C	0.40	0.10	0.54	0.02	0.60	0.008	0.28	0.25
Δ TG	0.28	0.26	0.35	0.15	0.31	0.22	0.25	0.31
Δ LDL-C	0.07	0.77	0.41	0.09	0.31	0.21	0.13	0.60
Δ non-HDL-C	0.26	0.30	0.56	0.02	0.44	0.07	0.37	0.13

BMI: body mass index, WC: waist circumference, HbA1c: hemoglobin A1c, GA: glycated albumin, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, non-HDL-C: non high-density lipoprotein cholesterol. Δ is defined as the change in values between admission and discharge.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: