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Increased cholesterol efflux from cultured fibroblasts to plasma from hypertriglyceridemic type 2 diabetic patients: Roles of pre β-HDL, phospholipid transfer protein and cholesterol esterification

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Abstract

We tested whether hypertriglyceridemia associated with type 2 diabetes mellitus is accompanied by alterations in pre β-HDL, which are considered to be initial acceptors of cell-derived cholesterol, and by changes in the ability of plasma to promote cellular cholesterol efflux. In 28 hypertriglyceridemic and 56 normotriglyceridemic type 2 diabetic patients, and in 56 control subjects, we determined plasma lipids, HDL cholesterol and phospholipids, plasma pre β-HDL and pre β-HDL formation, phospholipid transfer protein (PLTP) activity, plasma cholesterol esterification (EST) and cholesteryl ester transfer (CET) and the ability of plasma to stimulate cholesterol efflux out of cultured human fibroblasts. HDL cholesterol and HDL phospholipids were lower, whereas plasma PLTP activity, EST and CET were higher in hypertriglyceridemic diabetic patients than in the other groups. Pre β-HDL levels and pre β-HDL formation were unaltered, although the relative amount of pre β-HDL (expressed as % of total plasma apo A-I) was increased in hypertriglyeridemic diabetic patients. Cellular cholesterol efflux to plasma from hypertriglyceridemic diabetic patients was increased compared to efflux to normotriglyceridemic diabetic and control plasma, but efflux to normotriglyceridemic diabetic and control plasma did not differ. Multiple linear regression analysis demonstrated that cellular cholesterol efflux to plasma was positively and independently related to pre β-HDL formation, PLTP activity and EST (multiple $r = 0.48$), but not to the diabetic state. In conclusion, cholesterol efflux from fibroblasts to normotriglyceridemic diabetic plasma is unchanged. Efflux to hypertriglyceridemic diabetic plasma is enhanced, in association with increased plasma PLTP activity and cholesterol esterification. Unaltered pre β-HDL formation in diabetic hypertriglyceridemia, despite low apo A-I, could contribute to maintenance of cholesterol efflux.

Keywords: ATP-binding cassette transporter type 1; Cholesterol efflux; Human fibroblasts; Pre β-HDL; Reverse cholesterol transport; Triglycerides; Type 2 diabetes mellitus

1. Introduction

Efflux of cholesterol from peripheral cells to extracellular acceptors is considered to be an important early step in the anti-atherogenic reverse cholesterol transport (RCT) system, whereby excess cholesterol is transported from the arterial wall back to the liver for metabolism and excretion in the bile [1–3]. A number of pathways, including aqueous diffusion, transport of cholesterol via the ATP-binding cassette transporters (ABCA1 and ABCG1) and scavenger receptor class B type 1 (SR-BI)-mediated efflux are supposed to play

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a role in cellular cholesterol removal [4–8]. The importance of the ABCA1 system for protection against the development of atherosclerosis is underscored by the observation that cardiovascular risk is elevated in subjects with genetic defects in ABCA1 [9,10]. Small lipid poor and lipid free apolipoprotein (apo) A-I-containing particles, designated pre β-HDL, are recognized to be initial acceptors of cell-derived cholesterol via the ABCA1 system [11], while larger sized HDL may promote cellular cholesterol efflux via ABCG1 and SR-BI [12–14].

Several intravascular mechanisms are involved in HDL metabolism, and may affect the ability of plasma to stimulate cellular cholesterol efflux. Phospholipid transfer protein (PLTP) facilitates transfer of phospholipids towards HDL during lipolysis of triglyceride-rich lipoproteins, and is able to convert HDL into larger and smaller particles, thereby giving rise to pre β-HDL [15,16]. Esterification of free cholesterol by lecithin:cholesterol acyltransferase (LCAT) contributes to HDL maturation, increases HDL particle size and decreases pre β-HDL levels [1]. Subsequently, cholesteryl ester transfer protein (CETP) action enables transfer of cholesteryl ester from HDL to lipoproteins of lower density, making HDL particles to become cholesteryl ester depleted and triglyceride enriched [17–23]. Such particles are more suitable for hydrolysis by HL. Combined CETP and hepatic lipase (HL) activity may also contribute to the generation of pre β-HDL [19].

High plasma triglycerides and low HDL cholesterol are well known features of dyslipidemia in type 2 diabetes mellitus, and it is increasingly appreciated that high plasma PLTP activity, elevated plasma cholesterol esterification (EST) as well as cholesteryl ester transfer contribute to abnormalities in HDL metabolism in diabetes-associated hypertriglyceridemia as well [17,20–23]. Some studies that were carried out mainly in non-diabetic subjects have suggested that pre β-HDL, either expressed as its concentration or expressed as percentage of total plasma apo A-I, may be increased in hypertriglyceridemia [24–26], but little is known about pre β-HDL in type 2 diabetic patients with or without hypertriglyceridemia.

Using Fu5AH rat hepatoma cells, which have a high expression of SR-BI but lack functional ABCA1, it has been documented that the ability of type 2 diabetic plasma to stimulate cellular cholesterol efflux is decreased or unaltered [27,28]. An early study with cultured human fibroblasts, a cell system which abundantly expresses ABCA1 [29], has shown that the capacity of plasma from severely hypertriglyceridemic type 2 diabetic patients to promote cellular cholesterol efflux is diminished [30], but it is uncertain whether glycation of HDL could impair its cellular cholesterol efflux-stimulating ability [31,32]. On the other hand, the ability of plasma from non-diabetic hypertriglyceridemic subjects to promote ABCA1-mediated cholesterol efflux out of J774 macrophages [26] and of type 1 diabetic patients to stimulate cholesterol efflux out of both Fu5AH cells and human fibroblasts is enhanced [29]. Thus, evidence concerning abnormalities in the ability of diabetic plasma to stimulate cell-derived cholesterol removal is limited, and it is unclear at present whether this early step in the RCT process is affected by hypertriglyceridemia.

In the present study we questioned whether, in type 2 diabetes, hypertriglyceridemia is not only accompanied by decreased HDL but also by alterations in pre β-HDL, and in the ability of plasma to stimulate cellular cholesterol efflux. Therefore, we compared the ability of plasma from hypertriglyceridemic and normotriglyceridemic type 2 diabetic patients and control subjects to promote cholesterol efflux out of human cultured fibroblasts. Moreover, we assessed the relationships of cellular cholesterol efflux with plasma pre β-HDL, HDL cholesterol and phospholipids, PLTP activity, cholesterol esterification and cholesteryl ester transfer.

2. Research design, subjects and methods

The medical ethics committee of the University Medical Center Groningen approved the study protocol, and written informed consent was obtained from each participant. Three groups of participants were recruited: type 2 diabetic patients with plasma triglycerides >2.0 mmol/l; type 2 diabetic patients with plasma triglycerides ≤2.0 mmol/l and non-diabetic control subjects with plasma triglycerides ≤2.0 mmol/l. Type 2 diabetes mellitus was previously diagnosed using blood glucose cut-off values as defined by the WHO, and patients were treated with diet alone or in combination with oral glucose lowering agents. Insulin treatment was an exclusion criterion. All participants were aged >18 years. Current or previous smoking and the use of lipid lowering drugs were exclusion criterions. Participants did not have a history of cardiovascular disease or proliferative diabetic retinopathy. None of the participants had liver function abnormalities or thyroid dysfunction. Subjects with micro- or macro-albuminuria defined as urinary albumin >20 mg/l were also excluded. Maximal alcohol intake was 3 beverages per day. All participants were evaluated after an overnight fast. BMI was calculated as weight (kg) divided by height (m) squared. Blood pressure was measured after 15 min rest at the left arm in sitting position using a sphygmomanometer. Mean arterial pressure (MAP) was calculated as 1/3 × systolic blood pressure + 2/3 × diastolic blood pressure.

Fifty-six normotriglyceridemic control subjects, 56 normotriglyceridemic type 2 diabetic patients and 28 hypertriglyceridemic diabetic patients participated in the study. Gender distribution ($p = 0.79$) and age ($p = 0.16$) were not significantly different between the three groups. Sixteen normolipidemic control women, 22 normotriglyceridemic diabetic women and 9 hypertriglyceridemic diabetic women were postmenopausal ($p = 0.17$). Of the postmenopausal women, only two in the control group, one in the normotriglyceridemic diabetic group and none