

Vascular Disease Is Associated With the Expression of Genes for Intestinal Cholesterol Transport and Metabolism

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Context: Intestinal cholesterol metabolism is important in influencing postprandial lipoprotein concentrations, and might be important in the development of vascular disease.

Objective: This study evaluated associations between expression of intestinal cholesterol metabolism genes, postprandial lipid metabolism, and endothelial function/early vascular disease in human subjects.

Design/Patients: One hundred patients undergoing routine oesophago-gastro-duodenoscopy were recruited. mRNA levels of Nieman-Pick C1-like 1 protein (NPC1L1), ABC-G5, ABC-G8, ABC-A1, microsomal tissue transport protein (MTTP), and sterol-regulatory element-binding protein (SREBP)-2 were measured in duodenal biopsies using quantitative reverse transcription polymerase chain reaction. Postprandially, serum lipid and glycemic profiles were measured, endothelial function was assessed using fasting, and postprandial flow-mediated dilatation (FMD) and carotid intima-media thickness (IMT). Subjects were divided into those above and below the median value of relative expression of each gene, and results were compared between the groups.

Results: There were no between-group differences in demographic variables or classical cardiovascular risks. For all genes, the postprandial triglyceride incremental area under the curve was greater ($P < 0.05$) in the group with greater expression. Postprandial apolipoprotein B48 (ApoB48) levels were greater ($P < 0.05$) in groups with greater expression of NPC1L1, ABC-G8, and SREBP-2. For all genes, postprandial but not fasting FMD was lower ($P < 0.01$) in the group with greater expression. Triglyceride and ApoB48 levels correlated significantly with postprandial FMD. Carotid artery IMT was greater ($P < 0.05$) in groups with greater expression of MTTP, ABC-A1, and SREBP-2.

Conclusion: Intestinal cholesterol metabolism gene expression is significantly associated with postprandial increment in triglycerides, intestinal ApoB48, and reduced postprandial FMD. Some genes were also associated with increased IMT. These findings suggest a role of intestinal cholesterol metabolism in development of early vascular disease. (*J Clin Endocrinol Metab* 102: 326–335, 2017)

The theory that postprandial hyperlipidemia has a significant influence on the development of atherosclerosis was first postulated by Zilversmit in 1979 (1). The field of postprandial hyperlipidemia has once again

become an area of interest, with recent research highlighting the role that chylomicrons and chylomicron remnants play in the atherogenic postprandial milieu (2–4). The control mechanisms influencing postprandial

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Abbreviations: ABC, adenosine triphosphate-binding cassette; ApoB48, apolipoprotein B48; AUC, area under the curve; BMI, body mass index; CIMT, carotid artery IMT; CVD, cardiovascular disease; FMD, flow-mediated dilatation; GI, gastrointestinal; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HOMA-IR, Homeostasis Model Assessment - Insulin Resistance; iAUC, incremental AUC; IMT, intima-media thickness; LDL-C, calculated low-density lipoprotein; MTTP, microsomal tissue transport protein; NPC1L1, Nieman-Pick C1-like 1 protein; SREBP, sterol-regulatory element-binding protein; TG, triglyceride.

serum lipoprotein levels are becoming increasingly well understood, with multiple intestinal processes becoming clearer in the last 10 years (5).

Nieman-Pick C1-like 1 protein (NPC1L1) is now known to be the principal cholesterol transporter present on the brush border of proximal intestinal enterocytes, responsible for the influx of cholesterol from the lumen, either of dietary or hepatobiliary origin (6,7). The discovery of this transporter stems from work into the cholesterol-lowering agent, ezetimibe, which has NPC1L1 as its primary target (8,9). Conversely, members of the adenosine triphosphate-binding cassette (ABC) transporter family, ABC-G5 and ABC-G8, form a heterodimer on the apical cellular surface that is responsible for net cholesterol efflux from both intestinal and hepatic sources (10). Cholesterol and triglycerides (TG) within enterocytes, either taken up from the lumen from dietary or hepatobiliary sources or derived from the endogenous production via HMG CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) synthesis, are packaged with apolipoproteins apolipoprotein B48 (ApoB48) and apolipoprotein CII to form chylomicrons for secretion into the lymph. This process is controlled by the enzyme microsomal tissue transport protein (MTTP), present within the endoplasmic reticulum of enterocytes (11). Together these three processes contribute significantly to the flux of cholesterol and TG into the bloodstream following a meal. A further pathway at the basolateral membrane, involving another adenosine triphosphate-binding cassette protein, ABC-A1, is responsible for the efflux of cholesterol from within the enterocyte to the apolipoprotein A1 moiety, with the generation of nascent high-density lipoprotein (HDL) (12). Sterol-regulatory element-binding protein (SREBP) type 2, a ubiquitously expressed membrane-bound transcription factor, plays a central role in endogenous lipid synthesis via the action of cholesterologenic genes (13).

Although there has been extensive research into the delineation of these pathways, evidence of a direct link between intestinal gene expression and cardiovascular risk is lacking. Markers of early atherosclerosis, such as common carotid artery intima-media thickness (CIMT), and features of early endothelial dysfunction, such as brachial artery flow-mediated dilatation (FMD), are well validated (14) and provide an opportunity to relate these processes in human subjects to potential risk factors, both classic and novel, and associated physiological and pathophysiological mechanisms.

This study was designed to explore, in a heterogeneous human cohort, associations between intestinal cholesterol metabolism gene expression, postprandial lipid metabolism, and recognized markers of early atherosclerosis—brachial artery FMD and CIMT.

Methods

Subjects

This study conformed to local ethics committee guidelines and obtained full ethical approval. Postprandial and vascular studies were carried out according to protocols previously described by our group (15). Subjects were randomly recruited from the routine hospital upper gastrointestinal (GI) endoscopy waiting list. Subjects were eligible if they were between 30 and 60 years of age, had no underlying chronic GI disorder likely to potentially influence cholesterol absorption (celiac disease, inflammatory bowel disease, previous upper GI surgery), and were willing to accept the collection of two biopsy specimens from the second part of the duodenum for the study. Subjects also had to be able and willing to attend for the second part of the study (as described later) within 1 month of the endoscopic procedure. Subjects were excluded if they were taking ezetimibe or any other medication likely to affect intestinal absorption, corticosteroids, or other immune-modulatory agents. Diabetic subjects were excluded if they required insulin therapy, or were on GLP-1 agonists or DPP-4 inhibitors.

Postprandial study day

All study subjects attended the Diabetes Research Centre for the clinical study day within 1 mo following the completion of the upper GI endoscopy. Subjects attended following an overnight fast of 12 hours, with all normal medication taken at the usual time. Subjects were asked to refrain from alcohol or significant exercise for the 24 hours prior to attendance and to refrain from cigarette smoking for the duration of the day.

Mixed meal

Following the collection of fasting data, all subjects were given the same meal of mixed nutritional content. Nutritional content of this meal was a total of 940 kcal, constituting 27 g protein, 140 g carbohydrate (40 g of which was sugars), and 36 g fat. Subjects were asked to consume this within a 15-minute period, and the timing of postprandial measurements was taken from the completion of the meal.

Laboratory analysis

Blood samples were drawn from all subjects fasting, and at intervals of 15 minutes for the first 2 hours following the study meal, then 2 hourly up to 8 hours. Serum insulin, plasma glucose, and full lipid profile, including total cholesterol, HDL cholesterol (HDL-C), TG, and calculated low-density lipoprotein (LDL-C), were measured fasting and at 120-minute intervals, as was serum for the measurement of ApoB48 via enzyme-linked immunosorbent assay (Gentaur, Kampenhout, Belgium). Glucose was measured by an enzymatic (hexokinase) method on the Roche P Module (Roche, Stockholm, Sweden), and insulin was measured by electrochemiluminescence immunoassay on the Roche E Module (coefficients of variation <5% for both). Total cholesterol, TG, and HDL-C were measured using standard laboratory techniques (coefficient of variation <5%). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedwald equation. Values for postprandial lipid levels were calculated as area under the curve (AUC), incremental (above baseline, representing the postprandial effect) AUC (iAUC), and peak values for appropriate comparison. Insulin resistance was calculated from fasting plasma glucose and insulin values using the Homeostasis Model

Assessment computer algorithm (16) and expressed as HOMA-IR (Homeostasis Model Assessment - Insulin Resistance).

Vascular studies

Measurements of vascular disease were carried out according to consensus guidelines (17) on a single ultrasonographic machine (Sonosite Micromaxx Ultrasound System; Sonosite, Bothell, WA) by one of the authors (W.M.W.). Carotid IMT was measured on all subjects 120 minutes postprandially to ensure standardization. Measurements were taken from each common carotid artery, within 1 cm of the carotid bulb, with three views of each artery measured and averaged to form a composite measurement. FMD of the brachial artery was completed at two time points during the study: fasting and at 240 minutes postprandially. FMD was carried out on the subjects' right arm, with measurements of the brachial artery diameter and flow parameters taken at rest, and at 30-second intervals for 2 minutes after the performance of a hyperemic maneuver. A sphygmomanometer cuff was inflated to at least 20 mmHg greater than systolic pressure on the upper arm for 5 minutes before release, with subsequent images taken at the time points mentioned previously. Calculation of percentage change of the arterial diameter was calculated based on resting diameter compared with the maximal increase seen over these time points.

Duodenal biopsy and genetic studies

Subjects were consented for the collection of two additional duodenal biopsies (D2) at the time of their upper GI endoscopy. Samples were collected by the performing gastroenterologist and immediately rinsed in sterile water and transferred into RNAlater (Ambion; ThermoFisher Scientific, Waltham, MA) storage media by the principal investigator (W.M.W.). Samples were kept at 4°C for 24 hours before being frozen at –80°C for storage: subsequent defrosting also occurred in RNAlater. Sample disruption and homogenization used the TissueRuptor rotor-stator homogenizer in QIAzol Lysis Reagent, with RNA extraction performed using the RNeasy mini kit (Qiagen, Hilden, Germany). Postextraction RNA integrity was assessed using the Agilent 2100 Bioanalyser (Agilent Technologies Santa Clara, CA). Reverse transcription was carried out on a PTC-200 DNA engine thermal cycler platform (MJ Research; GMI Inc, Waltham, MA), utilizing Bioscript reverse transcription (Bioline Reagents, London, United Kingdom), dNTPs, random primers, and RNaseOut ribonuclease inhibitor (Invitrogen; ThermoFisher Scientific), with 1 mcg mRNA used for conversion to cDNA for each patient. Polymerase chain reaction was performed on a 7900HT Fast

Realtime PCR Light-Cycler System (Applied Biosystems; ThermoFisher Scientific). Gene primer probes for *NPC1L1*, *ABCG5*, *ABCG8*, *ABCA1*, *MTTP*, *SREBP2*, and *18S* (Applied Biosystems) were purchased, and real-time quantitative polymerase chain reaction was performed using TaqMan mastermix (Applied Biosystems). Data were analyzed utilizing the $2^{-\Delta\Delta C_t}$ method. Gene expression was standardized internally against a ubiquitously expressed control gene (18S RNA), and the gene expression was calculated and tabulated relative to a randomly determined baseline subject designated the expression value of 1 for all genes. These genetic analyses were carried out using methodologies previously described by our group (18).

Statistical analysis

Statistical analysis was carried out using SPSS for Windows Version 11.5 (SPSS, Chicago, IL). For analysis, subjects were divided into those above and below the median of relative expression of each gene, and results were compared between the groups using the chi-squared test for categorical and unpaired Student's *t* test for continuous data. Univariate correlations were calculated using Pearson's product moment correlation coefficient. Multivariate analysis utilizing standard multiple linear regression was also carried out. Nonnormally distributed data were log-transformed prior to analysis. Results were considered significant for all analyses at $P < 0.05$.

Results

Baseline characteristics

One hundred and forty-nine subjects consented to participation in the study and were enrolled at the time of upper GI endoscopy. Of these, 49 were unable to complete all aspects of the study, and were therefore not included in the final analysis: no duodenal biopsy obtained, 7; inability to attend for postprandial study day, 39; inability to complete postprandial study day, 3 (unable to complete meal, 1; presyncopal response to cannulation, 1; previously undiagnosed severe hypertriglyceridemia detected, 1).

A final total of 100 subjects completed all aspects of the study and are included in the results described later. Reasons for undertaking the endoscopy were as follows: investigation of dyspeptic symptoms, 40; follow-up of known gastro-esophageal reflux or peptic ulcer disease,

Table 1. Baseline Demographic and Biochemical Data of Full Cohort

Age (years)	50.3 [0.8]	HbA1C (% DCCT)	5.6 [0.07]
BMI (kg/m ²)	29.5 [0.58]	FPG (mmol/L)	5.4 [0.14]
Waist hip ratio	0.9 [0.01]	HOMA-IR	1.6 [0.15]
Systolic BP (mmHg)	135.5 [1.53]	Fasting total cholesterol (mmol/L)	5.0 [0.1]
Diastolic BP (mmHg)	86.2 [1.1]	Total cholesterol AUC (mmol/L)	40.1 [0.77]
Gender (m:f)	57:43	Fasting triglyceride (mmol/L)	1.8 [0.14]
Smoker (%)	26	Triglyceride AUC (mmol/L)	19.4 [1.1]
Diabetes (%)	10	Triglyceride iAUC (mmol/L)	5.0 [0.45]
Statin (%)	28	Fasting ApoB48 (μg/mL)	13.4 [1.1]
Aspirin (%)	20	ApoB48 AUC (μg/mL)	139.2 [8.0]

Values are shown mean [standard error of the mean].

Abbreviations: BP, blood pressure; DCCT, Diabetes Control and Complications Trial; FPG, fasting plasma glucose; HbA1C, hemoglobin A1C.

29; investigation of other GI symptoms (abdominal pain, change in bowel habit), 15; surveillance for Barrett's esophagus, 11; investigation of Fe-deficient anemia, 5). None of the included subjects had any condition found that would influence the obtained results. Demographic data for these 100 subjects are presented in Table 1.

Association of gene expression with demographic, biochemical, and vascular variables

As described previously, subjects were divided into those above and below the median value of relative expression of each gene, and all variables were compared between the two groups for each gene. Mean fold change was significantly different between the two groups for each gene (NPC1L1 1.9 *versus* 8.6, ABC-G5 1.5 *versus* 12.7, ABC-G8 1.3 *versus* 8.3, ABC-A1 1.2 *versus* 5.6, MTTP 1.6 *versus* 10.9, SREBP-2 1.6 *versus* 11.2; $P < 0.01$ for all).

Demographic and biochemical variables

For all of the genes studied, there were no significant differences in age, body mass index (BMI), gender, presence of diabetes, or statin use between subjects with gene expression below or above the median value. There were no significant between-group differences for HOMA-IR, hemoglobin A1C (HbA1C), or plasma glucose (fasting, peak, or AUC). There were no significant between-group differences for total cholesterol, LDL-C, HDL-C, or TG levels (fasting, peak, or AUC) between subjects with gene expression below or above the mean value for any of the genes studied. Low-density lipoprotein was not included in

postprandial analysis due to significant loss of data due to postprandial TG levels >4 mmol/L.

As an example, data for the gene NPC1L1 are presented in Table 2. Results are similar for all of the other genes studied.

Fasting ApoB48 levels did not differ between groups for any of the genes studied. Postprandial ApoB48 AUC levels were greater ($P < 0.05$) in the groups with greater expression of NPC1L1, ABC-G5, ABC-G8, and SREBP-2, whereas there was a nonsignificant trend in the other two genes. Results are shown in Fig. 1.

For all genes, TG iAUC was greater ($P < 0.05$) in the group with greater gene expression (Fig. 2). Correlation analysis confirmed significant association between TG iAUC and the expression of NPC1L1 and MTTP ($R = 0.247$ and 0.201 , respectively, $P < 0.05$), whereas other genes showed a nonsignificant trend ($R = 0.141$ – 0.163 , $P < 0.15$).

All of the analyses mentioned previously were repeated with 1) subjects with diabetes; 2) subjects on statin therapy removed from the dataset. These adjustments did not affect the outcome of the initial analysis.

Gene expression versus vascular data

For all genes, postprandial but not fasting FMD was lower ($P < 0.01$) in the group with greater gene expression. Results are shown in Fig. 3. CIMT was greater ($P < 0.05$) in the groups with greater expression of MTTP, ABC-A1, and SREBP-2.

Correlation analysis between gene expression, biochemical variables, and vascular markers

There were highly significant positive intercorrelations between all genes (Table 3). All genes correlated with postprandial FMD, whereas all except NPC1L1 correlated with IMT. Age, BMI, systolic blood pressure, fasting plasma glucose, HOMA-IR, and HbA1c all correlated negatively with postprandial FMD and positively with CIMT, whereas waist-hip ratio also correlated negatively with postprandial FMD. TG and ApoB48 (baseline, peak, and AUC for both) levels correlated significantly with postprandial FMD, whereas TG (baseline, peak, and AUC) also correlated with IMT.

Multiple regression analysis

Multiple regression analysis was performed to further analyze the predictive effect of demographic, biochemical, and genetic variables (independent variables), and IMT and postprandial FMD (dependent variables). Independent variables included were those that showed significant correlation with each of the dependent variables in univariate analysis. Due to marked intercorrelation between gene expression levels, only the gene that correlated most closely with each of the dependent variables was included in

Table 2. Baseline Demographic and Biochemical Data of Divided Cohort for Example Gene, NPC1L1

Variable	Below Median	Above Median	P
Age (years)	49.8 [1.1]	50.7 [1.1]	NS
Gender (% male)	50	50	NS
BMI (kg/m ²)	29.3 [0.9]	29.8 [0.8]	NS
Waist hip ratio	0.9 [0.01]	0.9 [0.02]	NS
Systolic BP (mmHg)	135.7 [2.2]	135.4 [2.2]	NS
HbA1C (% DCCT)	5.5 [0.1]	5.6 [0.1]	NS
FPG (mmol/L)	5.4 [0.2]	5.4 [0.1]	NS
HOMA-IR	1.7 [0.3]	1.5 [0.1]	NS
TC fasting (mmol/L)	5.1 [0.1]	4.9 [0.1]	NS
TC AUC (mmol/L)	40.9 [1.2]	39.3 [1.0]	NS
HDL fasting (mmol/L)	1.3 [0.05]	1.2 [0.04]	NS
HDL AUC (mmol/L)	10.2 [0.4]	9.3 [0.3]	NS
TG fasting (mmol/L)	1.9 [0.3]	1.7 [0.1]	NS
TG AUC (mmol/L)	18.8 [1.9]	20.0 [1.3]	NS
TG iAUC (mmol/L)	3.7 [0.6]	6.2 [0.6]	<0.01
ApoB48 fasting	12.3 [1.4]	14.5 [1.6]	NS
ApoB48 AUC	121.6 [11.1]	157.6 [11.0]	<0.05

Values are shown mean [standard error of the mean].

Abbreviations: BP, blood pressure; DCCT, Diabetes Control and Complications Trial; FPG, fasting plasma glucose; NS, not significant; TC, total cholesterol.

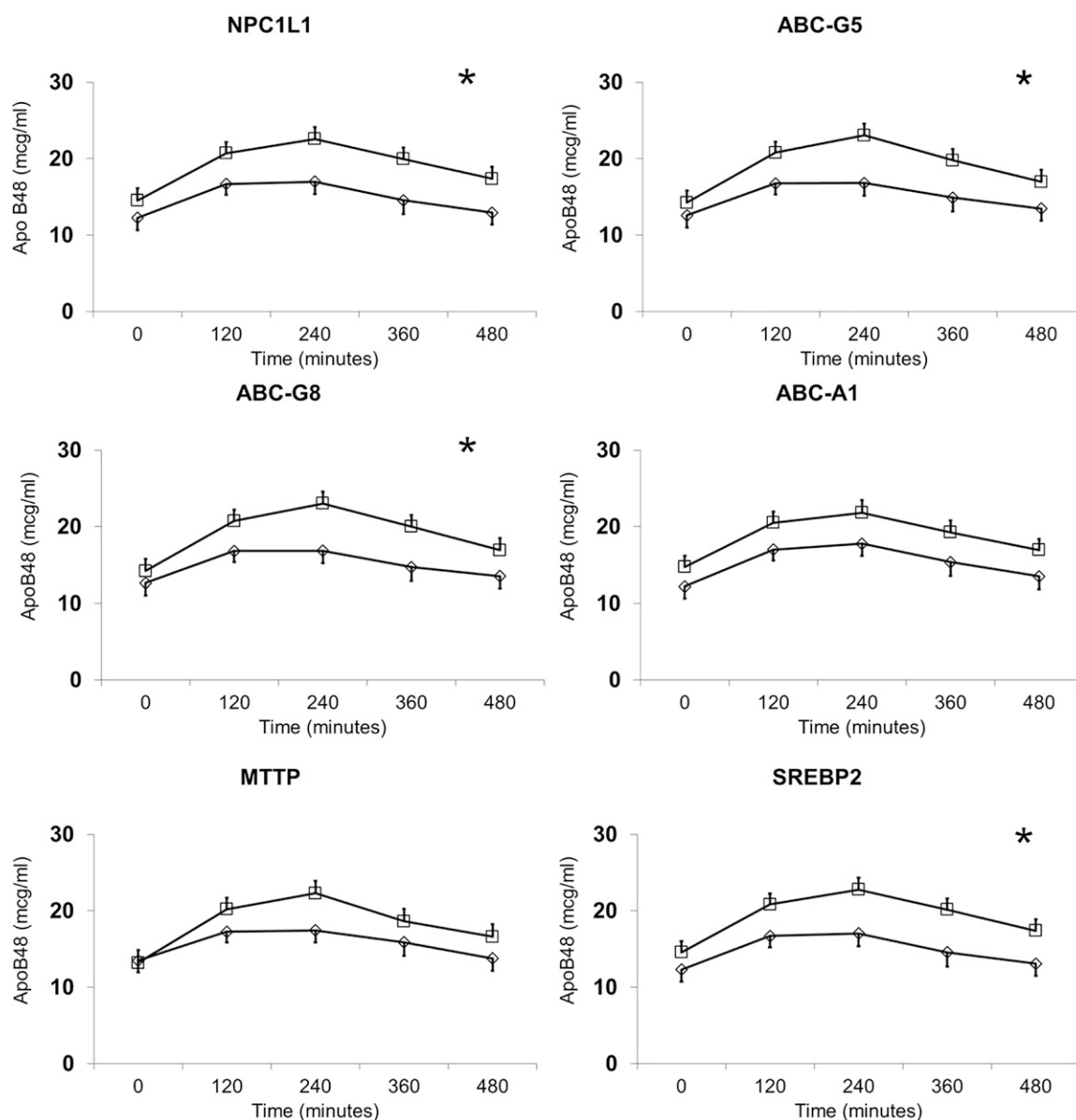


Figure 1. Fasting and postprandial ApoB48 in subjects greater than (open squares) and lower than (open diamonds) the median for expression of intestinal expression of six genes involved in cholesterol transport. * $P < 0.05$ between groups for ApoB48 AUC.

analysis. Similarly, where other independent variables were closely intercorrelated (e.g., glycemic variables), only the independent variable that correlated most closely with each of the dependent variables was included in analysis. Postprandial FMD multivariate analysis (R^2 for model = 0.28, $P = 0.017$) revealed independent significance of the expression of ABC-A1 ($R = -0.361$). The analysis revealed independent significance of age ($R = 0.46$), BMI ($R = 0.30$), and expression of ABC-G5 ($R = 0.32$) for CIMT (R^2 for model = 0.51, $P < 0.001$).

Discussion

This study demonstrates an association between the level of expression of intestinal cholesterol metabolism genes

and markers of early vascular disease in a heterogeneous cohort of human subjects. The association is most consistent across genes for brachial artery FMD but is also clearly demonstrated in carotid IMT, and suggests an increase in both early endothelial dysfunction and more established atherosclerotic change in subjects with higher levels of gene expression. This association is independent of all demographic parameters as well as measures of glycemic status and insulin resistance. Analysis of postprandial lipid levels reveals increased levels of ApoB48 in subjects with higher expression of four genes (NPC1L1, ABC-G5, ABC-G8, and SREBP-2) and a nonsignificant trend in the other two genes, whereas increased postprandial TG levels, measured as the iAUC (AUC above baseline fasting levels), are associated with the higher

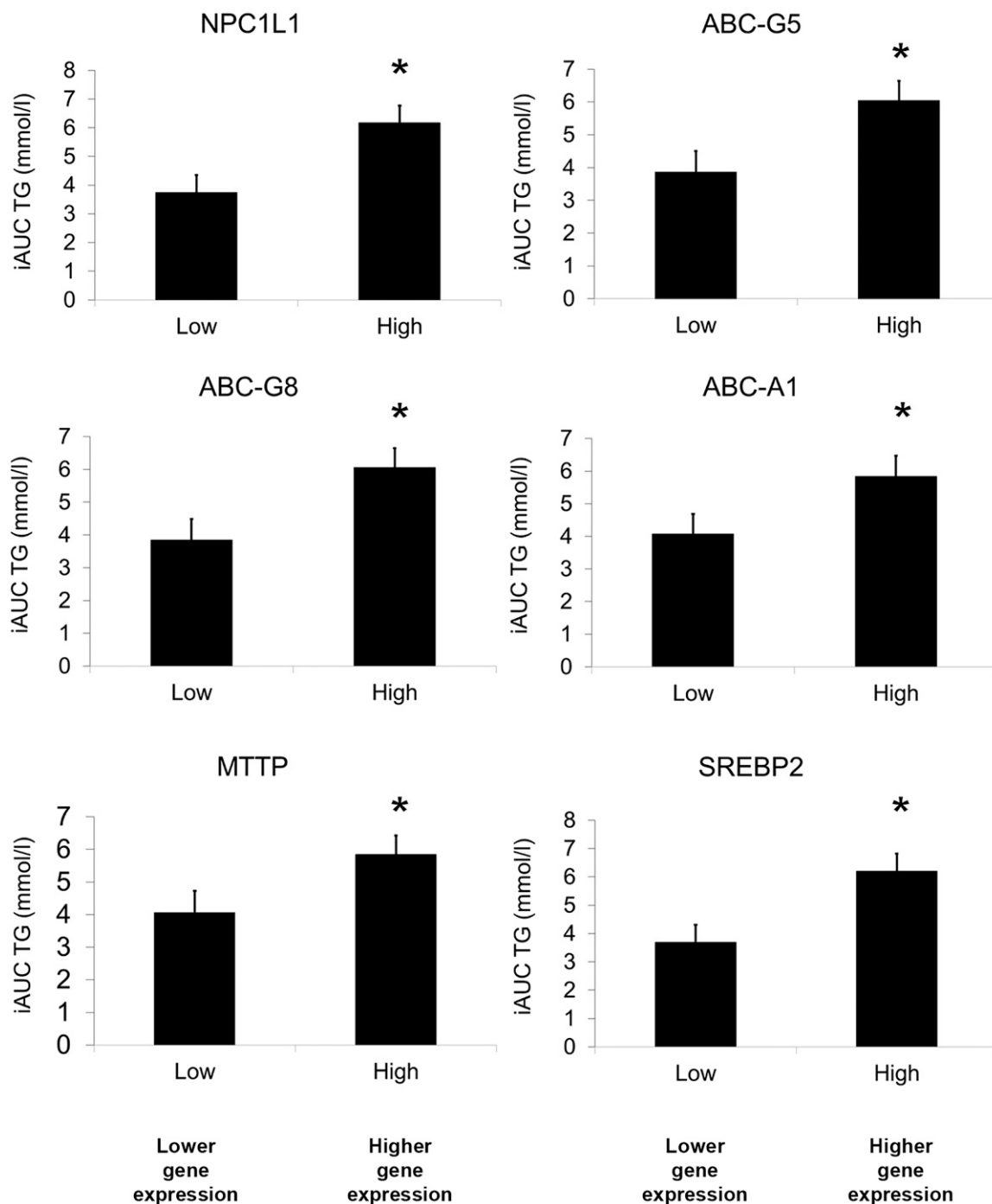


Figure 2. iAUC TG (signifying AUC above the fasting value) in subjects greater than and lower than the median for expression of intestinal expression of six genes involved in cholesterol transport. * $P < 0.05$ between groups.

gene expression cohort for all genes studied. Fasting and total AUC levels of all other lipid fractions showed no association with gene expression.

These results are unique in the literature, in which the majority of study has focused on the description and delineation of the individual metabolic processes related to each gene and gene product. Evidence for the importance of intestinal cholesterol transport and metabolism in postprandial hyperlipidemia is fairly extensive in animal models, with NPC1L1 (19,20), ABC-G5/G8

(21,22), MTTP (23–25), and ABC-A1 (23) all clearly implicated. Direct relationships to more established atherosclerosis in animals are less clearly demonstrated, with conflicting evidence as to the role of the ABC transporter proteins (26–28) in the etiology or development of atherosclerosis depending on the model studied; NPC1L1, through the study of the effectiveness of ezetimibe, has a more convincing preclinical evidence base (29).

Evidence in humans comes initially from rare conditions of genetic under- or overexpression associated with

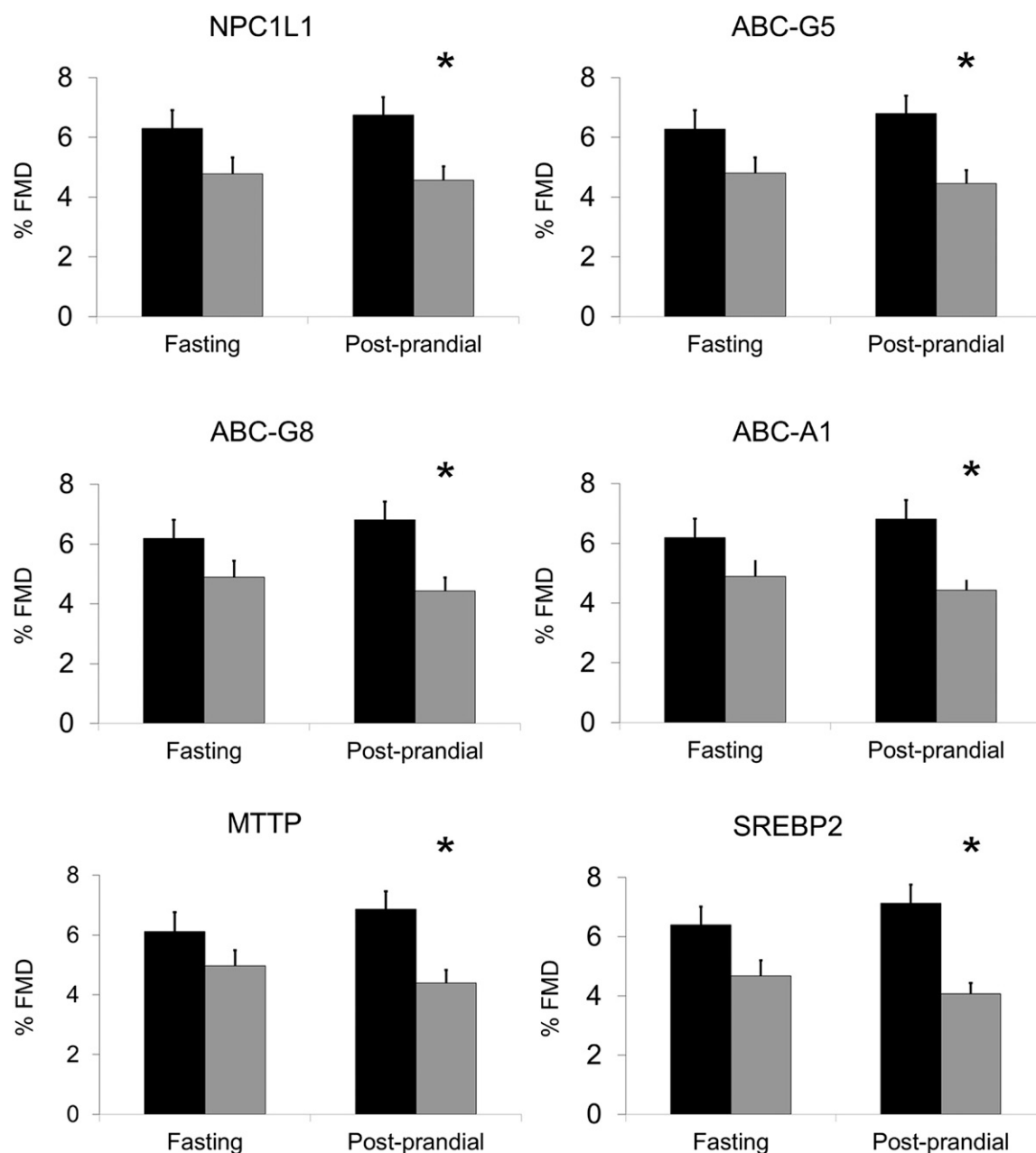


Figure 3. Fasting and postprandial percent FMD (%FMD) in subjects lower than (black columns) and higher than (gray columns) the median for expression of intestinal expression of six genes involved in cholesterol transport. * $P < 0.05$ between groups.

marked changes in plasma lipids and accelerated or enhanced risk of cardiovascular events; sitosterolemia for ABC-G5/G8 (30); abetalipoproteinemia for MTTP (31); and Tangier's disease for ABC-A1 (30). Although these rare monogenic syndromes carry evidence of cardiovascular sequelae, genome-wide studies have failed to show equivocal evidence of linkage between intestinal metabolic gene loci and cardiovascular risk in a more general population (32,33). Furthermore, evidence of a direct link between atherosclerosis and gene expression is lacking or controversial, with much of the literature in this area focused on NPC1L1 and ezetimibe. Although there have been several studies claiming an improvement

in cardiovascular parameters with the use of ezetimibe (34,35), this is offset by further literature showing no significant difference compared with statin therapy either alone or in combination with ezetimibe (36,37). There have been no studies published to date examining the other intestinal cholesterol metabolic proteins and direct cardiovascular markers in the human context.

An interesting, and unexpected, finding from this study is the strong intercorrelation between the genes studied, with clear upregulation of cholesterol metabolic pathways in some subjects compared with others. There is little in the literature regarding the interrelation of the genes studied; several small studies in both humans (38,39) and

Table 3. Correlations Between Relative Expression of Cholesterol Metabolism Genes

Gene	NPC1L1	ABC-G5	ABC-G8	MTTP	ABC-A1	SREBP-2
NPC1L1	—	0.66	0.71	0.78	0.66	0.75
ABC-G5	0.66	—	0.98	0.78	0.72	0.79
ABC-G8	0.71	0.98	—	0.79	0.75	0.8
MTTP	0.78	0.78	0.79	—	0.74	0.93
ABC-A1	0.66	0.72	0.75	0.74	—	0.93
SREBP-2	0.75	0.79	0.8	0.93	0.93	—

R values for Pearson's product moment correlation coefficient.

P < 0.01 for all correlations.

mice (25) have demonstrated a differential expression of NPC1L1, ABC-G5/G8, and MTTP in subjects with diabetes mellitus compared with nondiabetic controls. Our data did not demonstrate differential expression in any demographic or comorbid group, rather illustrating a general up- or downregulation of the entire metabolic pathway, potentially suggesting tight regulation of cholesterol metabolism within the enterocyte to match absorption and synthesis to efflux. The strong intercorrelation seen in this study might be explained by the presence of further regulatory pathways to date unidentified or additional roles to those already known to exist [such as liver X receptor, a nuclear transcription factor responsible for the regulation of ABC-A1 and ABC-G1 expression (40)], which may keep a tight control on the opposing mechanisms of cholesterol flux to ensure cholesterol balance is preserved within the enterocyte.

As mentioned previously, this study demonstrates an association between the level of gene expression and early vascular disease. It is reasonable to postulate that processes intricately involved in the flux of lipids into and out of the body via the enterocyte and intestinal lumen should thus have a link to markers of vascular disease such as FMD and CIMT, as demonstrated in our results, with postprandial hyperlipidemia representing a plausible intermediary mechanism. With the subcellular localization and lipid-processing pathway described previously, each gene and their gene product plays a role in the balance of cholesterol flux across the intestinal border and thus potentially postprandial lipid and lipoprotein concentrations. This would be suggested by the higher levels of ApoB48 (present on a 1:1 ratio with intestinally derived chylomicrons) in the cohort of subjects with higher levels of expression of NPC1L1, ABC-G5, G8, and SREBP-2; total cholesterol, TG, and HDL levels were not associated with gene expression in our data. Additionally, the higher expression cohort of all genes was associated with increased postprandial levels of TG, as measured by the iAUC.

The finding in this study that there is a strong association between cholesterol metabolic gene expression and vascular disease markers demonstrates an important relationship that requires further scrutiny. It is made all the

more striking given the heterogeneous nature of the cohort studied, making it all the more relevant to a general population. The strength of the association in this study, as evidenced by the relative correlation coefficients in the univariate analysis, is comparable to the role of more traditional cardiovascular disease (CVD) risk factors such as blood pressure, plasma lipids, and glycemic status. In particular, for endothelial dysfunction, the strongest correlation was seen with ABC-A1, which was shown to be the only independent predictor following multivariate analysis. The strength of the association seen in this study would suggest a potential benefit to be derived from pursuing therapeutic options targeting the metabolic processes in the intestine as further CVD risk-modifying strategies.

It must be noted that the association documented in this study is only that, an association, and it is difficult to draw strong conclusions from data such as these. Although the cohort studied was heterogeneous and reasonably representative of a general population, there are potential confounding factors within this cohort that may influence the degree of association. As noted in previous studies, the degree to which an association is demonstrated depends on the comorbidities and disease groups studied, and therefore comparison between studies is difficult when the cohorts differ dramatically. In truth, the degree of heterogeneity demonstrated in our demographics makes the strength of the relationship between gene expression and vascular disease more significant, but also means that it is impossible to state categorically that there were no unforeseen confounders influencing the results.

Conclusion

This study has demonstrated a significant association between the expression of genes encoding intestinal cholesterol transport proteins and the degree of early vascular disease markers present in a heterogeneous cohort of subjects. This association appears largely independent of traditional cardiovascular risk factors and is not previously documented in the literature. Strong intercorrelation between genes suggests tight regulation in the expression profiles within the enterocyte to maintain

cholesterol balance and flux, and the combination of results suggests the potential for therapeutic strategies to target these pathways above and beyond what is already known for potential CVD risk reduction. Although the association appears independent of traditional risk factors like fasting hyperlipidemia, blood pressure, and glycemia, future study could focus on elucidating less traditional factors such as postprandial lipids and inflammation. Factors involved in determining and regulating the genetic expression could also be important potential targets for therapeutics, and therefore will be a focus of further research in this area.

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