Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 430–438
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Genes that affect cholesterol synthesis, cholesterol absorption, and chylomicron assembly: The relationship between the liver and intestine in control and streptozotosin diabetic rats

Sean Lally, Daphne Owens, Gerald H. Tomkin*

Department of Diabetes and Endocrinology, Trinity College, Dublin 18, Ireland

Received 17 July 2006; accepted 13 October 2006

Abstract

Chylomicrons and very low-density lipoproteins (VLDLs) are abnormal in diabetes. The aim of this study was to compare the expression of *Niemann-Pick C1-like1 (NPC1L1)*, *adenosine triphosphate–binding cassette (ABC) proteins G5 and G8, microsomal triglyceride transfer protein (MTP)*, and *3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA)* reductase in the fasting and fed states in nondiabetic Sprague-Dawley rats fed a high-fat/cholesterol diet and to examine the messenger *RNA* (mRNA) expression of these proteins in the liver and intestine of diabetic and control animals using streptozotosin diabetic cholesterol-fed rats. Chylomicron and VLDL concentrations were significantly lower after a 12-hour fast in fasted compared with fed rats ($P < .02$). There was no change with fasting in *mRNA* expression of any of the genes in the intestine, but *MTP* level was significantly lower in the liver after the 12-hour fast ($P < .01$). There was a positive correlation between intestinal *NPC1L1 mRNA* and chylomicron cholesterol ($P < .01$) and between hepatic *NPC1L1 mRNA* and VLDL cholesterol ($P < .01$). The diabetic rats had significantly higher chylomicron and VLDL cholesterol, triglyceride, and apolipoprotein B-48 and B-100 levels compared with control rats ($P < .0001$). They had significantly increased *NPC1L1* and *MTP mRNA* in both liver and intestine ($P < .05$ and $P < .0005$, respectively), and *ABCG5* and *ABCG8 mRNA* were significantly reduced ($P < .05$). *HMGCoA reductase mRNA* was increased in diabetic animals ($P < .01$). In conclusion, fasting intestinal gene expression reflects the fed state. In diabetes, intestinal and hepatic gene expression correlates with abnormalities in chylomicron and VLDL cholesterol.

1. Introduction

Abnormal cholesterol metabolism plays a major part in the development of atherosclerosis in diabetes. Cholesterol absorption through the intestinal villi is controlled to some extent by the *Niemann-Pick C1-Like1 (NPC1L1)* protein and inhibition of this protein has been shown to significantly reduce absorption [1,2]. Cholesterol absorbed in the intestine comes from both dietary cholesterol and biliary cholesterol as part of the enterohepatic circulation. Absorption of cholesterol newly synthesized in the intestine for which *3-hydroxy-3-methylglutaryl coenzymeA (HMGCoA)* reductase is the rate-limiting enzyme may also require the *NPC1L1* protein, although this remains to be proven. *NPC1L1* is also found in the liver but its role there has not been clearly defined. Increased cholesterol absorption has been shown in streptozotosin diabetic rats [3], although we were unable to confirm this [4]. Increased cholesterol absorption has also been described in patients with type 1 diabetes mellitus [5]. We have recently described an increase in intestinal *NPC1L1* in human subjects with type 2 diabetes mellitus [6], but there is no information on *NPC1L1* in the liver in diabetes.

Adenosine triphosphate–binding cassette (ABC) proteins G5 and G8 regulate cholesterol homeostasis. They work in tandem to re-excrete some of the absorbed cholesterol and virtually all plant sterols back into the intestine from the intestinal villi. In the liver, they regulate cholesterol excretion into the bile [7,8]. Streptozotosin-induced diabetes in rats has been shown to reduce these intestinal and hepatic cholesterol transporters, and insulin supplementation was found to normalize the changes [9,10]. In human type 2 diabetes mellitus, intestinal *ABCG5* and *ABCG8* messenger *RNA* (mRNA) is reduced [6].

* Corresponding author. Diabetes Research Foundation, Co Dublin, Ireland. Tel.: +353 1 2390658; fax: +353 1 2721395.
E-mail address: gerald.tomkin@tcd.ie (G.H. Tomkin).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.10.028

Microsomal triglyceride transfer protein (MTP) is responsible for the assembly of the chylomicron particle in the intestine, and very low-density lipoprotein (VLDL) in the liver. We have demonstrated an increase in *MTP mRNA* in both liver and intestine of diabetic rabbits [10] and in nondiabetic obese Zucker rats [11]. In streptozotosin diabetic rats, we found the increase only in the intestine [12]. Lewis et al [13] found increased *MTP* expression in isolated enterocytes from fructose-fed hamsters, a model of insulin resistance, and this was associated with oversecretion of apolipoprotein (apo) B-48. In human studies, we have shown an increase in *MTP mRNA* in the intestine of fasting type 2 diabetic patients [6,14].

NPC1L1, *ABCG5/ABCG8*, *HMGCoA reductase*, and *MTP* all have a sterol response element in the promoter region. *NPC1L1 mRNA* was shown to be down-regulated by cholesterol feeding in experiments in mice [15], whereas in hamsters, *NPCIL1 mRNA* was not altered by cholesterol feeding, but *ABCG5* and *ABCG8 mRNA* appear to be sensitive to cholesterol feeding in the hamster [16]. The *MTP* gene promoter is positively regulated by cholesterol [17,18] and *HMGCoA reductase* is down-regulated [19].

The metabolic defect in diabetes is to a large extent a postprandial phenomenon. The mechanism of the disturbance in the lipoprotein cascade from chylomicron through VLDL to LDL in diabetes is poorly understood. The diurnal variation of the above newly described genes has not been examined, and there has been little investigation into the relationship between intestine and liver in the control of cholesterol homeostasis in diabetes. The present study investigates the effect of fasting on the above gene expression in normal rats fed a high-cholesterol diet. The second aim of the study was to explore the relationship between the expression of genes regulating cholesterol synthesis, absorption, and lipoprotein assembly in the liver and intestine using the streptozotosin diabetic rat as a model of diabetes.

1.1. Study protocol

Forty Sprague-Dawley rats were examined in this study and were divided into 4 groups. All animals were fed a high-fat (40% fat), high-cholesterol (0.5%) chow diet (Harlan, Oxford, UK) for 10 days before being killed. We used a high-fat, high-cholesterol diet to stimulate chylomicron production. Twenty percent of the fat was palmitic acid, 25% stearic acid, 29% oleic acid, and 26% linoleic acid, with 0.5% cholesterol.

To investigate the effect of feeding on the intestinal and hepatic expression of *NPC1L1*, *ABCG5/ABCG8*, *HMGCoA reductase*, and *MTP mRNA*, 3 groups of 10 nondiabetic Sprague-Dawley rats were examined after 10 days of ad-lib feeding. Ten of the rats were killed after the feeding period at 8 AM, 10 after a 6-hour fast, and 10 after a 12-hour fast (one of the rats from the 6-hour–fast group died before experimentation). The rats were exsanguinated and chylomicrons and VLDL were isolated from the plasma. The liver was removed and samples were stored in RNAlater. The intestine was washed, the mucosa was scraped from the jejunum, and samples were stored in RNAlater for *NPC1L1*, *ABCG5* and *ABCG8*, *MTP*, and *HMGCoA reductase mRNA* determination.

To examine the effect of diabetes on genes influencing chylomicron and VLDL formation, diabetes was induced in 10 of the rats by subcutaneous injection of streptozotocin (60 mg/kg, in sodium citrate buffer). The rats were allowed to stabilize for 24 hours. Diabetes was confirmed within 30 hours by a blood glucose level greater than 20 mmol/L (2 rats did not survive). Diabetic control was monitored in tail vein blood samples. Food and water intake of each rat was monitored before and after the induction of diabetes, and each animal was weighed on a daily basis. Animals had confirmed diabetes for 10 days before the experiment and were fed the above high-fat diet during this period. Rats were killed at the end of the feeding phase at 8 AM. The rats were exsanguinated and chylomicrons and VLDL were isolated from the plasma. The liver was removed and samples were stored in RNAlater, and the intestine was washed, mucosa was scraped, and samples were stored in RNAlater for *NPC1L1*, *ABCG5* and *ABCG8*, *MTP*, and *HMGCoA reductase mRNA* determination.

1.2. Lipoprotein isolation

After separation of plasma, the following preservatives were added to prevent oxidation and degradation of apo B: phenylalanyl-prolyl-arginyl-chloromethyl ketone (1 mmol/L), phenylmethylsulfonyl fluoride (0.1 mmol/L), sodium azide (0.02% wt/vol), aprotinin (0.05 TIU), and EDTA (0.1%). Chylomicrons and VLDL were isolated from the plasma by sequential ultracentrifugation as previously described [20]. Plasma was overlaid with a solution of density 1.006 g/mL and centrifuged at 20 000 rpm at 4°C for 30 minutes in a Beckman L7-55 ultracentrifuge using a fixed-angle rotor. Chylomicrons were carefully removed from the top of the tube with a stretched Pasteur pipette. The infranate was topped up with 1.006-g/mL solution and was centrifuged at 40 000 rpm at 4°C for 18 hours to isolate VLDL. Lipoprotein fractions were stored at 4°C and lipoproteins and apoproteins were measured within 1 week.

1.3. Biochemical analyses

Capillary blood glucose level was measured with a glucometer (Boehringer Mannheim, Mannheim, Germany). Chylomicron cholesterol and triglyceride were measured by an enzymatic colorimetric method using kits from Boehringer Mannheim.

1.4. Chylomicron and VLDL apo B-48 and B-100 determination

Chylomicron and VLDL apo B-48 and apo B-100 were separated by sodium dodecyl sulfate–polyacrylamide gel

Table 1
Effect of fasting on chylomicron and VLDL composition

	Chylomicron			VLDL		
	Fed	6-h Fast	12-h Fast	Fed	6-h Fast	12-h Fast
n	10	9	10	10	9	10
Triglyceride (mg/mL plasma)	91 ± 30	78 ± 35	53 ± 24	99 ± 43	90 ± 35	62 ± 12
Cholesterol (mg/mL plasma)	45 ± 12	42 ± 8	29 ± 8	58 ± 27	52 ± 27	30 ± 9
Apo B-48 (μg/mL plasma)	19 ± 9	17 ± 6	7 ± 2**	9 ± 5	9 ± 4	4 ± 2*
Apo B-100 (μg/mL plasma)	17 ± 8	19 ± 8	6 ± 3*,†	27 ± 11	24 ± 6	14 ± 4**

Values are mean ± SD.
* $P < .05$, different from fed rats.
** $P < .01$, different from fed rats.
† $P < .05$. different from 6-hour–fasted rats.

electrophoresis using 4% to 15% gradient gels (Biorad, Hercules, CA) as previously described [20]. The bands were quantified by densitometry using Vilber Lourmat equipment (Vilber Lourmat Biotechnology, Marne La Valee, France) and Bio1D v6.32 software (Vilber) for analysis.

1.5. Total RNA extraction

Ten milligrams of liver or intestine that had been stored in RNAlater was homogenized in RLT buffer (Qiagen, Crawley, UK) using a Mixer-Mill 300 (Qiagen) and 5-mm stainless steel beads. The *RNA* from this homogenized sample was then extracted using the RNeasy Mini Isolation Kit (Qiagen). The sample was treated with a ribonuclease-free deoxyribonuclease set to eliminate any contaminating *DNA*. The *RNA* was then eluted into 50 μL ribonuclease-free H_2O, aliquoted, and stored.

1.6. RNA quantification

RNA was quantified by using the QuantiT Ribogreen *RNA* Assay Kit (Molecular Probes, Eugene, OR). An *RNA* standard curve was constructed by using dilutions of a supplied, known amount of RNA and read on a dual-band plate reader with an excitation wavelength of 500 nm and an emission wavelength of 525 nm. Samples were calculated by linear regression from this standard curve.

1.7. Reverse transcription

RNA was reverse transcribed as part of the 2-step real-time reverse transcription-polymerase chain reaction (RT-PCR) to complementary DNA (cDNA) using TaqMan Reverse Transcription Reagents (Applied Biosystems, Dublin, Ireland). A GeneAmp 2400 PCR System (Applied Biosystems) was used and the conditions were as follows: 10 minutes at 25°C, 30 minutes at 48°C, and 5 minutes at 95°C. Eight hundred nanograms of each sample in 100μL was reverse transcribed to cDNA, with 80 ng of the cDNA to be used in the next step.

1.8. Real-time PCR

Ten microliters of the cDNA containing 80 ng of cDNA was used in the real-time PCR analysis. The rat-specific primers and probes for the genes of interest were purchased from Applied Biosystems using the Assay-on-Demand system. Thermal cycling conditions and volumes of ingredients are already optimized using this system. The thermal cycling conditions used were as follows: stage 1 at 50°C for 2 minutes, stage 2 at 95°C for 10 minutes, stage 3 at 40 repetitions of 95°C for 15 seconds alternating with 60°C for 1 minute. A sample volume of 25μL was used in each well. Primers and probes were used from a 20× stock solution and the final concentration was 1×. A 2× master mix solution was added to each well at a final concentration of 1×. Two microliters cDNA containing 80 ng was added separately into each well and an SD on CT value of less than 0.3 was accepted. *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* was used as the housekeeping gene and all reactions were done as single-plex reactions on a 96-well plate. A TaqMan Universal PCR Master Mix was used

Table 2
Effect of fasting on hepatic and intestinal *mRNA* expression (AU)

mRNA	Intestine			Liver		
	Fed	6-h Fast	12-h Fast	Fed	6-h Fast	12-h Fast
n	10	9	10	10	9	10
NPC1L1	51 ± 68	104 ± 64	36 ± 43	15 ± 5	15 ± 5	11 ± 3
ABCG5	84 ± 35	82 ± 41	106 ± 58	72 ± 28	77 ± 22	72 ± 22
ABCG8	4 ± 4	3.5 ± 2	3.4 ± 1.6	66 ± 34	70 ± 14	64 ± 21
HMGCoA reductase	45 ± 27	51 ± 24	46 ± 23	95 ± 26	76 ± 12	80 ± 13
MTP	47 ± 40	53 ± 27	48 ± 27	78 ± 12	61 ± 10	41 ± 9**

Mean + SD.
** $P < .001$ different from fed rats.

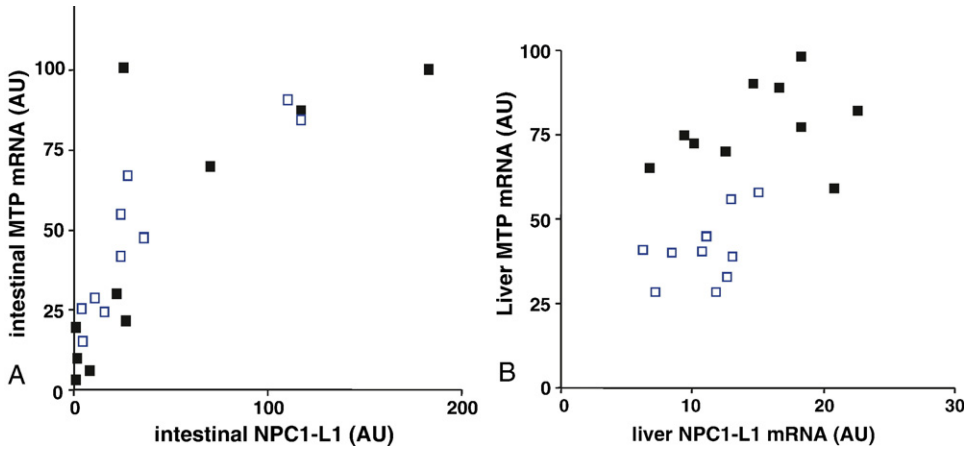

Fig. 1. Correlation in nondiabetic animals (n = 10) between (A) intestinal *MTP* and *NPC1L1 mRNA* in the fed (■, $r = 0.76$, $P < .001$) and fasting (12 hours) (□, $r = 0.84$, $P < .001$) states and (B) between hepatic *MTP* and *NPC1L1 mRNA* in the fed (■, $r = 0.30$, not significant) and fasting (6 hours) (□, $r = 0.66$, $P < .05$) states.

(Applied Biosystems) as the mix for the rest of the PCR ingredients. Analysis was run on an ABI-prism 7000 and the $2^{-\Delta\Delta CT}$ method of relative gene expression was used. Results were expressed as arbitrary units (AU) in comparison to *GAPDH*.

1.9. Statistical analysis

Statistical analysis was performed using 1-way analysis of variance for multiple comparisons with a post hoc Tukey honestly significantly different test to compare groups in the time course study. Student *t* test (unpaired) was used to compare diabetic with control rats. Nonparametric tests were used for triglyceride analysis. Correlation coefficients were measured by linear regression analysis (Microsoft Excel) and figures were made using Cricket Graph. Data are expressed as the mean ± SD or mean ± SEM in graphs. A *P* value of less than .05 was regarded as statistically significant.

2. Results

2.1. Effect of feeding on lipoprotein composition in control rats

There was no difference in the mean weight of the 3 groups of control rats (408 ± 30, 410 ± 17, and 412 ± 36 g). Chylomicron and VLDL composition after feeding and after 6- and 12-hour fasts is shown in Table 1. There was very little difference between fed and 6-hour–fasting lipoprotein values but there was a significant reduction in chylomicron and VLDL triglyceride, cholesterol, apo B-48, and apo B-100 ($P < .02$) after the 12-hour fast.

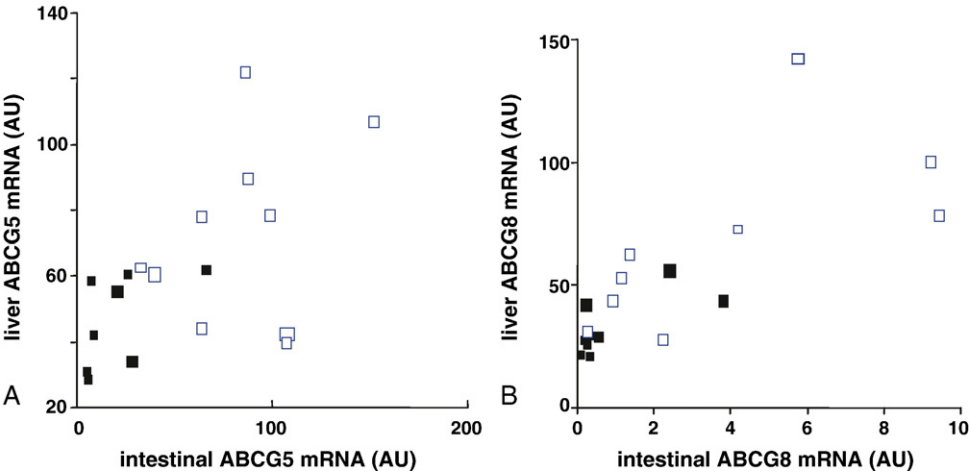

Fig. 2. A, Correlation between *ABCG5 mRNA* in the intestine and liver in the combined group (n = 18) of diabetic (■) and control (□) animals (n = 10, $r = 0.54$, $P < .05$) (no correlation when the diabetic and control animals were analyzed separately). B, Correlation between intestinal and liver *ABCG8 mRNA* in the combined group (n = 18) ($r = 0.68$, $P < .001$) and in the diabetic (■, n = 8) ($r = 0.63$, $P < .05$) and control (□, n = 10, $r = 0.64$, $P < .05$) animals analyzed separately.

Table 3
Effect of diabetes on lipoprotein composition

	Chylomicron		VLDL	
	Control	Diabetic	Control	Diabetic
n	10	8	10	8
Triglyceride (mg/mL plasma)	91 ± 30	496 ± 206***	99 ± 43	835 ± 242***
Cholesterol (mg/mL plasma)	45 ± 12	357 ± 141***	58 ± 27	398 ± 139***
Apo B-48 (μg/mL plasma)	19 ± 9	208 ± 74***	9 ± 5	108 ± 39***
Apo B-100 (μg/mL plasma)	17 ± 8	158 ± 43***	27 ± 11	376 ± 132***

*** $P < 0001$, different from control rats.

Table 2 shows the intestinal and liver *mRNA* at the various time points. There was no significant difference between the fed state and 12-hour fasting for intestinal *NCP1-L1*, *ABCG5/ABCG8*, *HMGCoA reductase*, or *MTP mRNA*. There was no change in liver *NPC1L1*, *HMGCoA reductase*, or *ABCG5/ABCG8 mRNA* over the 12-hour period. Hepatic *MTP mRNA* expression fell significantly in stepwise fashion after 6 and 12 hours and was significantly reduced at 12 hours ($P < .01$).

2.2. Relationships between ABCG5, ABCG8, NPC1L1, and MTP in the fed and fasted state

As expected there was a strong positive correlation between intestinal *ABCG5* and *ABCG8 mRNA* ($r = 0.75$, $P < .001$) and between *ABCG5* and *ABCG8 mRNA* in the liver ($r = 0.92$, $P < .0001$).

There was a strong positive correlation between intestinal *NPC1L1* and *MTP mRNA* in both the fed ($r = 0.76$, $P < .01$) and fasting ($r = 0.84$, $P < .001$) states and between liver *NPC1L1* and *MTP mRNA* in the fasting state (6 hours) ($r = 0.66$, $P < .05$) but no correlation in the fed animals (Fig. 1).

2.3. Relationship between intestinal and hepatic mRNA expression of ABCG5/ABCG8, HMGCoA reductase, MTP, and NPC1L1

In the fed state, when the diabetic and control rats were combined, there was a significant positive correlation between intestinal and hepatic *ABCG5 mRNA* ($r = 0.54$, $P < .05$) but no correlation in the control and diabetic animals when analyzed separately (Fig. 2). There was also a significant positive correlation between intestinal and hepatic *ABCG8 mRNA* in the combined group ($r = 0.68$, $P < .001$) and in the diabetic ($r = 0.63$, $P < .05$) and control animals ($r = 0.64$, $P < .05$) analyzed separately (Fig. 2).

In control animals, there was no correlation in the fasting state between intestinal and hepatic *ABCG5* or *ABCG8* and there was no correlation between intestinal and hepatic *NPC1L1*, *HMGCoA reductase*, or *MTP mRNA* in either the fed or fasting states.

Table 4
Effect of diabetes on intestinal and hepatic *mRNA* expression (AU) (fed)

mRNA	Intestine		Liver	
	Control	Diabetic	Control	Diabetic
n	10	8	10	8
NPC1L1	51 ± 68	231 ± 181*	15 ± 5	24 ± 9*
ABCG5	84 ± 35	20 ± 19***	72 ± 28	47 ± 14*
ABCG8	4 ± 4	0.9 ± 1.4*	66 ± 34	33 ± 12*
HMGCoA reductase	45 ± 27	67 ± 23	95 ± 26	164 ± 71**
MTP	47 ± 40	266 ± 156***	78 ± 12	320 ± 79***

Values are mean ± SD.
* $P < .05$, different from control rats.
** $P < .01$, different from control rats.
*** $P < .0005$, different from control rats.

2.4. Effect of diabetes on chylomicron and VLDL composition and on intestinal and hepatic mRNA expression

The diabetic rats were examined in the fed state and compared with fed control animals. Diabetic rats had a mean blood glucose of 32 ± 4mmol/L compared with 5.3 ± 1.2 mmol/L for control rats and they were significantly lighter than control animals (260 ± 41 vs 428 ± 30g) when killed. Triglyceride, cholesterol, apo B-48, and apo B-100 levels in both the chylomicron and VLDL fractions were very significantly higher in the diabetic rats ($P < .0001$) (Table 3).

Table 4 compares *mRNA* expression in the liver and intestine of the diabetic and control rats. *NPC1L1 mRNA* was increased significantly in both intestine and liver in the diabetic animals ($P < .05$), whereas *ABCG5* and *ABCG8 mRNA* were reduced in both intestine ($P < .005$ and $P < .05$, respectively) and liver ($P < .05$). Intestinal and hepatic *HMGCoA reductase mRNA* were increased in the diabetic rats compared with controls, but the increase was significant only in the liver ($P < .01$). *MTP* expression was significantly increased in both the intestine and the liver ($P < .0005$).

Table 5
Correlations between chylomicron cholesterol and intestinal and hepatic *mRNA* (AU) in the fed state

	Whole group		Diabetic		Control	
	Chylomicron cholesterol (r)	$P <$	Chylomicron cholesterol (r)	$P <$	Chylomicron cholesterol (r)	$P <$
Intestine						
NPC1L1	0.76	.001	0.63	.05	0.80	.01
ABCG5	−0.74	.001	−0.62	.05	−0.50	NS
ABCG8	−0.66	.01	−0.42	NS	0.41	NS
HMGCoA reductase	0.47	.05	0.07	NS	0.55	NS
MTP	0.77	.001	0.68	.05	0.88	.001
Liver						
NPC1L1	0.55	.05	0.24	NS	0.29	NS
ABCG5	−0.51	.05	0.45	NS	0.16	NS
ABCG8	−0.56	.05	0.33	NS	0.19	NS
HMGCoA reductase	0.47	NS	0.01	NS	0.18	NS
MTP	0.88	.001	0.55	NS	0.15	NS

NS indicates not significant.

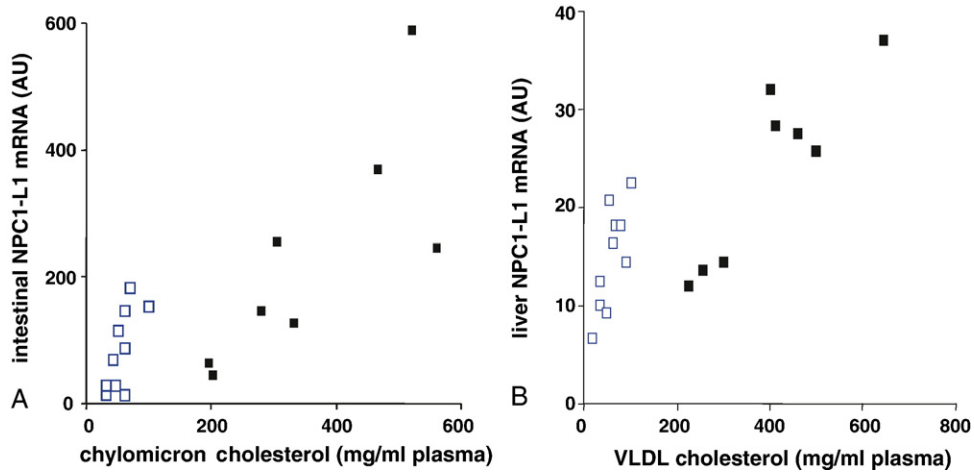

Fig. 3. Relationship between (A) chylomicron cholesterol and intestinal *NPC1L1 mRNA* in diabetic (■, n = 8, $r = 0.63$, $P < .05$) and control (□, n = 10, $r = 0.80$, $P < .01$) animals and in the combined group (n = 18, $r = 0.76$, $P < .001$) and between (B) VLDL cholesterol and liver *NPC1L1 mRNA* in diabetic (■, $r = 0.90$, $P < .001$) and control (□, $r = 0.79$, $P < .01$) animals and in the combined group ($r = 0.81$, $P < .001$).

2.5. Correlation between lipoprotein cholesterol and intestinal and hepatic mRNA expression in diabetic and control rats

There was a strong positive correlation in the combined diabetic and control rats between intestinal *NPC1L1* and chylomicron cholesterol ($P < .01$) (Table 5). These correlations remained when the diabetic and control groups were analyzed separately ($P < .05$ and $P < .01$, respectively) (Fig. 3). Intestinal *ABCG5* and *ABCG8* correlated negatively with chylomicron cholesterol in the combined group ($P < .01$); in the diabetic rats, chylomicron cholesterol correlated with *G5* ($P < .05$). The relationship with *G5* or *G8* was not significant in the control animals. There was a positive correlation between intestinal *HMGCoA reductase* and chylomicron cholesterol when both groups were analyzed together ($P < .001$), but this significance was lost when the groups were analyzed separately. Intestinal *MTP* in the combined group strongly correlated with chylomicron cholesterol ($P < .001$); this significance remained when the diabetic and control groups were analyzed separately ($P < .05$ and $P < .001$, respectively).

There was a strong positive correlation between liver *NPC1L1* and VLDL cholesterol ($P < .001$) (Table 6) that was also present in the diabetic ($P < .001$) and control ($P < .01$) groups (Fig. 3). There was a negative correlation between both intestinal and hepatic *ABCG5* and *ABCG8* and VLDL cholesterol in the combined group ($P < .01$). In the diabetic rats, the correlation remained in the intestine and in the liver for *ABCG5* ($P < .05$), but there was no correlation in the control group (Table 6). There was no correlation between hepatic *HMGCoA reductase* and VLDL cholesterol. There was a significant correlation in the combined groups between hepatic *MTP* and VLDL cholesterol ($P < .001$), which remained when the diabetic and control groups were analyzed separately ($P < .01$ and $P < .05$, respectively) (Fig. 4).

3. Discussion

The atherogenicity of postprandial lipoproteins is well recognized and may be of particular importance in diabetes. Interrelationship between *mRNA* expression of genes affecting cholesterol metabolism in the liver compared to the intestine and the effect of fasting on gene expression is poorly described in the literature. Studies examining human intestinal biopsies are usually done in the fasting state, but genes regulating the postprandial phase may be up-regulated by food.

Table 6
Correlations between VLDL cholesterol and intestinal and hepatic *mRNA* (AU)

	Whole group		Diabetic		Control	
	VLDL cholesterol (r)	$P <$	VLDL cholesterol (r)	$P <$	VLDL cholesterol (r)	$P <$
Intestine						
NPC1L1	0.51	.05	0.01	NS	0.47	NS
ABCG5	−0.83	.001	−0.65	.05	−0.13	NS
ABCG8	−0.78	.001	−0.72	.05	−0.77	.01
HMGCoA reductase	0.30	NS	−0.32	NS	0.22	NS
MTP	0.6	.01	0.14	NS	0.34	NS
Liver						
NPC1L1	0.81	.001	0.90	.001	0.79	.01
ABCG5	−0.70	.001	−0.64	.05	−0.64	.05
ABCG8	−0.70	.001	−0.36	NS	−0.67	.05
HMGCoA reductase	−0.34	NS	−0.44	NS	−0.56	NS
MTP	0.77	.001	0.76	.01	0.66	.05

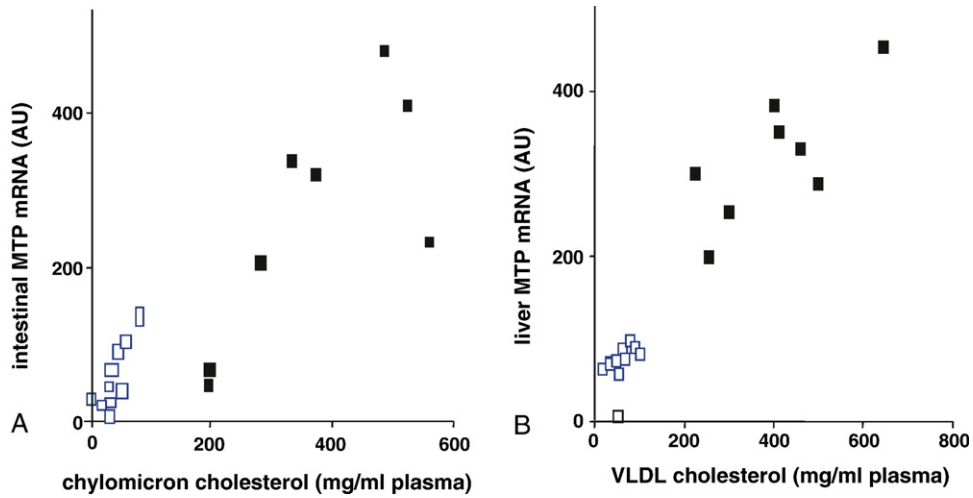

Fig. 4. Relationship between (A) chylomicron cholesterol and intestinal *MTP mRNA* in diabetic (■, n = 8, $r = 0.68$, $P < .05$) and control (□, n = 10 $r = 0.88$, $P < .001$) rats and in the combined group (n = 18, $r = 0.77$, $P < .001$) and between (B) VLDL cholesterol and liver *MTP mRNA* in diabetic (■, $r = 0.76$, $P < .01$) and control (□, $r = 0.66$, $P < .05$) animals and in the combined group ($r = 0.77$, $P < .001$).

We examined the effect of fasting for 6 and 12 hours in nondiabetic cholesterol-fed rats and demonstrated a significant decrease in both chylomicron and VLDL protein and lipid after 12-hour fast. There was no change in intestinal mRNA levels of *HMGCoA reductase*, *MTP*, *ABCG5* and *ABCG8*, or *NPC1L1* during the 12-hour fast. This was a short-term experiment, but Field et al [16] reported experiments in the hamster and showed, over a 14-day period, that adding cholesterol to the chow diet did not alter *NPC1L1*, whereas *ABCG5* and *ABCG8* were increased. In mice, it has been shown that cholesterol feeding together with cholate for 7 days resulted in a reduction in intestinal *NPC1L1* with no significant change in *ABCG5* and *ABCG8* [15]. Thus, the regulation of *NPC1L1* and the *ABCG5* and *ABCG8* may vary depending on the animal model used. We measured the *mRNA* for *HMGCoA reductase*, the rate-limiting enzyme for cholesterol synthesis and found no change after a 12-hour fast, although *HMGCoA reductase* is known to be up-regulated by cholesterol depletion. MTP regulates chylomicron assembly, but intestinal *mRNA* levels were unchanged by the fast in our study. Cholesterol is known to regulate *MTP* expression through its sterol-binding domain [17,18]; however, because dietary cholesterol is trapped by the enterocyte and biliary cholesterol is continually available to the intestine for reabsorption together with de novo synthesized cholesterol, it was perhaps to be expected that *HMGCoA reductase* and *MTP mRNA* would not change in this short period of fasting. The present study demonstrates that in this animal model, the 12-hour fasting state reflects the fed state in the intestine.

This study gave us the opportunity to examine *mRNA* changes in the liver during a 12-hour fast. The results were similar to those found in the intestine except that there was a stepwise reduction in *MTP* in the liver. This was accompanied by a reduction in VLDL. The implications of this finding are unclear because the change in the chylomicrons over the 12 hours was very similar to that found in the VLDL. It is possible that a longer fast would have been necessary to see a reduction in MTP in the intestine particularly because dietary cholesterol may be trapped in the intestinal villi for some time. Previous studies have suggested that both cholesterol and triglyceride drive MTP [14,18,21], although we found that cholesterol appeared to have the stronger effect [14]. It could be that the diminishing delivery of chylomicron cholesterol to the liver resulted in the down-regulation of hepatic *MTP mRNA*. MTP inhibition leads to steatosis both in the intestine and in the liver [22]. Steatosis also occurs frequently in obesity, suggesting that there are limits to MTP up-regulation.

We have previously shown, in animal models, a relationship between *HMGCoA reductase* in the intestine and liver [19]. In the present study, there was no correlation in the fasting state between intestinal and hepatic *mRNA* levels of any of the genes measured. In the postprandial phase only *ABCG5* and *ABCG8 mRNA* in the intestine correlated significantly with levels in the liver ($P < .05$) (Fig. 2). The study suggests that, in the rat, the *mRNA* of *NPC1L1*, and *MTP* are independently regulated in the intestine and liver. Chylomicron cholesterol closely correlated with the intestinal *NPC1L1 mRNA* levels as expected because *NPC1L1* regulates cholesterol absorption (Fig. 3). *NPC1L1* is probably involved in the absorption of not only dietary cholesterol, but also biliary cholesterol and endogenously synthesized cholesterol [2,23]. In the present study, there was a positive correlation between hepatic *NPC1L1* and VLDL cholesterol, suggesting an important role for hepatic *NPC1L1* in the delivery of cholesterol to the VLDL particle. An *NPC1L1* knockout mouse has been described. The animals displayed an increase in *HMGCoA reductase*, but despite this increase, VLDL cholesterol was significantly reduced, confirming an important role for *NPC1L1* in the

delivery of hepatic cholesterol into the circulation [15]. We did not find any correlation between *NPC1L1* and *HMGCoA reductase* in either the liver or intestine, but it would be interesting to examine the relationship in animals on a chow diet without added cholesterol.

Postprandial dyslipidemia is a feature of diabetes, and in this study, diabetes resulted in significant increase in the number of chylomicron particles. The increased *MTP mRNA* in the intestine in the diabetic animals suggests that an increase in particle synthesis is a major cause for this dyslipidemia. In the diabetic rabbit model we have also shown an increase in particle number [10], whereas in the streptozotosin diabetic rats we have demonstrated an increase in chylomicron lipid and cholesterol without an increase in particle number [12]. An increase in chylomicron particle production has previously been demonstrated in the insulin-resistant fructose-fed hamster [13]. The present study suggests that an increase in cholesterol absorption through increased NCPC1L1 and decreased excretion by reduced ABCG5 and ABCG8 both play a part in delivery of excess cholesterol for chylomicron assembly by MTP in diabetes.

The considerable up-regulation of *NPC1L1* gene expression in the liver of the diabetic rat and the close correlation between *mRNA* level and VLDL cholesterol suggest an important role of the gene in the dysregulation of VLDL metabolism in diabetes. We have shown up-regulation of human intestinal *NPC1L1* in diabetes [6], and it would be interesting to know if human diabetes is also associated with up-regulation of *NPC1L1* in the liver. This may be of particular importance because an inhibitor of NPC1L1 is now available [2,24].

Recently it has been suggested that because of differential localization of ABCG5 and ABCG8 they may have independent functions, as well as functioning as a heterodimer [25]. Cholesterol absorption has been difficult to measure, but it has been suggested that it is not altered in type 2 diabetes mellitus [26], whereas it is increased in type 1 diabetes mellitus [5] Streptozotocin diabetic rats are a model of type 1 diabetes mellitus and it is of interest that our findings suggest a net increase in cholesterol absorption both through an up-regulation of *NPC1L1* and through a down-regulation of *ABCG5* and *ABCG8* in the diabetic animals in both liver and intestine. Bloks et al [27] also found reduced levels of *ABCG5* and *ABCG8* in streptozotozin diabetic rats and they calculated that cholesterol absorption was increased in their rats. The correlations between intestinal and hepatic *ABCG5* and *ABCG8* have been explored in nondiabetic rats, and cholesterol feeding appears to down-regulate *ABCG5* and *ABCG8* in the liver and increase levels in the ileum [28]. In our rat model of diabetes, both liver and intestine seem to be sensitive to the effects of diabetes. The differences in correlation of *ABCG5* and *ABCG8* with chylomicron and VLDL cholesterol support the concept that the 2 proteins may have separate functions as well as acting as heterodimers in the role of cholesterol excretion.

In this study, we did not measure either protein or activity. In previous studies, we have shown a correlation between *MTP mRNA* and activity [11]. *HMGCoA reductase mRNA* has also been shown to reflect activity [29]. To our knowledge, there is no information on the relationship between ABCG5/ABCG8 activity and *mRNA* and we were unable to discover whether *NPC1L1 mRNA* reflects activity. The significant correlations with lipoprotein cholesterol do suggest that *NPC1L1* gene expression does reflect activity. *ABCG5/ABCG8* correlations are less supportive because they were only present in the combined diabetic and control group.

In conclusion, *MTP*, *NPC1L1*, and *ABCG5* and *ABCG8* expression do not change in the intestine at the end of a 12-hour fast, suggesting that it is legitimate to measure gene expression in either the fasting or fed state. Hepatic *MTP mRNA* expression decline significantly at the end of the fast ($P < .05$). In the streptozotocin diabetic rat model, *NPC1L1* and *MTP mRNA* are increased in both liver and intestine and *ABCG5/ABCG8 mRNA* are decreased ($P < .05$). These results were associated with the abnormalities in chylomicron and VLDL composition, which are typically found in diabetes. The correlations we have found in this study between NPC1L1, ABCG5/ABCG8 and MTP with chylomicron and VLDL cholesterol confirm the known function of these genes in regulating cholesterol composition of the chylomicrons and VLDL.

Acknowledgment

The authors thank the Meath Foundation, the Diabetes Federation of Ireland, and the Diabetes Research Foundation for their support of this project.

References

[1] Altmann SW, Davis Jr HR, Zhu LJ, et al. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science 2004;303:1201-4.

[2] van Heek M, Farley C, Compton DS, Hoos LM, Smith-Torhan A, Davis HR. Ezetimibe potently inhibits cholesterol absorption but does not affect acute hepatic or intestinal cholesterol synthesis in rats. Br J Pharmacol 2003;138:1459-64.

[3] Young NL, Lopez DR, McNamara DJ. Contributions of absorbed dietary cholesterol and cholesterol synthesized in small intestine to hypercholesterolemia in diabetic rats. Diabetes 1988;37:1151-6.

[4] Gleeson A, Owens D, Collins P, Johnson A, Tomkin GH. The relationship between cholesterol absorption and intestinal cholesterol synthesis in the diabetic rat model. Int J Exp Diabetes Res 2000;1:203-10.

[5] Gylling H, Tuominen JA, Koivisto VA, Miettinen TA. Cholesterol metabolism in type 1 diabetes. Diabetes 2004;53:2217-22.

[6] Lally S, Tan CY, Owens D, Tomkin GH. mRNA levels of genes involved in dysregulation of postprandial lipoproteins in type 2 diabetes: the role of Niemann Pick C1-like 1, ABCG5 and G8 and microsomal triglyceride transfer protein. Diabetologia 2006;49:1008-16.

[7] Berg KE, Tian H, Graf GA, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000;290:1771-5.

[8] Yu L, Gupta S, Xu F, Liverman AD, Moschetta A, Mangelsdorf DJ, et al. Expression of ABCG5 and ABCG8 is required for regulation of biliary cholesterol secretion. J Biol Chem 2005;280:8742-7.
[9] van Waarde WM, Henkjan J, Verkade H, et al. Differential effects of streptozotocin-induced diabetes on expression of hepatic ABC-transporters in rats. Gastroenterology 2002;122:1842-52.
[10] Phillips C, Anderton K, Bennett A, et al. Intestinal rather than hepatic microsomal triglyceride transfer protein as a cause of postprandial dyslipidaemia in diabetes. Metabolism 2002;51:847-52.
[11] Phillips C, Owens D, Collins P, Tomkin GH. Microsomal triglyceride transfer protein: does insulin resistance play a role in the regulation of chylomicron assembly? Atherosclerosis 2002;160:355-60.
[12] Gleeson A, Anderton K, Owens D, et al. The role of microsomal triglyceride transfer protein and dietary cholesterol in chylomicron production in diabetes. Diabetologia 1999;42:944-9.
[13] Lewis GF, Uffelman K, Naples M, et al. Intestinal lipoprotein overproduction, a newly recognized component of insulin resistance, is ameliorated by the insulin sensitizer rosiglitazone: studies in the fructose-fed Syrian Golden hamster. Endocrinology 2005;146:247-55.
[14] Phillips C, Mullan K, Owens D, Tomkin GH. Intestinal microsomal triglyceride transfer protein in type 2 diabetic and non-diabetic subjects: the relationship to triglyceride-rich postprandial lipoprotein composition. Atherosclerosis 2006;187:57-64.
[15] Davis Jr HR, Zhu LJ, Hoos LM, et al. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J Biol Chem 2004;279:33586-92.
[16] Field FJ, Born E, Mathur SN. Stanol esters decrease plasma cholesterol independently of intestinal ABC sterol transporters and Niemann-Pick C1-like 1 protein gene expression. J Lipid Res 2004;45:2252-9.
[17] Bennett AJ, Bruce JS, Salter AM, White DA, Billett MA. Hepatic microsomal triglyceride transfer protein messenger RNA concentrations are increased by dietary cholesterol in hamsters. FEBS Lett 1996;394:247-50.
[18] Linn MC, Gordon D, Wetterau JR. Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. J Lipid Res 1995;36:1073-81.
[19] O'Meara N, Devery R, Owens D, Collins P, Johnson A, Tomkin GH. Serum lipoproteins and cholesterol metabolism in two hypercholesterolaemic rabbit models. Diabetologia 1991;34:139-43.
[20] Phillips C, Murugasu G, Owens D, Collins P, Johnson A, Tomkin GH. Improved metabolic control reduces the number of postprandial apolipoprotein B48–containing particles in type 2 diabetes. Atherosclerosis 2000;148:283-91.
[21] Qiu W, Taghibiglou C, Avramoglu RK, Van Iderstine SC, Naples M, Ashrafpour H, et al. Oleate-mediated stimulation of microsomal triglyceride transfer protein (MTP) gene promoter: implications for hepatic MTP overexpression in insulin resistance. Biochemistry 2005;44:3041-9.
[22] Chandler CE, Wilder DE, Pettini JL, Savoy YE, Petras SF, Chang G, et al. CP-346086: an MTP inhibitor that lowers plasma cholesterol and triglycerides in experimental animals and in humans. J Lipid Res 2003;44:1887-901.
[23] Yu L, Bharadwaj S, Brown JM, Ma Y, Du W, Davis MA, et al. Cholesterol-regulated translocation of Niemann-pick C1-like 1 to the cell surface facilitates free cholesterol uptake. J Biol Chem 2006;281:6616-24.
[24] Garcia-Calvo M, Lisnock J, Bull HG, et al. The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). Proc Natl Acad Sci U S A 2005;102:8132-7.
[25] Klett EL, Lee MH, Adams DB, Chavin KD, Patel SB. Localization of ABCG5 and ABCG8 proteins in human liver, gall bladder and intestine. BMC Gastroenterol 2004;4:21.
[26] Gylling H, Miettinen TA. Cholesterol absorption, synthesis and LDL metabolism in NIDDM. Diabetes Care 1997;20:90-5.
[27] Bloks VW, Bakker-Van Waarde WM, Verkade HJ, et al. Down-regulation of hepatic and intestinal Abcg5 and ABCG8 expression associated with altered sterol fluxes in rats with streptozotocin-induced diabetes. Diabetologia 2004;47:104-12.
[28] Dieter MZ, Maher JM, Cheng X, Klaassen CD. Expression and regulation of the sterol half-transporter genes ABCG5 and ABCG8 in rats. Comp Biochem Physiol C Toxicol Pharmacol 2004;139:209-18.
[29] Feingold KR, Wilson DE, Wood LC, Kwong LK, Moser AH, Grunfeld C. Diabetes increases hepatic hydroxymethyl glutaryl coenzyme A reductase protein and mRNA levels in the small intestine. Metabolism 1994;43:450-4.