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Intensive insulin therapy reduces small dense low-density lipoprotein particles in patients with type 2 diabetes mellitus: relationship to triglyceride-rich lipoprotein subspecies

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Abstract

It remains unclear whether insulin improves dyslipidemia in patients with type 2 diabetes mellitus. Small dense low-density lipoprotein (sd-LDL) particles are recognized as a powerful risk factor for coronary heart disease and are often elevated in type 2 diabetes mellitus. We examined the effect of intensive insulin therapy on sd-LDL particles and triglyceride (TG)–rich lipoprotein subspecies. Intensive insulin therapy (insulin aspart [NovoRapid, Tokyo, Japan] before each meal and isophane insulin suspension at bedtime) was given to poorly controlled type 2 diabetic patients (n = 46) who were on high doses of sulfonylureas. Fasting serum samples were collected before and 14 days after the commencement of insulin therapy. Low-density lipoprotein size was measured by gradient gel electrophoresis, and the small dense LDL cholesterol (sd-LDL-C) concentration was measured by a new precipitation method. Chylomicrons (Svedberg flotation unit >400), very low-density lipoprotein 1 (VLDL1) (Sf, 60-400), and VLDL2 (Sf, 20-60) were separated by ultracentrifugation. Serum apolipoprotein B-48 and lipoprotein lipase levels were measured by the enzyme immunoassay method. Serum glucose and glycoalbumin levels were substantially decreased by insulin treatment. The LDL size increased (25.8-26.0 nm, $P < .05$) and the sd-LDL-C level was significantly reduced (44-34 mg/dL, $P < .005$). Apolipoproteins B-48 and C-III were decreased, whereas lipoprotein lipase was increased. Triglyceride levels in chylomicrons, VLDL1, and VLDL2 all showed a decrease. Changes of sd-LDL-C or LDL size were associated with changes of the TG levels in the major TG-rich lipoprotein subspecies. These results suggest that intensive insulin therapy decreases atherogenic sd-LDL particles by reducing TG in TG-rich lipoproteins. We did not find any specific relationship between VLDL1 and sd-LDL during insulin treatment.

1. Introduction

Type 2 diabetes mellitus is a common disorder that is accompanied by numerous metabolic abnormalities leading to a high risk of coronary heart disease (CHD). The United Kingdom Prospective Diabetes Study confirmed that intensive glycemic control delays the onset and retards the progression of microvascular disease, and possibly CHD, in patients with type 2 diabetes mellitus [1]. Insulin resistance plays a major role in the development of hyperglycemia and dyslipidemia in type 2 diabetes mellitus. However, when pancreatic beta-cell function declines, insulin therapy is required to achieve optimal glycemic control in type 2 as well as type 1 diabetes mellitus. Although many patients with type 2 diabetes mellitus are on insulin therapy, few studies have investigated the influence of insulin therapy on dyslipidemia in type 2 diabetes mellitus. It is important to investigate how insulin therapy affects lipid metabolism in patients with type 2 diabetes mellitus because there is a possibility that it could cause hyperinsulinemia, which has the potential to exacerbate lipid abnormalities.

An increase in triglyceride (TG), decrease in high-density lipoprotein cholesterol (HDL-C), and increase in small dense low-density lipoprotein (sd-LDL) particles represent a common dyslipidemic pattern in individuals who have insulin resistance, such as patients with type 2 diabetes mellitus, obesity, and metabolic syndrome [2]. In particular, sd-LDL has attracted attention as a very strong risk factor for CHD beyond LDL cholesterol (LDL-C) [2,3]. The recent Quebec cardiovascular study clearly demonstrated that an increase of sd-LDL-C predicts a higher risk of CHD events,

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Table 1
General profile of type 2 diabetic subjects before commencement of intensive insulin therapy

n	46 (F/M, 29/17)
Age (y)	63 ± 2
BMI	23.4 ± 0.5
HbA_{1c} (%)	10.4 ± 0.3
FPG (mg/dL)	221 ± 9.3
2-h Glucose (mg/dL)	328 ± 16
Fasting C-peptide (ng/mL)	2.3 ± 0.2
2-h C-peptide (ng/mL)	4.9 ± 0.3
Fasting insulin (μU/mL)	6.1 ± 0.6

Data are expressed as mean ± SE. Glucose and C-peptide were measured 2 hours after breakfast. BMI indicates body mass index; HbA_{1c}, hemoglobin A_{1c}; FPG, fasting plasma glucose.

whereas a change of large buoyant LDL-C does not [4]. Griffin and Packard [5,6] demonstrated that large TG-rich very low-density lipoprotein 1 (VLDL1) is a precursor of sd-LDL particles, and VLDL1 is preferentially produced in the liver after insulin resistance developed. This explains the intimate association between sd-LDL particles and insulin resistance. Very recently, the same group reported that hyperglycemia stimulates VLDL1 production in type 2 diabetes mellitus [7]. Accordingly, we speculated that control of hyperglycemia with insulin could reduce sd-LDL particles by specifically suppressing VLDL1 secretion.

In the present study, we investigated how insulin therapy changes LDL size and the sd-LDL concentration in type 2 diabetes mellitus and explored the relationship between sd-LDL and TG-rich lipoprotein (TGRL) subspecies. We measured VLDL1 (large VLDL), VLDL2 (small VLDL), and chylomicrons (CMs) after separation by ultracentrifugation, as well as serum apolipoprotein (apo) B-48 (a marker of CMs and its remnants). Because CMs are not a precursor of LDL, it is interesting to investigate the relationship between sd-LDL particles and CMs to elucidate the specific association between VLDL1 and sd-LDL particles.

2. Methods

Forty-six patients with type 2 diabetes mellitus were enrolled in this study. All of the patients were admitted to the Showa University Hospital to receive insulin therapy. They had been treated with high doses of sulfonylureas (>7.5 mg glibenclamide or 4 mg glimepiride per day), but hemoglobin A_{1c} had remained more than 7.5% for at least 3 months before admission. Fourteen patients were being treated with α-glucosidase inhibitors in addition to sulfonylureas. Dietary therapy was supervised by a dietitian, and exercise therapy was prescribed by a physician for at least 3 months before and during the study according to the recommendations of the Japanese Diabetes Association. Table 1 shows the profile of the subjects on the first day of admission. Type 1 diabetes mellitus was excluded by no detention of glutamic acid decarboxylase (GAD) antibody and a significant increase of C-peptide at 2 hours after breakfast. Intensive insulin therapy (insulin aspart [NovoRapid, Tokyo, Japan] before each meal and isophane insulin suspension at bedtime) was given for 3 to 5 days after hospitalization. The total dose of insulin was 12 to 56 U/d (mean, 26 ± 11 U/d). All oral hypoglycemic agents were withdrawn 1 day before starting insulin administration. Ten patients were being treated with statins for hypercholesterolemia, and they were allowed to continue this medication during the study. Fasting serum samples were collected before and 14 days after the commencement of insulin therapy. Written informed consent was obtained from all subjects, and this study was approved by the ethics committee of the Showa University.

Low-density lipoprotein size was measured by gradient gel electrophoresis [8], and the sd-LDL-C concentration was measured by a newly developed precipitation method, as

Table 2
Changes in serum concentrations before and after intensive insulin therapy

	Pretreatment	Posttreatment	Difference	% Change
FPG (mg/dL)	207 ± 12	126 ± 6.8	−86.8 ± 14****	−32.6 ± 7.6****
Glycoalbumin (%)	32.0 ± 1.7	26.1 ± 1.1	−5.7 ± 0.9****	−15.7 ± 1.9****
Triglyceride (mg/dL)	158 ± 12	123 ± 7.0	−38.7 ± 11***	−14.5 ± 4.6***
LDL-C (mg/dL)	140 ± 6.5	130 ± 6.1	−11.3 ± 4.5*	−5.8 ± 3.5
HDL-C (mg/dL)	51 ± 2.2	47 ± 1.9	−4.6 ± 1.3***	−7.0 ± 2.5**
ApoA-I (mg/dL)	130 ± 4.7	119 ± 4.0	−11.8 ± 3.3***	−7.7 ± 2.2***
ApoB (mg/dL)	114 ± 5.2	104 ± 4.5	−10.2 ± 3.3***	−6.7 ± 3.1*
ApoC-III (mg/dL)	8.7 ± 0.6	5.7 ± 0.5	−3.2 ± 0.6***	−30.0 ± 0.5***
ApoE (mg/dL)	4.8 ± 0.7	4.2 ± 0.6	−0.7 ± 0.2****	−16.1 ± 2.8****
ApoB-48 (μg/ mL)	6.1 ± 0.8	4.6 ± 0.5	−1.7 ± 0.5*	−13.8 ± 5.0
LPL (ng/mL)	31.8 ± 2.2	37.2 ± 1.7	5.4 ± 1.2**	17.1 ± 2.1*
sd-LDL-C (mg/dL)	43.4 ± 3.9	34.5 ± 3.1	−10.4 ± 3.0***	−14.7 ± 5.4***
Mean LDL diameter (nm)	258.2 ± 0.8	260.1 ± 0.8	1.5 ± 0.8*	0.6 ± 0.3*

Data are expressed as mean ± SE. Difference represents posttreatment minus pretreatment.
* $P < .05$.
** $P < .01$.
*** $P < .005$.
**** $P < .0001$.