Diabetes Contributes to Cholesterol Metabolism Regardless of Obesity

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OBJECTIVE — To investigate cholesterol metabolism in obesity with and without diabetes.

RESEARCH DESIGN AND METHODS — We performed cross-sectional metabolic studies in obese individuals with and without type 2 diabetes. The study population consisted of 16 obese (BMI >30 kg/m^2) diabetic subjects with a mean age of 52 ± 2 years (SE) and 16 nondiabetic control subjects of similar age and weight. Cholesterol absorption efficiency was measured with peroral dual isotopes and cholesterol synthesis with sterol balance.

RESULTS — Serum total cholesterol did not differ between the groups, but LDL and HDL cholesterol were significantly lower and VLDL cholesterol and serum total and VLDL triglycerides were higher in the diabetic group than in the control group. Cholesterol absorption efficiency was 29 ± 1% in diabetic subjects vs. 42 ± 2% in the control subjects ($P < 0.01$). Cholesterol synthesis was higher (17 ± 1 vs. 14 ± 1 mg · kg^{-1} · day^{-1}; $P < 0.05$) and neutral sterol and bile acid excretion and cholesterol turnover tended to be higher in the diabetic group than in the control group. Blood glucose was positively related to cholesterol synthesis in the diabetic group ($r = +0.663$, $P < 0.01$) and in the control group ($r = +0.590$, $P < 0.05$), suggesting that the higher blood glucose level, the higher the cholesterol synthesis. In addition, blood glucose was significantly positively related to fecal neutral sterol excretion in both groups.

CONCLUSIONS — Cholesterol absorption efficiency was lower and cholesterol synthesis was higher in obese subjects with diabetes than in those without diabetes, suggesting that diabetes modulates cholesterol metabolism more than obesity alone.

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In patients with type 2 diabetes, cholesterol metabolism differs from nondiabetic patients because cholesterol synthesis is high (1–4) and is reduced by insulin (5,6). Low cholesterol absorption efficiency has been reported earlier in a limited number of diabetic subjects with mild hyperlipidemia (4) and in moderately overweight, markedly hypertriglyceridemic subjects including both type 1 and type 2 diabetes (7). Also, serum plant sterol levels, indicators of cholesterol absorption efficiency (8,9) are low in type 2 diabetes (10) and even in subjects with high-to-normal blood glucose levels (11). Accordingly, cholesterol metabolism mimics that observed in obesity (12–15).

We have shown previously that cholesterol absorption efficiency was increased by weight reduction, and the variables of glucose metabolism improved in obese diabetic subjects, suggesting that low cholesterol absorption is associated with insulin resistance and metabolic syndrome (16), an association found earlier nondiabetic subjects (11). The question now is whether overweight, which is frequently associated with diabetes, is responsible for the alterations observed in cholesterol metabolism in diabetes, or does diabetes have any independent role in regulating cholesterol metabolism.

To this end, we studied cholesterol absorption efficiency and sterol balance in obese subjects with and without diabetes, the latter selected by BMI from a population-based cohort.

RESEARCH DESIGN AND METHODS

Study population

The study population consisted of 16 obese diabetic patients (BMI >30 kg/m^2), 13 men and 3 women, with a mean age of 52 ± 2 (SE) years, recruited from the outpatient clinics of Helsinki University Hospital (diabetic group; Table 1). Diabetes (fasting blood glucose >7.0 mmol/l) had been recently diagnosed (<2 years). None of the patients had insulin therapy, diabetic nephropathy, hepatic or thyroid disease, unstable angina pectoris, or myocardial infarction or invasive coronary treatment within a year. All women (including the control subjects) were postmenopausal without hormone replacement therapy. Diabetes was treated with diet in 10 patients, 3 had glibenclamide, and 3 had a combination therapy of glibenclamide and biguanide. There was no difference in variables of serum and lipoprotein lipids and variables of cholesterol metabolism between these treatment groups.

From a random population-based age-cohort of 50-year-old men (9) and women (17), 16 healthy normoglycemic subjects (10 men and 6 women), with BMI and age similar to the diabetic group, were recruited as control subjects. The exclusion criteria, except insulin therapy and diabetic nephropathy, were the same as in the diabetic group.Their health status was determined with medical examination and laboratory tests. All subjects volunteered to the study and gave informed consent. The study protocol had been accepted by the Ethics Committee of the 2nd Department of Medicine, University of Helsinki.

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Abbreviations: apo, apoprotein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Study population*

Variables	Diabetic group	Control group
n	16	16
M/F	13/3	10/6
Age (years)	52.2 ± 1.8	50.8 ± 0.5
Weight (kg)	93.2 ± 3.7	96.4 ± 3.3
Height (m)	1.70 ± 0.02	1.70 ± 0.01
BMI (kg/m^2)	32.2 ± 1.0	33.3 ± 0.9
Blood glucose (mmol/l)	8.4 ± 0.6*	4.6 ± 0.2
Cholesterol intake (mg/day)	351 ± 47	455 ± 49
Fat intake (g/day)	92 ± 9	101 ± 9
Plant sterol intake (mg/day)	356 ± 31	352 ± 34

Data are means ± SE, derived from Student's *t* test. *Significantly different from control subjects.

Study design

The diabetic group had been counseled to consume a low-fat, low-cholesterol diet with carbohydrates up to 55% energy, with preference of carbohydrates with low glycemic index and simple carbohydrates comprising less than one-third of the total amount, whereas the control subjects consumed their normal ad libitum home diet. The subjects visited the outpatient clinic twice, a week apart, when two blood samples were collected after a 12-h fast.The subjects kept a food record for 7 days, from which the dietary constituents were calculated (18). Also, in order to measure cholesterol absorption, they were given a capsule containing 4-^{14}C-cholesterol (4,500 ± 19 dpm), 22,23-^{3}H-β-sitostanol (11,588 ± 42 dpm), and Cr_2O_3 (200 mg) 3 times a day with their regular meals during the 7-day period. Stool was collected on the last 3 days of the week. The samples were pooled, and fecal elimination of cholesterol and bile acids and cholesterol absorption efficiency were measured from these fecal samples.

Methods

Serum total cholesterol and triglycerides were measured with Boehringer Diagnostica commercial kits. Serum lipoproteins were separated by ultracentrifugation into the following density classes: VLDL <1.006 g/ml, LDL 1.019–1.063 g/ml, and HDL 1.063–1.210 g/ml (19). Blood glucose was analyzed using the hexokinase method. Apoprotein (apo) E phenotypes were analyzed with isoelectric focusing from serum (20). Fecal cholesterol as fecal neutral sterols (cholesterol, coprostanol, and coprostanone), bile acids, and plant sterols were quantitated by gas-liquid chromatography from nonsaponifiable material on a 50-m capillary column (21–23), correcting fecal flow by the Cr_2O_3 measurement (24).

Calculations

Cholesterol absorption efficiency (fractional absorption of dietary cholesterol) was calculated by the altered ^{14}C-to-^{3}H ratio in stools as compared with the fed ratio (25).

Cholesterol synthesis was determined as difference between the fecal steroids (neutral and acidic) of cholesterol origin and dietary cholesterol. Total intestinal cholesterol pool was calculated as fecal neutral steroids/(1 − cholesterol absorption efficiency). The absorbed mass of total, dietary, and biliary cholesterol was calculated as respective fluxes multiplied by cholesterol absorption efficiency. Cholesterol turnover equaled cholesterol synthesis plus dietary cholesterol absorbed.

Statistics

Statistical analyses of data were performed using the Biomedical Data Processing Program and Microsoft Excel version 6. The hypothesis testing was performed using Student's two-sided *t* test and the χ^2 test and by calculating Pearson's correlation coefficients. Logarithmic transformations were used with skewed distributions. A *P* value <0.05 was considered statistically significant.

RESULTS — No sex difference was found for different variables between the diabetic and control groups (data not shown). In the diabetic group, treatment of diabetes had no effect on the different variables. The two groups were similar according to demographic variables (Table 1) and apoE phenotype distribution (data not shown), but blood glucose level was higher in the diabetic group than in the control group. The average serum insulin level was 19.7 ± 1.2 mU/l in the diabetic group and 8.0 ± 0.8 mU/l in the control group (measured randomly in six subjects; *P* <0.001 from the diabetic group). The reference values of our hospital laboratory were 2–20 mU/l. The dietary variables, fat intake, dietary plant sterols, and cholesterol intake did not differ between the study groups.

Serum total cholesterol was similar between the groups, whereas LDL and HDL cholesterol levels were lower and VLDL cholesterol was higher in the diabetic group than in the control group (Table 2). Serum total and VLDL triglycerides were higher and LDL triglycerides were lower in the diabetic group than in the control group.

Percent cholesterol absorption and the absorbed mass of dietary, total, and biliary cholesterol were ~30% (*P* < 0.01) lower in the diabetic group than in the control group (Table 3). Cholesterol synthesis was significantly higher in the diabetic group than in the control group. Cholesterol excretion as neutral and total

Table 2—*Serum and lipoprotein lipids*

Variables	Diabetic group	Control group
n	16	16
Serum cholesterol	5.9 ± 0.2	6.2 ± 0.3
VLDL cholesterol	1.4 ± 0.2*	0.6 ± 0.2
LDL cholesterol	3.2 ± 0.2*	4.0 ± 0.3
HDL cholesterol	0.85 ± 0.05*	1.25 ± 0.07
Serum triglycerides	3.8 ± 0.6*	1.9 ± 0.4
VLDL triglycerides	3.1 ± 0.5*	1.1 ± 0.3
LDL triglycerides	0.31 ± 0.02*	0.45 ± 0.05
HDL triglycerides	0.18 ± 0.01	0.19 ± 0.01

Data are means ± SE, expressed as mmol/l, and derived from Student's *t* test. *Significantly different from control subjects.

Table 3—*Cholesterol metabolism*

Variables	Diabetic group (n = 16)	Control group (n = 16)
Cholesterol absorption		
Efficiency (%)	29.5 ± 1.3*	41.7 ± 2.3
Dietary cholesterol absorbed mg · kg^{-1} · day^{-1}	1.09 ± 0.14*	1.95 ± 0.23
Biliary cholesterol absorbed mg · kg^{-1} · day^{-1}	4.80 ± 0.42*	6.66 ± 0.67
Total cholesterol absorbed mg · kg^{-1} · day^{-1}	5.89 ± 0.48*	8.61 ± 0.65
Fecal steroids mg · kg^{-1} · day^{-1}		
Bile acids	7.00 ± 0.64	6.53 ± 0.90
Neutral sterols	13.96 ± 0.87	11.88 ± 0.63
Total steroids	20.96 ± 1.12	18.40 ± 1.30
Intestinal cholesterol pool	19.85 ± 1.23	20.49 ± 0.92
Cholesterol synthesis	17.25 ± 0.93*	13.73 ± 1.50
Cholesterol turnover	18.33 ± 0.96	15.68 ± 1.38

Data are means ± SE, derived from Student's t test. *Significantly different from control subjects.

steroids, bile acid synthesis, and cholesterol turnover tended to be higher in the diabetic group than in the control group.

In the diabetic group, percent cholesterol absorption was unrelated to serum or LDL cholesterol concentrations, but tended to relate inversely to serum and VLDL triglyceride levels ($r = -0.486$ for serum and $r = -0.492$ for VLDL, $P = 0.06$ for both). Cholesterol absorption efficiency and the total mass of cholesterol absorbed were significantly related to serum total and HDL cholesterol only in the control group (HDL: $r = +0.7098$ and $r = +0.7187$, $P < 0.01$ for both). Cholesterol absorption efficiency was negatively associated with fecal neutral sterols only in the control group ($r = -0.641$, $P < 0.01$), less consistently with cholesterol synthesis (diabetic group: $r = -0.242$ control group: $r = -0.331$, NS for both). Blood glucose was associated with fecal bile acids and fecal neutral sterols ($r = +0.603$ and $r = +0.501$, $P < 0.05$ for both) and cholesterol synthesis (Fig. 1); in the control group, it was associated with fecal neutral steroids ($r = +0.551$, $P < 0.05$), but was unrelated to cholesterol absorption efficiency (diabetic group: $r = -0.210$; control group: $r = -0.140$, NS for both).

CONCLUSIONS — The two groups were of similar age, sex, weight, BMI, apoE phenotype distribution, and dietary intakes, and the study groups were well comparable, with the exception of blood glucose levels. Serum and LDL cholesterol levels in both groups exceeded the recent recommendations (26), but none of the subjects received lipid-lowering medication. In addition, all women were postmenopausal without hormone replacement therapy. Despite similar serum cholesterol levels, HDL cholesterol was lower and triglyceride contents in serum and VLDL were higher in the diabetic group than in the control group, revealing the typical lipoprotein lipid profile of diabetes.

From among the possible confounding variables affecting the low cholesterol absorption efficiency in the diabetic group, apoE phenotype distribution, dietary cholesterol, and plant sterol intakes were similar, and none of the subjects in the diabetic group had any symptoms of gastroparesis. In addition, antidiabetic drugs had no consistent effect on the cholesterol absorption, and diabetes had been recently diagnosed.

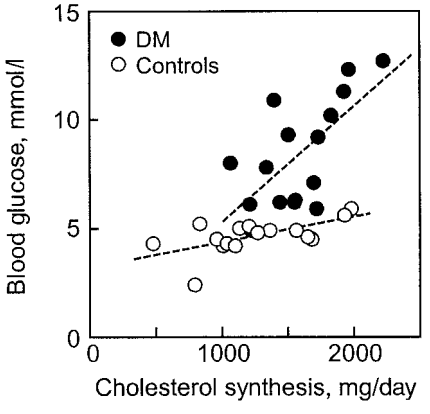

Figure 1—*Correlation between blood glucose and cholesterol synthesis. Diabetic group:* y = *0.005*x − *0.042*, r = *0.663*, P < *0.01; control group:* y = *0.001*x + *3.296*, r = *0.590*, P < *0.05*.

According to our results, diabetes seems to either upregulate cholesterol synthesis or downregulate cholesterol absorption efficiency as compared with the respective nondiabetic state, when obesity is not a confounding factor. Our results convincingly show for the first time the additional effects of diabetes on cholesterol absorption and synthesis in obese subjects. The question then arises, what is initially responsible for the altered cholesterol metabolism in diabetes? Because it has been found that efficient weight loss in diabetic individuals improves cholesterol absorption efficiency and markers of insulin resistance (16), cholesterol absorption efficiency might be the variable being affected primarily. However, in the present article, there only was a trend of a negative correlation between cholesterol absorption and blood glucose level, but a significant association between cholesterol synthesis and blood glucose level in both groups. Figure 1 actually shows that the increase of blood glucose within the normal limits markedly increases cholesterol synthesis, its increment being less in relation to grossly enhanced diabetic glucose values. According to cholesterol homeostasis, when cholesterol synthesis is increased, cholesterol absorption is decreased. However, increased cholesterol synthesis lowers fractional mass of absorbed cholesterol (albeit, inconsistently) because the intestinal cholesterol pool is expanded by effective biliary cholesterol secretion.

According to previous studies, the role of diabetes on cholesterol synthesis is unclear (1,2,4,7). The effects on cholesterol metabolism of normalizing blood glucose levels with insulin have varied, ranging from decreased (5,6) to unchanged (1) cholesterol synthesis. The univariate association between blood glucose and cholesterol synthesis in the present study could suggest that lowering of blood glucose levels would diminish cholesterol synthesis and increase cholesterol absorption; all of these changes could actually be seen after effective weight reduction in diabetic individuals (16).

Cholesterol synthesis will be upregulated if the hepatic cholesterol pool is diminished. This results from diminished hepatic cholesterol influx from the tissues or from intestine. When cholesterol influx from the tissues was considered, HDL cholesterol level was observed to be lower

in diabetic individuals, suggesting that reverse cholesterol transport might be interfered. However, even if it were, its significance in upregulating cholesterol synthesis remains open. The earlier studies in the nondiabetic population (15,27) have shown an association between serum total, LDL, and HDL cholesterol level and cholesterol absorption, suggesting that the higher the cholesterol absorption, the higher serum cholesterol level, though not all studies support that finding (28). In the present study, however, this association was significant only in the control group.

In the present study, diabetes was clearly associated with high cholesterol synthesis and with mildly elevated serum and lipoprotein triglyceride levels. Insulin-resistant fat cells release large amounts of free fatty acids to the circulation, which are taken up by the liver. Lipoprotein synthesis is increased, and large amounts of VLDL are secreted from the liver to the circulation. It is not known, however, whether the increased lipogenesis can activate sterol regulatory element-binding protein 2, which is necessary to upregulate cholesterol biosynthesis (29). In the present study, there was no significant correlation between serum or lipoprotein triglyceride levels and cholesterol synthesis. In transgenic mice, biliary cholesterol concentration was inversely correlated with percent cholesterol absorption (30), suggesting that large amounts of biliary cholesterol are able to saturate the micellar cholesterol pool and thereby resulting in poor absorption of dietary sterols. Effective biliary cholesterol secretion could also enlarge the intestinal cholesterol pool and dilute the dietary cholesterol absorption concentration. In obese human subjects, elevated cholesterol synthesis increases the biliary secretion of cholesterol (15), suggesting that one or the other of these mechanisms could explain the low cholesterol absorption efficiency also in the present diabetic subjects. However, it has recently been shown that of the ATP triphosphate-binding cassette transporter family, the expression of ABCG5 and ABCG8 mRNAs in mice intestine and liver can be increased by high-fat diet resulting in inhibited absorption of cholesterol and plant sterols (31). In phytosterolemia, their expression is depressed by mutation of the ABCG5 and ABCG8 genes (31), resulting in high intestinal absorption and decreased biliary secretion of sterols, two factors causing phytosterolemia (32). It could be assumed that obesity and diabetes by some unknown mechanism could increase the expression of these genes similarly to high-fat diet in mice and explain the low absorption efficiency of cholesterol in diabetic individuals.

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