Insulin sensitivity regulates cholesterol metabolism to a greater extent than obesity: lessons from the METSIM Study[1]

Helena Gylling,[2,*] Maarit Hallikainen,[*] Jussi Pihlajamäki,[†] Piia Simonen,[§] Johanna Kuusisto,[†] Markku Laakso,[†] and Tatu A. Miettinen[§]

Department of Clinical Nutrition,* University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland; Department of Medicine,† University of Eastern Finland, P.O. Box 1777, FI-70211 Kuopio, Finland; and Department of Medicine,§ Division of Internal Medicine, University of Helsinki, P.O. Box 700, FI-00029 HUS, Finland

Abstract **Cholesterol synthesis is upregulated and absorption downregulated in insulin resistance and in type 2 diabetes. We investigated whether alterations in cholesterol metabolism are observed across the glucose tolerance status, from normoglycemia through impaired glucose tolerance to type 2 diabetes, in 781 randomly selected men 45 to 70 years of age from a population-based Metabolic Syndrome in Men Study. Cholesterol metabolism was assayed using surrogate serum markers, squalene, and noncholesterol sterols. The study population was classified into subgroups according to glucose tolerance as follows: normoglycemia, impaired fasting glucose, impaired glucose tolerance, and type 2 diabetes. LDL cholesterol did not differ between the groups. Cholesterol synthesis markers were lowest and absorption markers highest in normoglycemia. Sitosterol was lower in subjects with impaired fasting glucose compared with normoglycemic subjects (113 ± 7 vs. 136 ± 3 10^2 μmol/mmol of cholesterol, $P < 0.05$). LDL cholesterol was not associated with lathosterol/sitosterol ratio, a marker of cholesterol metabolism. Peripheral insulin sensitivity evaluated by the Matsuda index was associated with the lathosterol/sitosterol ratio in the entire population ($r = -0.457$, $P < 0.001$) and with that of lathosterol/cholestanol independently of obesity. In conclusion, cholesterol metabolism was altered already from subjects with impaired fasting glucose. Upregulated cholesterol synthesis was associated with peripheral insulin resistance independent of obesity.**—Gylling, H., M. Hallikainen, J. Pihlajamäki, P. Simonen, J. Kuusisto, M. Laakso, and T. A. Miettinen. **Insulin sensitivity regulates cholesterol metabolism to a greater extent than obesity: lessons from the METSIM Study.** ***J. Lipid Res.*** **2010.** 51: **2422–2427.**

Supplementary key words Metabolic Syndrome in Men • lathosterol • sitosterol • cholestanol • type 2 diabetes • insulin resistance

Cholesterol metabolism is regulated mainly by intestinal absorption, hepatic synthesis, and biliary excretion of cholesterol. The negative interaction between cholesterol synthesis and absorption combined with the regulation of LDL receptor activity control the cellular cholesterol contents (1). Depletion of cellular cholesterol activates sterol regulatory element-binding protein 2 (SREBP2) resulting in increased cholesterol synthesis, whereas excess cholesterol activates the liver X receptor (LXR)-system, resulting in increased bile acid synthesis and increased biliary transport of cholesterol (1). Furthermore, glucose metabolism and insulin action are associated with cholesterol metabolism with unknown mechanism(s). In type 2 diabetes (T2D), cholesterol metabolism is disturbed so that cholesterol absorption efficiency is low (2–4), and cholesterol synthesis is elevated (2–6). Even in subjects without diabetes, low absorption efficiency and high synthesis of cholesterol are related to high-normal serum glucose level, insulin resistance, and obesity (7–9). We wanted to investigate whether the alterations in cholesterol metabolism are continuous from normoglycemia through impaired glucose tolerance to type 2 diabetes. Accordingly, we assayed serum cholesterol precursors (squalene, cholestenol, desmosterol, and lathosterol), markers of cholesterol synthesis in subjects without (10) and with T2D (4), and serum plant sterols and cholestanol, markers of cholesterol ab-

The study was supported by grants from the Academy of Finland.

Manuscript received 5 March 2010 and in revised form 1 May 2010.

Published, JLR Papers in Press, May 1, 2010
DOI 10.1194/jlr.P006619

Abbreviations: BMI, body mass index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LXR, liver X receptor; METSIM, Metabolic Syndrome in Men; NGT, normal glucose tolerance; NS, nonsignificant; OGTT, oral glucose tolerance test; SREBP, sterol regulatory element-binding protein; T2D, type 2 diabetes.

[1] This paper was presented in part at the 3rd International Congress on Prediabetes and the Metabolic Syndrome, Nice, France, and published as an abstract (*J. Diab.* 2009. 1 Suppl 1:A47).

[2] To whom correspondence should be addressed.
e-mail: helena.gylling@uef.fi

sorption in subjects without (10) and with T2D (4) in a large population-based sample of middle-aged men.

MATERIALS AND METHODS

Study population

From an ongoing population-based METSIM (Metabolic Syndrome in Men) Study, a random sample of 781 men without statin treatment were selected from about 6,500 men examined in the present study. The participants of the METSIM Study are men aged from 45 to 70 years old and selected from the population registry of Kuopio town, Eastern Finland (11). The mean (± SE) age of the participants was 57.8 ± 0.2 years, and body mass index (BMI) 27.2 ± 0.1 kg/m^2.

The subjects were examined at an outpatient visit at the Clinical Research Unit at the University of Kuopio. Detailed medical history and current drug treatments were collected in an interview, and glucose tolerance and cardiovascular risk factors were evaluated. Eighty-nine subjects had a β-blocking agent, 49 had diuretics, 38 had calcium channel blockers, 73 had angiotensin converting enzyme, and 60 had angiotensin receptor blocking agents. Thirty-eight subjects with T2D had sulphonylurea or metformin, and seven had evening insulin. None of the drug treatments were related to lipid or sterol variables. All subjects were on their normal habitual diet. Blood samples after 12 h fast were drawn followed by an oral glucose tolerance test (OGTT).

The subjects gave their written informed consent. The study was performed according to the Declaration of Helsinki, and it was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital.

Methods

Height and weight were measured to the nearest 0.5 cm and 0.1 kg. Waist was measured at the midpoint between the lateral iliac crest and lowest rib to the nearest 0.5 cm. A 2 h oral OGTT (75 g glucose) was performed, and samples for plasma glucose were drawn at 0, 30, and 120 min. Glucose tolerance was evaluated based on the OGTT as follows: normal glucose tolerance (NGT) = fasting plasma glucose <6.1 mmol/l and 2 h plasma glucose <7.8 mmol/l; impaired fasting glucose (IFG) = fasting plasma glucose 6.1–6.9 mmol/l and 2 h plasma glucose <7.8 mmol/l; impaired glucose tolerance (IGT) = fasting plasma glucose <6.1 mmol/l and 2 h plasma glucose between 7.8–11.0 mmol/l; T2D = fasting plasma glucose ⩾7.0 mmol/l and/or 2 h plasma glucose ⩾11.1 mmol/l (11). The insulin sensitivity index (Matsuda index) was calculated as described (12).

Laboratory analyses

Plasma glucose was measured with an enzymatic hexokinase photometric assay (Konelab System Reagents, Thermo Fisher Scientific, Vantaa, Finland). Insulin was determined with immunoassay (Siemens ADVIA Centaur®, Siemens Medical Solutions Diagnostics, Tarrytown, NY), apoproteins with immunoturbidimetry (Konelab System Reagents, Thermo Fisher Scientific, Vantaa, Finland), and serum total, LDL, and HDL cholesterol and serum triglycerides with commercial kits (Konelab System Reagents, Thermo Fisher Scientific, Vantaa, Finland).

Serum cholesterol, cholesterol precursors (squalene, desmosterol, and lathosterol), campesterol and sitosterol (plant sterols), and cholestanol, a metabolite of cholesterol, were quantified from nonsaponifiable serum material by capillary GC (Agilent 6890N Network GC System, Agilent Technologies, Wilmington, DE) equipped with a 50 m long Ultra 2 capillary column (5% Phenyl-methyl siloxane) (Agilent Technologies, Wilmington, DE) (13). The CV % was as follows: cholesterol 3.9%, cholestanol 4.4%, desmosterol 7.1%, lathosterol 5.0%, and sitosterol 3.0%, respectively. The serum values were expressed as concentrations (μg/dl) but also in terms of 10^2 μmol/mmol of cholesterol (called ratio in the text) by dividing squalene and noncholesterol sterol concentrations with the cholesterol value of the same GC run in order to eliminate the changing concentrations of sterol transporters, mainly LDL. The ratios to cholesterol of serum cholesterol precursors reflect whole-body cholesterol synthesis and those of plant sterols and cholestanol cholesterol absorption. We also calculated lathosterol/sitosterol and lathosterol/cholestanol ratios to depict cholesterol metabolism with one variable.

Statistics

Statistical analyses were performed with SPSS for Windows 14.0 statistics program (SPSS, Chicago, IL). Normality and homogeneity of variance were checked before further analyses. Logarithmic modifications were used with skewed distributions. Univariate ANOVA was used to compare the groups. ANCOVA was used to adjust age and BMI for all analyses. Pearson or Spearman correlation coefficients were calculated. To investigate whether obesity or insulin sensitivity explained the variability of cholesterol metabolism, linear regression analyses were calculated with lathosterol to cholesterol (cholesterol synthesis), sitosterol and cholestanol to cholesterol (cholesterol absorption), and lathosterol/sitosterol and lathosterol/cholestanol (cholesterol metabolism) as dependent variables. The results are given as mean ± SE. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the study population, 549 had NGT, 100 had IFG, 56 had IGT, and 76 had T2D, respectively (**Table 1**). Height did not differ between the glucose tolerance groups, but age and BMI differed so that the results were age- and BMI-corrected. Waist circumference was larger in IFG and T2D than in NGT. Similarly, plasma glucose and serum insulin concentrations were higher and the Matsuda index lower in IFG, IGT [plasma glucose non-significant (NS)], and T2D than in NGT.

Serum and LDL cholesterol concentrations did not differ between the groups (Table 1). HDL cholesterol concentration was lower and serum triglycerides higher in subjects with IGT than in subjects with NGT. Serum apoprotein A1 and apoprotein B levels differed between the groups.

Concentrations of serum squalene and noncholesterol sterols

Serum cholesterol measured with GC differed slightly between the groups ($P = 0.036$) (**Table 2**). The concentration of squalene was similar, but those of desmosterol and lathosterol differed between the groups so that the lowest values were present in subjects with NGT. Unadjusted desmosterol concentration was significantly higher already in subjects with IFG compared with subjects with NTG (192 ± 5 vs. 175 ± 2 μg/dl, $P < 0.05$).

Plant sterol concentrations were highest in subjects with NGT (Table 2). From subjects with IFG to those with T2D, the unadjusted plant sterol concentrations were lower than those of subjects with NGT. Unadjusted cholestanol concentration was lower in subjects with IGT and T2D

TABLE 1. Characteristics of the study population of 781 middle-aged men, aged from 45 to 70 years

Variables	NGTn = 549	IFGn = 100	IGTn = 56	T2Dn = 76	*P* Adjusted for Age and BMI
Age, years	57.3 ± 0.2	58.0 ± 0.5	59.7 ± 0.9*	59.6 ± 0.6*	<0.001
Height, cm	175.7 ± 0.3	175.7 ± 0.6	174.7 ± 0.8	175.4 ± 0.7	0.731
Weight, kg	81.6 ± 0.5	87.8 ± 1.5*	87.4 ± 1.4*	94.2 ± 2.2*	<0.001
BMI, kg/m^2	26.4 ± 0.1	28.4 ± 0.4*	28.6 ± 0.4*	30.6 ± 0.7*	<0.001
Waist, cm	95.3 ± 0.4	101.5 ± 1.1*	101.9 ± 1.2(*)	107.9 ± 1.8*	<0.001
Plasma glucose, mmol/l	5.41 ± 0.02	6.33 ± 0.02*	5.56 ± 0.05	7.46 ± 0.23*	<0.001
Serum insulin, mU/l	6.5 ± 0.2	9.5 ± 0.6*	11.2 ± 1.1*	15.6 ± 1.8*	<0.001
Matsuda index	52.6 ± 27.1	31.4 ± 16.4*	26.6 ± 14.1*	22.2 ± 11.6*	<0.001
Serum cholesterol, mmol/l	5.63 ± 0.04	5.79 ± 0.09	5.50 ± 0.12	5.57 ± 0.16	0.281
LDL cholesterol, mmol/l	3.65 ± 0.03	3.75 ± 0.08	3.60 ± 0.11	3.54 ± 0.13	0.397
HDL cholesterol, mmol/l	1.54 ± 0.02	1.57 ± 0.04	1.27 ± 0.04*	1.50 ± 0.05	<0.001
Serum triglycerides, mmol/l	1.30 ± 0.03	1.44 ± 0.07	1.77 ± 0.13*	1.65 ± 0.10(*)	0.002
Serum apoprotein A1, g/l	1.45 ± 0.01	1.49 ± 0.02	1.36 ± 0.03(*)	1.47 ± 0.04	0.005
Serum apoprotein B, g/l	1.08 ± 0.01	1.13 ± 0.03	1.18 ± 0.04	1.12 ± 0.04	0.143

Mean ± SE. NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes; BMI, body mass index. *$P < 0.05$ from NGT. (*) Non-significant after correction of age and BMI.

than in subjects with NGT, but the adjusted concentrations were not different between the groups. Lathosterol/sitosterol and lathosterol/cholestanol ratios differed between the groups so that the ratio was lowest in subjects with NGT. Neither the synthesis nor absorption markers differed between the IFG, IGT, and T2D groups.

Serum squalene and noncholesterol sterol ratios to cholesterol

The squalene to cholesterol ratio did not differ between groups (Table 2). The desmosterol to cholesterol ratio was lowest in subjects with NGT, and especially the unadjusted levels showed that this ratio was significantly increased already in subjects with IFG compared with subjects with NGT (96 ± 2 vs. 90 ± 1 10^2 μmol/mmol of cholesterol, $P < 0.05$). The lathosterol to cholesterol ratios were different among the groups with lowest values in subjects with NGT. The plant sterol to cholesterol ratios were lower in subjects with IFG than in subjects with NGT, as well as the unadjusted ratios in subjects with IGT and T2D. The cholestanol to cholesterol ratios differed between the groups ($P = 0.038$), but not after the adjustment for age and BMI. Neither the precursor nor absorption marker ratios differed between the IFG, IGT, and T2D groups.

Correlations

Age was not correlated with any of the metabolic variables (data not shown). The correlations between squalene and desmosterol varied from $r = 0.419$ to $r = 0.538$, $P < 0.001$, and those of squalene and lathosterol from $r = 0.265$ (NS) to $r = 0.487$ ($P < 0.001$) in different groups. The respective correlations between desmosterol and lathosterol varied from $r = 0.577$ to $r = 0.714$, $P < 0.001$. The correlations between absorption markers were significant in different groups (r-values from 0.463 to 0.925, $P < 0.001$ for all). To evaluate the maintenance of homeostatic regula-

TABLE 2. Serum sterols and squalene in 781 middle-aged men, aged from 45 to 70 years

Variables	NGT n = 549	IFG n = 100	IGT n = 56	T2D n = 76	*P* Adjusted for Age and BMI
Serum cholesterol, mg/dl[a]	195 ± 1	200 ± 3	188 ± 4	189 ± 5	0.042
Synthesis markers					
Squalene, μg/dl	37 ± 1	37 ± 1	37 ± 2	35 ± 2	0.698
Desmosterol, μg/dl	175 ± 2	192 ± 5(*)	194 ± 6(*)	187 ± 7	0.028
Lathosterol, μg/dl	281 ± 5	307 ± 13	320 ± 16	281 ± 13	0.013
Absorption markers					
Campesterol, μg/dl	541 ± 11	461 ± 23(*)	433 ± 27(*)	431 ± 25(*)	0.049
Sitosterol, μg/dl	263 ± 6	224 ± 13(*)	209 ± 12(*)	213 ± 14(*)	0.045
Cholestanol, μg/dl	279 ± 3	282 ± 7	252 ± 8(*)	260 ± 10(*)	0.072
Lathosterol/cholestanol	1.01 ± 0.53	1.09 ± 0.57	1.27 ± 0.55(*)	1.08 ± 0.66	0.040
Lathosterol/sitosterol	1.36 ± 0.04	1.80 ± 0.14(*)	1.81 ± 0.12(*)	1.80 ± 0.17	0.023
Ratios to cholesterol, 10^2 μmol/mmol of cholesterol					
Synthesis markers					
Squalene	19 ± 0	18 ± 1	20 ± 1	19 ± 1	0.787
Desmosterol	90 ± 1	96 ± 2(*)	104 ± 3*	99 ± 3(*)	0.001
Lathosterol	146 ± 2	154 ± 6	171 ± 8(*)	152 ± 8	0.018
Absorption markers					
Campesterol	278 ± 5	231 ± 12*	229 ± 13(*)	232 ± 13(*)	0.027
Sitosterol	136 ± 3	113 ± 7*	111 ± 6(*)	115 ± 7(*)	0.025
Cholestanol	143 ± 1	141 ± 3	134 ± 3	137 ± 3	0.490

Mean ± SE. NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes. * $P < 0.05$ from NGT. (*) Non-significant after correction of age and BMI.

[a] analyzed with gas-liquid chromatography.

TABLE 3. Correlations between cholesterol metabolism indicated with lathosterol/sitosterol ratio and BMI, waist circumference, lipids, plasma glucose, serum insulin, and the Matsuda index in 781 middle-aged men, aged from 45 to 70 years

	Serum Lathosterol/Sitosterol, μg/μg			
Variables	NGT n = 549	IFG n = 100	IGT n = 56	T2D n = 76
BMI, kg/m^2	0.453***	0.349***	0.362*	0.439***
Waist circumference, cm	0.417***	0.348***	0.462***	0.396***
LDL cholesterol, mmol/l	0.054	0.115	−0.098	−0.010
HDL cholesterol, mmol/l	−0.302***	−0.293*	−0.159	−0.355**
Serum triglycerides, mmol/l	0.360***	0.152	0.131	0.217
Plasma glucose, mmol/l	0.203***	0.011	0.127	−0.063
Serum insulin, mU/l	0.435***	0.214	0.513***	0.082
Matsuda index	−0.453***	−0.256*	−0.495***	−0.453*

NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes; BMI = body mass index. *$P < 0.05$;**$P < 0.01$***$P < 0.001$.

tion, the correlations between lathosterol and sitosterol were calculated (NGT $r = -0.318$, $P < 0.001$, IFG $r = -0.248$, $P = 0.05$; IGT $r = -0.111$, NS, T2D $r = -0.366$, $P = 0.004$).

The lathosterol/sitosterol ratio was significantly associated with BMI and waist circumference in all groups (**Table 3**). The ratio was negatively associated with HDL cholesterol level in all groups (in the IGT group nonsignificantly), and positively with serum triglycerides in subjects with NGT. LDL cholesterol levels were not related with the lathosterol/sitosterol ratio in any of the groups. Plasma glucose in subjects with NTG and serum insulin in subjects with NGT and IGT were related to the lathosterol/sitosterol ratio. The Matsuda index was inversely related to the lathosterol/sitosterol ratio in all subjects, individual values shown in **Fig. 1**.

Linear regression model

To further evaluate whether obesity or insulin sensitivity was associated with cholesterol metabolism, a linear regression analysis was carried out with BMI, waist circumference, and the Matsuda index as the independent variables (**Table 4**). Logarithmic transformations of the variables did not change the results. The Matsuda index, independent of obesity, was significantly associated with lathosterol and cholestanol to cholesterol, and the lathosterol/cholestanol ratio explaining 14.4% of the variability of the lathosterol/cholestanol ratio. However, the Matsuda index and waist circumference were both significantly associated with sitosterol level and the lathosterol/sitosterol ratio. With this model, 26.8% of the variation of the lathosterol/sitosterol ratio could be explained.

DISCUSSION

The novel finding of the present study including subjects with varying degrees of glucose tolerance was that cholesterol metabolism, assayed with surrogate serum markers, was perturbed already in subjects with IFG so that cholesterol synthesis was upregulated and cholesterol absorption downregulated. Furthermore, insulin sensitivity measured with the Matsuda index was associated with cholesterol synthesis independent of obesity. If cholesterol metabolism was assayed with the lathosterol/sitosterol ratio, both waist circumference and the Matsuda index were associated with the metabolic variable, but the Matsuda index alone independent of obesity was associated with the lathosterol/cholestanol ratio. The difference between plant sterols and cholestanol as absorption markers is that serum plant sterol levels reflect their dietary amount, whereas the serum cholestanol content is not dependent on dietary intake. Serum squalene does not consistently behave as a cholesterol synthesis marker, but in this study population, it was associated with the other synthesis markers, better with desmosterol than with lathosterol, and the best association was observed in subjects with T2D ($r = 0.538$, $P < 0.001$).

In insulin resistance (8), and in insulin resistant states such as obesity (9), metabolic syndrome (14, 15), and T2D (2–6), cholesterol synthesis is upregulated and cholesterol absorption efficiency is low compared with controls. The present study showed that cholesterol metabolism was perturbed already in subjects with IFG and IGT, and did not essentially differ between subjects with IFG/IGT and T2D.

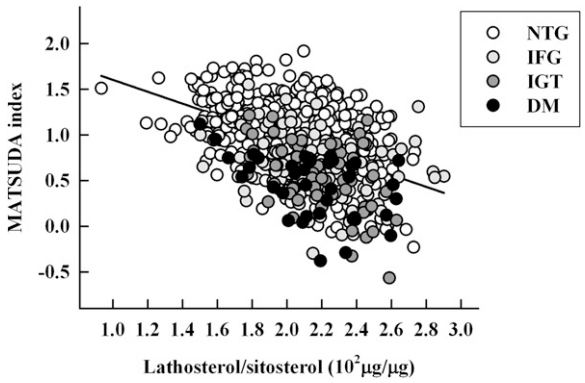

Fig. 1. The correlation between serum lathosterol/sitosterol (10^2 μg/μg) reflecting cholesterol metabolism and the Matsuda index reflecting peripheral insulin sensitivity in 781 middle-aged men, aged from 45 to 70 years. $r = -0.457$, $P < 0.001$, All: $y = -0.650 \times +2.254$. NTG: $y = -0.586 \times +2.206$; IFG: $y = -0.302 \times +1.349$; IGT: $y = -0.802 \times +2.298$; DM: $y = -0.534 \times +1.597$. NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D,= type 2 diabetes.

TABLE 4. Regression analysis showing the associations between the ratios of lathosterol, cholestanol, and sitosterol to cholesterol (10^2 μmol/mmol of cholesterol), and the lathosterol to cholestanol and lathosterol to sitosterol ratios (μg/μg) and BMI, waist circumference, and the Matsuda index in 781 middle-aged men, aged from 45 to 70 years

	Lathosterol/Cholesterol	Cholestanol/Cholesterol	Sitosterol/Cholesterol	Lathosterol/Cholestanol	Lathosterol/Sitosterol
Variables	Multivariate *p*-value				
BMI, kg/m^2	0.584	0.471	0.675	0.659	0.501
Waist, cm	0.344	0.729	<0.001	0.288	<0.001
Matsuda index	<0.001	<0.001	<0.001	<0.001	<0.001
R^2 of the model	0.121	0.108	0.220	0.144	0.268

BMI, body mass index.

Similarly, markers of cholesterol absorption, especially serum plant sterol concentrations and ratios to cholesterol, were decreased in subjects with IFG but remained comparable between subjects with IFG and T2D. Accordingly, cholesterol and sterol metabolism did not differ between subjects with IFG, IGT, and T2D, in contrast to insulin action, which differs across these glucose tolerance status groups (11). The predominant feature of subjects with IGT is impaired peripheral insulin sensitivity and impaired early-phase insulin release, whereas in IFG, impaired early and total insulin release and impaired hepatic insulin sensitivity was observed (11).

Peripheral insulin sensitivity, measured with the Matsuda index, is an accurate noninvasive indicator of overall insulin sensitivity (11) applicable to population studies. BMI and waist circumference were associated positively with markers of cholesterol synthesis. In contrast, insulin sensitivity was inversely associated with markers of cholesterol synthesis but positively with markers of cholesterol absorption. We have shown earlier that in obese T2D subjects, weight reduction together with decreasing hyperinsulinemia increases cholesterol absorption efficiency and decreases cholesterol synthesis (16). Accordingly, upregulated cholesterol synthesis and downregulated cholesterol absorption is not an optimal combination with respect to the risk of atherosclerotic complications. Therefore, even subjects with IFG should be recommended to start effective lifestyle changes to return cholesterol metabolism to the level of normoglycemic individuals. The novel finding in the present study was, however, that insulin sensitivity regulates cholesterol metabolism to a greater extent than obesity.

The mechanism(s) by which hyperinsulinemia, induced by insulin resistance, regulates cholesterol metabolism remains open. Hyperinsulinemia enhances the biogenesis of SREBP-1c, which in liver increases the synthesis of fatty acids, the production of VLDL particles, and is responsible for the development of triglyceride-enriched fatty liver (17, 18). However, SREBP-2, which is responsible for de novo cholesterol synthesis, is activated by cellular cholesterol depletion but not by hyperinsulinemia or hyperglycemia. Accordingly, either cholesterol depletion develops in liver cells during enhanced VLDL production by hyperinsulinemia, or the reason for the activation of cholesterol synthesis resides in some other steps of the complicated transcriptional or post-transcriptional phases of cholesterol synthesis.

When the homeostatic regulation of cholesterol synthesis and absorption is intact, another possibility for altered cholesterol metabolism in hyperinsulinemia is depression in intestinal cholesterol absorption. In the present study, cholesterol homeostasis was intact. The essential membrane receptors involved in sterol absorption are Niemann-Pick C1 Like-1 and ABCG5 and ABCG8 proteins. Very little is known of their regulation except that in experimental animals, streptozotocin-induced insulin deficiency interfered with the sterol-secreting action of ABCG5 and ABCG8 receptors so that the absorption of cholesterol was increased several-fold (19). Clinical studies in type 1 diabetes confirm these results by showing that the serum levels of plant sterols and cholestanol are increased compared with controls or with T2D (20, 21). In addition, mutations in the ABCG5 and ABCG8 receptors cause phytosterolemia with increased absorption of cholesterol and especially those of plant sterols (22), but more detailed information of possible effects of insulin or glucose on these receptors is lacking. A polymorphism in the Niemann-Pick C1 Like-1 gene causes changes in cholesterol absorption and LDL cholesterol level (23), but there is no information of its role during hyperglycemia or insulin resistance.

In conclusion, cholesterol metabolism was altered already in subjects with IFG, so that markers of cholesterol synthesis were increased and those of cholesterol absorption were decreased. LDL cholesterol level was not associated with cholesterol metabolism, whereas HDL cholesterol was negatively and serum triglycerides positively associated with cholesterol synthesis. Insulin sensitivity was inversely associated with cholesterol synthesis, and the relationship was independent of obesity.

The expert assistance of Ms. Leena Kaipiainen and Ritva Nissinen is greatly acknowledged.

REFERENCES

1. Ikonen, E. 2008. Cellular cholesterol trafficking and compartmentalization. *Nat. Rev. Mol. Cell Biol.* **9:** 125–138.
2. Sutherland, W. H., R. S. Scott, C. J. Lintott, M. C. Robertson, S. A. Stapely, and C. Cox. 1992. Plasma non-cholesterol sterols in patients with non-insulin dependent diabetes mellitus. *Horm. Metab. Res.* **24:** 172–175.

3. Simonen, P. P., H. K. Gylling, and T. A. Miettinen. 2002. Diabetes contributes to cholesterol metabolism regardless of obesity. *Diabetes Care.* **25:** 1511–1515.
4. Simonen, P., H. Gylling, and T. A. Miettinen. 2008. The validity of serum squalene and non-cholesterol sterols as surrogate markers of cholesterol synthesis and absorption in type 2 diabetes. *Atherosclerosis.* **197:** 883–888.
5. Briones, E. R., D. L. Steiger, P. J. Palumbo, W. M. O'Fallon, A. L. Langworthy, B. R. Zimmerman, and B. A. Kottke. 1986. Sterol excretion and cholesterol absorption in diabetics and nondiabetics with and without hyperlipidemia. *Am. J. Clin. Nutr.* **44:** 353–361.
6. Andersen, E., P. Hellström, and K. Hellström. 1986. Cholesterol and bile acid metabolism in middle-aged diabetics. *Diabetes Metab.* **12:** 261–267.
7. Stranberg, T. E., V. Salomaa, H. Vanhanen, and T. A. Miettinen. 1996. Associations of fasting blood glucose with cholesterol absorption and synthesis in nondiabetic middle-aged men. *Diabetes.* **45:** 755–761.
8. Pihlajamäki, J., H. Gylling, T. A. Miettinen, and M. Laakso. 2004. Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. *J. Lipid Res.* **45:** 507–517.
9. Miettinen, T. A., and H. Gylling. 2000. Cholesterol absorption efficiency and sterol metabolism in obesity. *Atherosclerosis.* **153:** 241–248.
10. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesäniemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **131:** 20–31.
11. Stančáková, A., M. Javorský, T. Kuulasmaa, S. M. Haffner, J. Kuusisto, and M. Laakso. 2009. Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 Finnish men. *Diabetes.* **58:** 1212–1221.
12. Matsuda, M., and R. A. DeFronzo. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* **22:** 1462–1470.
13. Miettinen, T. A. 1988. Cholesterol metabolism during ketoconazole treatment in man. *J. Lipid Res.* **29:** 43–51.
14. Gylling, H., M. Hallikainen, M. Kolehmainen, L. Toppinen, J. Pihlajamäki, H. Mykkänen, J. T. Ågren, R. Rauramaa, M. Laakso, and T. A. Miettinen. 2007. Cholesterol synthesis prevails over absorption in metabolic syndrome. *Transl. Res.* **149:** 310–316.
15. Chan, D. C., G. F. Watts, P. H. Barrett, F. H. O'Neill, and G. R. Thompson. 2003. Plasma markers of cholesterol homeostasis and apolipoprotein B-100 kinetics in the metabolic syndrome. *Obes. Res.* **11:** 591–596.
16. Simonen, P., H. Gylling, A. N. Howard, and T. A. Miettinen. 2000. Introducing a new component of the metabolic syndrome: low cholesterol absorption. *Am. J. Clin. Nutr.* **72:** 82–88.
17. Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109:** 1125–1131.
18. Browning, J. D., and J. D. Horton. 2004. Molecular mediators of hepatic steatosis and liver injury. *J. Clin. Invest.* **114:** 147–152.
19. Bloks, V. W., W. M. Bakker-Van Waarde, H. J. Verkade, I. P. Kema, H. Wolters, E. Vink, A. K. Groen, and F. Kuipers. 2004. Down-regulation of hepatic and intestinal Abcg5 and Abcg8 expression associated with altered sterol fluxes in rats with streptozotocin-induced diabetes. *Diabetologia.* **47:** 104–112.
20. Järvisalo, M., O. Raitakari, H. Gylling, and T. A. Miettinen. 2006. Cholesterol absorption and synthesis in children with type 1 diabetes. *Diabetes Care.* **29:** 2300–2304.
21. Miettinen, T. A., H. Gylling, J. Tuominen, P. Simonen, and V. Koivisto. 2004. Low synthesis and high absorption of cholesterol characterize type 1 diabetes. *Diabetes Care.* **27:** 53–58.
22. Berge, K. E. 2003. Sitosterolemia: a gateway to new knowledge about cholesterol metabolism. *Ann. Med.* **35:** 502–511.
23. Cohen, J. C., A. Pertsemlidis, S. Fahmi, S. Esmail, G. L. Vega, S. M. Grundy, and H. H. Hobbs. 2006. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proc. Natl. Acad. Sci. USA.* **103:** 1810–1815.