

# Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men

Jussi Pihlajamäki,<sup>1,\*</sup> Helena Gylling,<sup>†</sup> Tatu A. Miettinen,<sup>§</sup> and Markku Laakso\*

Department of Medicine<sup>\*,†</sup> and Clinical Nutrition, <sup>†</sup> University of Kuopio, Kuopio, Finland; and Department of Medicine, Division of Internal Medicine,<sup>§</sup> University of Helsinki, Helsinki, Finland

**Abstract** Type 2 diabetes has been associated with high synthesis and low absorption of cholesterol independent of weight, indicating that insulin resistance may be a link between glucose and cholesterol metabolism. Therefore, we investigated the relationship of serum cholesterol precursors, reflecting cholesterol synthesis, and serum plant sterols and cholestanol, reflecting cholesterol absorption efficiency, with insulin sensitivity measured with the hyperinsulinemic euglycemic clamp in 72 healthy normoglycemic men. Men in the most insulin-resistant tertile had higher serum cholesterol precursor ratios ( $P < 0.05$ ), whereas no significant differences in serum absorption sterols were observed. In bivariate analysis, cholesterol synthesis markers correlated with fasting insulin ( $r = 0.36-0.46$ ,  $P < 0.01$ ) and the rates of insulin-stimulated whole-body glucose uptake (WBGU;  $r = -0.37-0.40$ ,  $P < 0.01$ ). Also, cholesterol absorption markers correlated with fasting insulin and WBGU ( $P < 0.05$ ). Fasting insulin correlated with desmosterol ( $r = 0.286$ ,  $P = 0.015$ ) and lathosterol ( $r = 0.248$ ,  $P = 0.037$ ) even when the rates of WBGU and body mass index (BMI) were controlled for. **■** We conclude that insulin resistance is linked to high cholesterol synthesis and decreased cholesterol absorption. Because fasting insulin correlated with cholesterol synthesis independent of the rates of BMI and WBGU, it is possible that regulation of cholesterol synthesis by hyperinsulinemia may be a link between insulin resistance and cholesterol metabolism.—Pihlajamäki, J., H. Gylling, T. A. Miettinen, and M. Laakso. **Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men.** *J. Lipid Res.* 2004. 45: 507–512.

**Supplementary key words** insulin sensitivity • hyperinsulinemic euglycemic clamp • cholestanol • campesterol • sitosterol • cholesterol • desmosterol • lathosterol • squalene

Insulin resistance and type 2 diabetes (T2DM) have been constantly associated with high triglyceride and low

HDL-cholesterol levels. Increased synthesis of VLDL particles in the liver has been proposed to be the main cause of increased concentrations of triglyceride-rich lipoproteins. This overproduction of VLDL and triglycerides in the liver has been proposed to be driven by high levels of serum FFAs in patients with insulin resistance. In T2DM, high levels of VLDL cholesterol have also been observed, and because of that, mildly increased levels of LDL cholesterol may occur [for review, see refs. (1, 2)]. These observations are in agreement with the findings that increased cholesterol synthesis has been observed in obese subjects (3) and in patients with the metabolic syndrome (4). Because similar findings have been observed in patients with T2DM independent of weight (5), insulin resistance could explain the increase in cholesterol synthesis in patients with obesity and T2DM. This hypothesis is supported by the finding that in subjects with normal glucose tolerance, high glucose is linked to increased synthesis of cholesterol (6). Increase in cholesterol synthesis is always accompanied by low rates of cholesterol absorption; therefore, it has been difficult to determine which of these two is primarily affected in subjects with obesity or T2DM.

In this study, we tried to answer the following questions. 1) Is directly measured insulin sensitivity linked to cholesterol metabolism in normoglycemic subjects? 2) Is cholesterol absorption or synthesis more strongly associated with insulin resistance? To this end, serum cholesterol precursors, reflecting cholesterol synthesis, and plant sterols and cholestanol, reflecting cholesterol absorption efficiency (7), were quantitated with gas-liquid chromatography in 72 healthy men. Insulin sensitivity was measured in these men using the hyperinsulinemic euglycemic clamp.

Abbreviations: BMI, body mass index; LXR, liver X receptor; T2DM, type 2 diabetes; WBGU, whole-body glucose uptake.

<sup>1</sup> To whom correspondence should be addressed.  
e-mail: pihlajam@uku.fi

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## Subjects

Study subjects were 72 healthy unrelated Finnish men without family history of diabetes or premature coronary heart disease randomly selected from the population living in the Kuopio University Hospital region. All subjects had normal glucose tolerance according to World Health Organization criteria (8) and did not have hypertension, symptoms or signs of coronary heart disease, or continuous drug treatment. Furthermore, they had normal liver, kidney, and thyroid function and no history of excessive alcohol intake. Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

## Metabolic studies

Insulin sensitivity was measured by the euglycemic clamp technique (9) after a 12 h fast as previously described (10). After the baseline blood drawing, a priming dose of insulin (Actrapid 100 IU/ml; Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min to increase plasma insulin concentration quickly to the desired level, at which it was maintained by a continuous insulin infusion of 480 pmol/m<sup>2</sup>/min. Under these study conditions, hepatic glucose production is completely suppressed in nondiabetic subjects (11). Blood glucose was clamped at 5.0 mmol/l for the next 180 min by the infusion of 20% glucose at varying rates according to the blood glucose measurements performed at 5 min intervals. The mean rates of glucose infusion during the last hour of the clamp were used to calculate the rates of insulin-stimulated whole-body glucose uptake (WBGU). Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) as previously described (12). Gas exchange and urinary nitrogen excretion were measured in the fasting state and during the last 30 min of the euglycemic clamp procedure. Data from the first 10 min of each measurement were discarded, and the mean values of data from the last 20 min were used in calculations. Respiratory quotient, the rates of glucose and lipid oxidation (or net synthesis during the hyperinsulinemic clamp procedure), and energy expenditure were calculated according to Ferrannini (13). The rates of nonoxidative glucose disposal during the euglycemic clamp procedure were estimated by subtracting the rates of glucose oxidation from the rates of WBGU.

## Analytical methods

Plasma glucose in the fasting state and after an oral glucose load and blood glucose during the euglycemic clamp procedure were measured by the glucose oxidase method (2300 Stat Plus; Yellow Springs Instrument Co., Inc., Yellow Springs, OH). For the determination of plasma insulin, blood was collected in EDTA-containing tubes, and after centrifugation, the plasma was stored at -20°C until analysis. Plasma insulin concentration was determined by a commercial double-antibody solid-phase radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation, as previously described (14). Cholesterol and triglyceride levels from whole serum and lipoprotein fractions were assayed by automated enzymatic methods (Boehringer-Mannheim, Mannheim, Germany). Apolipoprotein B levels were determined by a commercial immunoturbidometric method (Kone Instruments, Espoo, Finland), and serum FFAs from fresh frozen samples were determined by an enzymatic method (Wako Chemicals GmbH,

Neuss, Germany). Nonprotein urinary nitrogen was measured by an automated Kjeldahl method (15).

The serum cholesterol precursors squalene, cholesterol, desmosterol, and lathosterol, i.e., sterols reflecting cholesterol synthesis (7), and the plant sterols campesterol and sitosterol and cholestanol (a metabolite of cholesterol), i.e., sterols reflecting cholesterol absorption efficiency (7, 16), were quantitated with gas-liquid chromatography on a 50 m long capillary column (Ultra 1; Hewlett-Packard, Wilmington, DE) using 5 $\alpha$ -cholestane as an internal standard (17). The squalene and noncholesterol sterol values were expressed in terms of 10<sup>2</sup>× millimoles per mole of cholesterol (called ratio in the text), dividing the squalene and sterol values by the cholesterol value of the same run to eliminate the effects of different cholesterol concentrations.

## Statistical analysis

All basic calculations were performed with the SPSS/Win programs (version 10.0; SPSS, Inc.). The differences in the parameters among the tertiles of insulin sensitivity were tested by the ANOVA with age, gender, and body mass index (BMI) as covariates. Triglycerides, insulin, and FFA levels were logarithmically transformed to obtain a normal distribution before statistical analyses. A value of  $P < 0.05$  was considered statistically significant. All data are presented as means  $\pm$  SD. Factor analysis is a multivariate correlation method that is used to reduce a large set of intercorrelating variables, in this case plant sterols and cholesterol precursors, into a smaller set of latent underlying factors (18). The three main steps in factor analysis are as follows: 1) extraction of initial components; 2) rotation of components resulting in elucidation of factors; and 3) interpretation of factors. We used the principal components method for extraction of the initial components. The analysis was conducted with all plant sterols and cholesterol precursors. Varimax orthogonal rotation was then used to delineate two factors that are not correlated with each other. The resulting factor pattern was interpreted using factor loadings of  $\leq 0.4$ , i.e., variables with a loading of greater than 0.4 were taken in to that factor.

## RESULTS

The characteristics of study subjects divided into tertiles according to insulin sensitivity ( $n = 24$  in each tertile; WBGU cut-off points were 51.7 and 61.7  $\mu\text{mol/kg/min}$ ) are shown in **Table 1**. Subjects in the most insulin-resistant tertile had higher BMI, waist circumference, fasting plasma glucose and insulin, and total and VLDL triglycerides ( $P < 0.05$ ).

**Table 2** demonstrates that subjects in the most insulin-resistant tertile had higher serum cholesterol precursor ratios ( $P < 0.05$  for other than squalene), whereas the ratios of serum plant sterols or cholestanol tended to be lower in insulin-resistant subjects ( $P = 0.060$ – $0.598$ ). Factor analysis resulted in one factor each describing cholesterol absorption and synthesis. Serum squalene levels did not load significantly on either of the factors (**Table 3**). The factor score for cholesterol synthesis was higher in the most insulin-resistant tertile ( $P = 0.006$ ), whereas the score for cholesterol absorption did not differ among the tertiles ( $P = 0.656$ ). When adjusted for BMI or fasting, insulin differences in cholesterol synthesis between the tertiles disappeared.

TABLE 1. Characteristics of study subjects according to the tertiles of insulin sensitivity measured with the hyperinsulinemic euglycemic clamp

Variable	Insulin-Sensitive Tertile (n = 24)	Middle Tertile (n = 24)	Insulin-Resistant Tertile (n = 24)
Age (years)	53 ± 5	54 ± 5	55 ± 8
BMI (kg/m <sup>2</sup> )	24.9 ± 2.3	26.2 ± 2.7	28.6 ± 3.6 <sup>a</sup>
Waist (cm)	91 ± 7	94 ± 7	103 ± 8 <sup>a</sup>
Diastolic blood pressure (mmHg)	85 ± 7	85 ± 7	86 ± 6
Systolic blood pressure (mmHg)	133 ± 11	136 ± 14	136 ± 10
Fasting glucose (mmol/l)	5.2 ± 0.6	5.6 ± 0.4	5.9 ± 0.5 <sup>a</sup>
Fasting insulin (pmol/l)	37.5 ± 12.4	46.9 ± 16.8	90.3 ± 40.7 <sup>a</sup>
Total cholesterol (mmol/l)	6.05 ± 1.30	5.93 ± 0.88	5.99 ± 1.20
VLDL-cholesterol (mmol/l)	0.59 ± 0.29	0.60 ± 0.20	0.93 ± 1.17
LDL-cholesterol (mmol/l)	4.13 ± 1.19	4.06 ± 0.77	3.81 ± 1.02
HDL-cholesterol (mmol/l)	1.32 ± 0.33	1.25 ± 0.24	1.26 ± 0.27
Total triglycerides (mmol/l)	1.18 ± 0.40	1.33 ± 1.37	1.96 ± 1.86 <sup>b</sup>
VLDL-triglycerides (mmol/l)	0.61 ± 0.33	0.79 ± 0.30	1.31 ± 1.72 <sup>c</sup>
LDL-triglycerides (mmol/l)	0.36 ± 0.12	0.35 ± 0.11	0.41 ± 0.16
HDL-triglycerides (mmol/l)	0.21 ± 0.15	0.20 ± 0.08	0.24 ± 0.12
Free fatty acids (mmol/l)	0.42 ± 0.19	0.54 ± 0.18	0.49 ± 0.14

BMI, body mass index. Data shown are means ± SD.

<sup>a</sup>  $P < 0.001$  for the difference over the tertiles.

<sup>b</sup>  $P < 0.05$  for the difference over the tertiles.

<sup>c</sup>  $P < 0.01$  for the difference over the tertiles.

To determine which of the components of the insulin resistance syndrome (fasting hyperinsulinemia, obesity, or directly measured insulin sensitivity) had the strongest correlation with cholesterol synthesis, bivariate correlations between fasting insulin, BMI, WBGU, and cholesterol absorption markers and cholesterol precursors were calculated. The highest correlations were found between fasting insulin and cholesterol synthesis markers (Table 4). In addition, BMI and WBGU correlated with high levels of cholesterol precursors. Correlations between components of the insulin resistance syndrome and cholesterol absorption markers were less significant. Fasting insulin correlated with desmosterol ( $r = 0.286$ ,  $P = 0.015$ ) and lathosterol ( $r = 0.248$ ,  $P = 0.037$ ) even when WBGU and BMI were controlled. Other partial correlations after controlling for two other components of the insulin resistance syndrome were not significant. Figure 1 shows that the correlation between fasting insulin was higher with cholesterol synthesis factor ( $r = 0.416$ ,  $P < 0.001$ ) than

with cholesterol absorption factor ( $r = -0.253$ ,  $P = 0.068$ ).

No correlations were found between peripheral FFA levels in the fasting state or during the hyperinsulinemic clamp procedure and markers of cholesterol metabolism ( $r < 0.2$ ,  $P > 0.5$ ). However, the rates of lipid synthesis in the fasting state correlated positively with cholesterol synthesis factor ( $r = 0.332$ ,  $P = 0.004$ ). No association between lipid synthesis during the clamp procedure and cholesterol metabolism was observed ( $r = -0.017$ ,  $P = 0.887$ ).

## DISCUSSION

Obesity, T2DM, and the metabolic syndrome have been associated with increased cholesterol synthesis and decreased cholesterol absorption (3–5). In this study, we used surrogate markers (noncholesterol sterols) in serum

TABLE 2. Markers of cholesterol absorption and synthesis among study subjects according to the tertiles of insulin sensitivity measured with the hyperinsulinemic euglycemic clamp

Marker/Precursor	Insulin-Sensitive Tertile (n = 24)	Middle Tertile (n = 24)	Insulin-Resistant Tertile (n = 24)	<i>P</i>
Cholesterol absorption markers				
Cholestanol	1.36 ± 0.06	1.36 ± 0.06	1.18 ± 0.06	0.060
Campesterol	2.53 ± 0.17	2.34 ± 0.17	2.02 ± 0.25	0.204
Sitosterol	1.33 ± 0.08	1.31 ± 0.09	1.21 ± 0.11	0.598
Cholesterol precursors				
Cholestanol	0.09 ± 0.01	0.12 ± 0.01	0.15 ± 0.02	0.009
Desmosterol	0.65 ± 0.02	0.68 ± 0.04	0.77 ± 0.04	0.037
Lathosterol	1.54 ± 0.08	1.78 ± 0.12	2.01 ± 0.14	0.019
Squalene	0.28 ± 0.02	0.33 ± 0.02	0.31 ± 0.02	0.093

Data shown are means ± SD divided by serum cholesterol.

TABLE 3. Results of the factor analysis of cholesterol absorption markers and cholesterol precursors divided by serum cholesterol

Marker/Precursor	Factor 1 (Absorption Factor)	Factor 2 (Synthesis Factor)
Sitosterol	0.925	
Campesterol	0.862	
Cholestanol	0.652	-0.542
Cholestenol		0.914
Lathosterol		0.856
Desmosterol		0.748
Variance explained (%)	42.3	36.7

Extraction method, principal component analysis; rotation method, Varimax with Kaiser normalization. Rotation converged in three iterations. Squalene did not load significantly on either factor.

for the measurement of cholesterol synthesis and absorption. Thus, ratios to cholesterol of cholestenol, desmosterol, and lathosterol correlate positively with absolute cholesterol synthesis measured with the sterol balance technique, and those of cholestanol, campesterol, and sitosterol are positively related to absorption percentage of dietary cholesterol. The validity of these surrogate markers has been demonstrated in subjects with different degrees of insulin resistance, e.g., healthy subjects, obese subjects, and patients with T2DM (5, 6, 19). In this study, we demonstrated that insulin resistance itself was associated with high rates of cholesterol synthesis and low rates of cholesterol absorption but not with lipid levels except total and VLDL triglycerides. Fasting insulin had a stronger correlation with increased cholesterol synthesis than did BMI or the rates of WBGU, implying that fasting hyperinsulinemia, or hepatic insulin resistance, may be the link between insulin resistance and cholesterol metabolism.

Our main finding was that insulin resistance, determined by the hyperinsulinemic clamp procedure, is associated with high rates of cholesterol synthesis. Earlier, in patients with T2DM (5) and the metabolic syndrome (4), no clear determination could be made of whether cholesterol absorption or synthesis was primarily affected. Because both states are associated with insulin resistance, the

TABLE 4. Bivariate correlations of fasting insulin, BMI, and the rates of WBGU with different markers of cholesterol absorption and synthesis

Marker	Fasting Insulin	BMI	WBGU
Cholesterol absorption			
Cholestanol	-0.334 <sup>a</sup>	-0.273 <sup>a</sup>	-0.332 <sup>b</sup>
Campesterol	-0.335 <sup>a</sup>	-0.246 <sup>a</sup>	-0.336 <sup>b</sup>
Sitosterol	-0.333 <sup>a</sup>	-0.205	-0.240 <sup>a</sup>
Absorption factor	-0.253 <sup>a</sup>	-0.150	-0.198
Cholesterol synthesis			
Cholestenol	0.420 <sup>c</sup>	0.374 <sup>b</sup>	0.370 <sup>b</sup>
Desmosterol	0.462 <sup>c</sup>	0.268 <sup>a</sup>	0.397 <sup>b</sup>
Lathosterol	0.363 <sup>b</sup>	0.417 <sup>b</sup>	0.355 <sup>b</sup>
Squalene	0.160	-0.002	-0.294 <sup>a</sup>
Synthesis factor	0.416 <sup>c</sup>	0.379 <sup>b</sup>	0.395 <sup>b</sup>

WBGU, whole-body glucose uptake.

<sup>a</sup>  $P < 0.05$ .

<sup>b</sup>  $P < 0.01$ .

<sup>c</sup>  $P < 0.001$ .

possibility remained that insulin resistance would affect primarily either absorption or synthesis and then lead to compensatory changes in the other pathway, resulting in the observed changes in both cholesterol absorption and synthesis. Based on this study, we propose that a decrease in cholesterol absorption is secondary to increased cholesterol synthesis in subjects with insulin resistance for two reasons. First, the association of insulin resistance with markers of cholesterol synthesis was stronger than with markers of cholesterol absorption. Second, when we used factor analysis to separate two noncorrelating factors that describe cholesterol absorption and synthesis, insulin resistance was significantly linked to cholesterol synthesis factor and not to absorption factor.

Interestingly, cholesterol metabolism was more strongly correlated with fasting insulin than with the rates of WBGU. Although in our earlier studies obesity led to even more marked changes in cholesterol absorption and synthesis in patients with T2DM (19), the association between fasting insulin and cholesterol synthesis remained even after BMI was controlled. In addition, no significant

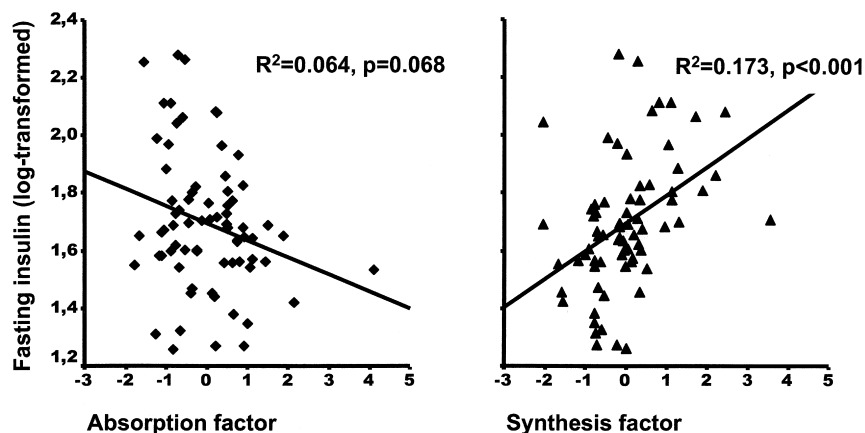


Fig. 1. Correlations between fasting insulin (log-transformed) and factors describing cholesterol absorption and synthesis (for factor analysis, see Subjects and Methods and Table 3).

correlation between serum FFAs, which are usually increased in obesity and insulin resistance, and cholesterol metabolism was observed. This indicates that insulin may have a direct action on cholesterol synthesis. Alternatively, an increase in portal FFA levels, which cannot be measured, could explain an increase in VLDL synthesis and VLDL cholesterol levels in insulin-resistant subjects (20). Insulin is known to stimulate liver X receptors (LXRs), which in turn upregulate lipogenesis and at least in part genes regulating cholesterol synthesis (21). This LXR-mediated coupled regulation of lipogenesis and cholesterol synthesis, possible via steroid regulatory element binding proteins (22), may also explain the positive correlation between the rates of lipid synthesis, based on indirect calorimetry, and cholesterol synthesis factor in our study.

Because cholesterol absorption markers correlate negatively with insulin resistance and fasting insulin, the question remains whether insulin resistance could directly affect cholesterol absorption. As indicated above, increased cholesterol synthesis is likely to result from high VLDL synthesis that is caused by hyperinsulinemia or high portal FFA levels in subjects with insulin resistance; hence, lower cholesterol absorption could be secondary to increased cholesterol synthesis. However, it is possible that hyperinsulinemia directly decreases cholesterol absorption. For example, stimulation of LXR by hyperinsulinemia could lead to increased expression of intestinal ABCG5 and ABCG8 genes (23) and hence lower cholesterol absorption. In that case, an increase in the rates of cholesterol synthesis might be secondary. This theory is supported by our earlier findings that weight loss, and decrease in insulin levels, lead to better cholesterol absorption without a significant change in cholesterol synthesis in obese patients with T2DM (19). Thus, a coordinate regulation, e.g., using the LXR pathway, of both cholesterol synthesis and absorption in insulin-resistant states may be the most likely explanation for our findings.

Finally, the possibility that changes in cholesterol synthesis or absorption could have an effect on insulin action exists. For example, inhibitors of cholesterol synthesis, statins, have been shown to improve insulin action (24, 25). In addition, an effect of statins on insulin action in the liver has been proposed in fructose-fed hamsters (26). Although this insulin-sensitizing effect of statins has been linked to their triglyceride-lowering effect, other mechanisms that link the effect of statins with insulin action may also exist. Finally, studies of the effect of cholesterol absorption changes, caused by either gene polymorphisms (e.g., ABCG genes) or drugs affecting cholesterol absorption, on insulin sensitivity are needed.

In conclusion, this study shows that cholesterol synthesis is increased and cholesterol absorption is decreased in insulin-resistant normoglycemic men. Fasting insulin was more strongly correlated with cholesterol synthesis than were BMI or the rates of WBGU, and no association of peripheral FFA levels with cholesterol metabolism was observed. These findings imply that the regulation of cholesterol metabolism by hyperinsulinemia, itself or as a

marker of hepatic insulin resistance, is the link between insulin resistance and cholesterol metabolism. **■**

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