Intestinal Rather Than Hepatic Microsomal Triglyceride Transfer Protein as a Cause of Postprandial Dyslipidemia in Diabetes

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Postprandial dyslipidemia may be a major cause of atherosclerosis in diabetes. Microsomal triglyceride transfer protein (MTP) is essential for the synthesis of the chylomicron particle in the intestine and very–low-density lipoprotein (VLDL) in the liver. The purpose of the present study was to examine the effect of diabetes on MTP mRNA expression in a rabbit model of diabetes, which develops atherosclerosis. Male New Zealand white rabbits were fed a 0.5% cholesterol diet. Diabetes was induced with alloxan monohydrate. The lymphatic duct was cannulated and lymph collected for isolation of chylomicrons by ultracentrifugation. Apolipoprotein B48 (apo B48) and apo B100 were separated by polyacrylamide gradient gel electrophoresis and quantified by densitometry. MTP mRNA was determined in liver and intestine by RNase protection analysis, and MTP activity was measured. Diabetic animals had significantly increased plasma triglyceride and decreased high-density lipoprotein (HDL) cholesterol ($P < .05$). They also secreted more lymph chylomicron apo B48 and apo B100 ($P < .05$) and more lymph chylomicron total and esterified cholesterol/h ($P < .05$). Lymph chylomicron particles in the diabetic animals contained significantly less lipid/apo B ($P < .05$). Intestinal MTP activity and mRNA were significantly higher in diabetic compared with control rabbits (0.07 ± 0.01 *v* 0.04 ± 0.015 fluorescent units/μg microsomal protein and 66 ± 21 *v* 37 ± 11 amol MTP mRNA/μg total RNA ($P < .005$). There was no difference in MTP activity or mRNA expression in the liver. This study suggests that MTP may play an important role in the postprandial dyslipidemia of diabetes.

DIABETES IS ASSOCIATED with a marked increase in atherosclerosis. Postprandial dyslipidemia is a feature of diabetes. Many lipoprotein abnormalities have been demonstrated in the fasting state, but recently attention has shifted to postprandial lipoprotein analysis, because the greatest metabolic disturbances in diabetes occurs postprandially. Interest in the postprandial state was stimulated by Zilversmit[1] when he suggested that chylomicron remnants might be particularly atherogenic. This suggestion was strengthened by the finding of a significant relationship between apolipoprotein B48 (apo B48), the intestinally-derived apolipoprotein, and progression of coronary artery atherosclerosis in nondiabetic subjects[2] and more recently in diabetic patients.[3] We have demonstrated an increase in intestinally-derived apo B48 in diabetic patients[4-6] and have shown that improved glycemic control reduced this level.[7] Human studies have shown cholesterol synthesis to be increased in diabetic patients.[8] In animals, diabetes has been shown to increase intestinal 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, the rate-limiting enzyme for cholesterol synthesis, activity, and mRNA expression[9-15] and to increase de novo cholesterol synthesis in the intestine.[14] Diabetes has also been shown to increase the rate of transport of endogenous, newly synthesized cholesterol from the intestine into the circulation.[16,17] There is dispute as to whether cholesterol absorption is increased, similar, or decreased in diabetes.[8,12,18] Intestinal cholesterol synthesis has been shown to be inhibited by cholesterol feeding in diabetic rats[19] and in nondiabetic rabbits.[20] Microsomal triglyceride transfer protein (MTP) is another protein, which is crucial to the synthesis of the chylomicron particle in the intestine and very–low-density lipoprotein (VLDL) in the liver.[21] MTP acts by transferring triglyceride to the nascent apo B polypeptide and facilitating the assembly of the triglyceride-rich lipoprotein particle.[22] MTP may also stimulate apo B translocation across the membrane since inactivation of MTP resulted in delayed removal of apo B48 and apo B100 from microsomal and golgi membranes of primary rat hepatocytes.[23] Insulin negatively regulates MTP gene expression in HepG2 cells,[24] and hepatic MTP has been described as being either increased or normal in diabetes.[25,26] Cholesterol has been shown to upregulate MTP expression via the sterol response element in the MTP gene promoter region,[27] and dietary fat also increases hepatic MTP expression.[28] The role of MTP in the intestine has been, to a large extent, ignored, but we have demonstrated a 4-fold increase in intestinal MTP mRNA in diabetic rats.[29] In the diabetic rat, a model that does not develop atherosclerosis, we found that the increase in MTP mRNA was associated with an increase in the size of the chylomicron particle with no change in apo B48, suggesting that in the diabetic rat, MTP and apo B expression are independent of each other. The purpose of the present study was to examine the effect of diabetes on MTP mRNA expression and activity in the liver and intestine of the cholesterol-fed, hypercholesterolemic, diabetic, rabbit, a model of diabetes that develops atherosclerosis, and to examine the relationship between intestinal MTP and chylomicron particle composition.

MATERIALS AND METHODS

Animals

Male New Zealand white rabbits (Harlan, Bichester, Oxon, UK) (n = 18) were housed individually in a reverse light cycle room (1:00

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Submitted September 4, 2001; accepted January 23, 2002.

Supported by the Health Research Board of Ireland and Bristol-Myers Squibb. D.W., A.B., and K.A. supported by project number 42/D06478 from the Biotechnology and Biological Sciences Research Council.

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0026-0495/02/5107-0014$35.00/0

doi:10.1053/meta.2002.33350

AM to 1:00 PM light, 1:00 PM to 1:00 AM dark). Rabbits were acclimatized for 1 week, with free access to standard chow and water. They were then fed a 0.5% cholesterol diet (Special Diets Service, Essex, UK) ad libitum for 6 weeks. Animals were housed under licence from the Department of Health, and experiments were performed according to Irish law as administered by the Department of Health.

Induction of Diabetes and Treatment

At the beginning of the fifth week, diabetes was induced by intravenous infusion of a 10% (wt/vol) solution of alloxan monohydrate (150 mg/kg) in physiologic saline through a catheter inserted via a marginal ear vein. To counteract hypoglycemia, caused by insulin release from necrotic β cells in the pancreas, the rabbits were provided with a 20% (wt/vol) solution of glucose for the first 24 hours. Blood glucose was determined throughout this period and when animals became hypoglycemic, a 50% (wt/vol) solution of glucose was given intragastrically. Diabetes was confirmed 48 hours later with blood glucose greater than 22 mmol/L as determined by glucotrend strip. Diabetic control was monitored daily by urinalysis using multistix reagent strips. The diabetic animals (n = 9) had significant glycosuria throughout the study, but ketonuria was prevented by daily subcutaneous injection of insulin (Ultratard; Novo Nordisk, Cambridge, UK). Food intake of each rabbit was also monitored. Animals were diabetic for at least 8 days prior to the experiment.

Lymph Duct Cannulation

Following a 12-hour fast, diabetic (n = 9) and control rabbits (n = 9) were given by gavage, 20 mL of an emulsion containing sunflower oil (75% vol/vol), H_2O (25% vol/vol) and phosphatidylcholine (5% wt/vol). Rabbits were returned to their cages with access to water. Five hours postgavage, rabbits were anesthetized by intramuscular injection of hypnorm (0.3 mL/kg) followed by intravenous injection of hypnovel (2 mg/kg). Rabbits were intubated and attached to a Penlon Nuffield anesthesia machine (Abingdon, UK) and administered oxygen and ethrane. Rabbits received 50 mL/h warm saline intravenously. A laparotomy was performed and the left kidney tied off and removed. The mesenteric lymph duct, which lies ventrally above the abdominal aorta, was tied off and cannulated by inserting a 2-mm portex tube. Tubing was exteriorized through a stab wound in the left side and positioned such that regular lymph flow occurred. Lymph was collected for 4 hours, blood was taken by cardiac puncture for determination of plasma lipids, and rabbits were killed by an overdose of euthane. The small intestine and liver were removed and weighed. Liver and intestine were washed with ice-cold phosphate-buffered saline (PBS). The liver was divided into portions and the intestinal mucosa scraped off; samples were then snap frozen in liquid nitrogen and stored at -70°C until required.

Preparation of Lymph Chylomicrons

The volume of lymph collected was measured and aprotinin (0.05 trypsin inhibitor unit [TIU]), NaN_3 (0.02% wt/vol), EDTA (0.1% wt/vol), D-phenylalanyl-L-propyl-L-arginine chlormethyl ketone di-hydrochloride (PPACK II) (1 mmol/L), and phenylmethyl sulfonyl fluoride (PMSF) (0.1 mmol/L) were added to protect the lymph from oxidation and degradation. Lymph was overlaid with a 1.006-g/mL density solution and centrifuged at 20,000 rpm at 10°C for 30 minutes in a Beckman L7-55 ultracentrifuge (Palo Alto, CA) using a fixed angle rotor. Chylomicrons were carefully removed from the top of the tube with a fine glass pipette, chylomicron volume was measured, and chylomicrons were stored at 4°C. Apo B48, B100, and lipids were determined within 1 week of lymph collection.

Lymph and Plasma Lipids

Plasma, lymph, and lymph chylomicron cholesterol were measured by enzymatic colorimetric methods using commercially available kits (Boehringer Mannheim GmBH, Mannheim, Germany). Triglycerides and phospholipids were measured with kits from BioMerieux (Charbonnieres les Bains, France). Plasma high-density lipoprotein (HDL) was measured by using a HDL-C reagent (Boehringer Mannheim).

Quantification of Apo B48 and Apo B100

Lymph and plasma chylomicron apo B48 and B100 were measured using a modification of the method described previously for human plasma apo B48 and B100.[7] Samples were partially delipidated by mixing with diethyl ether (1:6 vol/vol), centrifuged at 14,000 rpm for 15 minutes at room temperature, and the organic phase removed. The protein was dried down and redissolved in 25 μL denaturing buffer (2.0% [vol/vol], β-mercaptoethanol, 4.0% sodium dodecyl sulfate [SDS], 0.01% vol/vol bromophenol blue, 0.1 mol/l Tris-HCL pH 6.8, 20% [vol/vol] glycerol), heated for 4 minutes at 96°C, and loaded on a 4% to 15% polyacrylamide gel (BioRad, Herculas, CA). Electrophoresis was for 1 hour at 60 mA constant current in 0.019 mol/lTris, 0.192 mol/l glycine. Gels were stained for 1 hour with Coomassie Brilliant Blue (0.1% in methanol:acetic acid:water 4:1:5) and destained with several changes of the same solvent. Since the chromogenicity of apo B48 has been shown to be similar to that of apo B100,[30,31] a protein standard was prepared from LDL (density, 1.025 to 1.063 g/mL) of a single individual, was stored at -20°C and used throughout the study for quantification of apo B48 and apo B100. Apo B48 and apo B100 staining was linear within the range 0.1 to 2 μg of protein. Apo B100 protein standards (0.2, 0.6, 1.2, and 2 μg) were applied to all gels. The bands were quantified by densitometry using Vilber Lourmat equipment (Vilber Lourmat Biotechnology, Marne de Vallee, France). Video images of the gels were generated and imported into Bio1D v6.32 software (Vilber Lourmat) for analysis. Density values were assigned to the apo B100 bands of the human LDL and a standard curve constructed, the values were recalculated by linear regression, and curves with a correlation coefficient greater than .95 accepted. The concentrations of apo B48 and B100 were then determined from this standard. The intra- and interassay variations (n = 6) for apo B48 were 3.4% and 6.1% and for apo B100 5.9% and 8.3%.

MTP Activity

Microsomal fraction was prepared from rabbit liver and intestine and treated with deoxycholate to release MTP from the microsomal fraction as previously described.[32]

MTP assay was performed using the MTP assay kit (WAK-Chemie Medical, Bad Homburg, Germany). A total of 10 μg of microsomal protein was added to 10 μL donor lipid and 5 μL acceptor lipid in a final volume of 500 μL assay buffer (10 mmol/L Tris, 150 mmol/L NaCl, 2 mmol/L EDTA) pH 7.4. After incubation at 37°C for 12 hours, flourescence was determined in a Shimadzu RF-500 Spectrofluorophotometer (Shimadzu, UK) at an excitation wavelength of 465 nm and emmission wavelength of 535 nm. Results are given as arbitrary flourescence units per microgram of microsomal protein.

Total RNA Extraction

Total RNA was isolated using the Ultraspec RNA isolation system (Biotecx, Houston, TX). Liver and intestinal mucosa were homogenized in Ultraspec using a mortar and pestle, and chloroform extracted. RNA was precipitated with isopropanol and washed with 75% ethanol. Total RNA was quantified by absorbance at 260 nm assuming that 1 OD = 40 μg RNA/μL.

Transcription Vector Construction and Synthesis of Riboprobe and Synthetic mRNA Standard

Reverse transcription polymerase chain reaction (PCR) was used to amplify a 229-bp rabbit MTP cDNA fragment corresponding to nucleotides 365 to 594 of the human cDNA sequence. Primers 5′-TCAGCAGAGAGGAGAGAAGAGC-3′ and 3′ TATCTACCTCATTGGTGGTTCC-5′ were designed from regions of greatest homology between human, bovine, hamster, and mouse MTP nucleotide sequences. PCR was performed (50°C for 30 seconds, 72°C for 45 seconds, 93°C for 30 seconds) for 35 cycles and the product blunt-end cloned into the EcoRV site of pBluescript II KS vector (Stratagene, La Jolla, CA). The clone was then sequenced to confirm its identity and orientation. [^{32}P]-labeled cRNA probe and synthetic mRNA standard were synthesized using a T3/T7 transcription kit (Stratagene) according to the manufacturer's instructions. Unincorporated nucleotides were removed using Sephadex G50 spun columns. cRNA probes were counted by liquid scintillation, and mRNA standard was quantified by absorbance at 260 nm.

Quantitation of MTP mRNA by Nuclease Protection Assay

MTP mRNA levels were quantified by solution hybridization nuclease protection assay using an RPA III RNase protection kit (AMS Biotechnology, Abingdon, Oxon, UK). Total intestinal mucosa and hepatic RNA (8 μg) and synthetic mRNA (50 to 1000 amol) were hybridized overnight at 42°C with 1×10^5 cpm of cRNA in 10 μL hybridization buffer. Samples were then treated with RNase and precipitated in accordance with the manufacturer's instructions. The RNase-resistant products were separated by electrophoresis on a 6% acrylamide gel containing 7 mmol/L urea, 2 mmol/L EDTA, and 90 mmol/L Tris-borate, pH 8.0. Gels were dried down and analzsed on a Fugifilm Molecular Imager FLA-2000 (phosphorimager) system using Aida/2D densitometry version 2.0 (Raytest IsotopenmeβgerŠte, Benzstr Straubenhardt, Germany). Increasing amounts of synthetic mRNA were used to construct standard curves from which the amount of MTP mRNA in terms of amol MTP mRNA/μg total RNA were calculated. Results were corrected for variation in the mRNA content of total RNA samples by quantitation of poly A RNA using oligo dT_{18} hybridization. All mRNA values are expressed in amol MTP mRNA/microgram total RNA normalized to 30 ng poly A/microgram total RNA.

Statistical Analysis

Statistical analysis was performed by paired Student's *t* test using GraphPad Prism version 2.0b (GraphPad Software, San Diego, CA). Inter- and intra-assay variation are expressed as standard deviation/mean $\times$ 100. Results are expressed as mean $\pm$ SD. A *P* value of less than .05 was regarded as statistically significant.

RESULTS

Characteristics and lipid profiles of control and diabetic rabbits on the day of experiment are outlined in Table 1. Mean weight of the diabetic rabbits was similar to control rabbits. Hepatic and intestinal weights were similar, and average daily consumption of chow (day 2 to day 8) was not significantly different in the 2 groups. Examination of the plasma lipids at sacrifice showed the expected significant increase in plasma triglyceride ($P < .05$) and decrease in plasma HDL ($P < .05$) in the diabetic rabbits. Plasma-free cholesterol was significantly higher in the diabetic animals ($P < .05$).

The mean volume of lymph collected in 4 hours was similar for diabetic and control animals (5.7 $\pm$ 2.9mL *v* 5.2 $\pm$ 2.6 mL, respectively). Lymph from diabetic animals contained significantly more chylomicron apo B48 and B100/h compared with control animals ($P < .05$) (Table 2). Diabetic animals also secreted significantly more lymph chylomicron total cholesterol/hour ($P < .01$ and esterified cholesterol/hour ($P < .02$), while triglyceride and phospholipid secretion, although increased, were not significantly different between the groups. Lymph chylomicrons from the diabetic animals contained significantly less lipid/apo B ($P < .05$) than particles from control animals. The lymph chylomicron particle lipid and cholesterol composition was not significantly different between the 2 groups (Table 3).

Table 1. Diabetic and Control Rabbit Characteristics and Plasma Lipids

	Diabetic (n = 9)	Control (n = 9)
Body weight (kg)	2.9 $\pm$ 0.4	3.2 $\pm$ 0.3
Hepatic weight (g)	119 $\pm$ 25	127 $\pm$ 34
Intestinal weight (g)	67 $\pm$ 14	67 $\pm$ 9
Blood sugar (mmol/L)	20.5 $\pm$ 4.1*	5.8 $\pm$ 0.6
Insulin (U/d)	4.1 $\pm$ 3.5	—
Food intake (g/d)	146 $\pm$ 30	140 $\pm$ 30
Triglyceride (mmol/L)	5.7 $\pm$ 10.4†	0.9 $\pm$ 0.6
HDL cholesterol (mmol/L)	0.3 $\pm$ 0.15†	0.6 $\pm$ 0.3
Cholesterol (mmol/L)	33.8 $\pm$ 20.9	22.2 $\pm$ 12.7
Free cholesterol (mmol/L)	13.0 $\pm$ 7.3†	7.1 $\pm$ 4.8
Esterified cholesterol (mmol/L)	20.7 $\pm$ 14.5	15.1 $\pm$ 9.1
Apo B48 (nmol/L plasma)	21.8 $\pm$ 12.2†	5.9 $\pm$ 5.5
Apo B100 (nmol/L plasma)	25.0 $\pm$ 9.2†	8.6 $\pm$ 8.0

NOTE. Values are means $\pm$ SD.
*$P < .01$, †$P < .05$ different from control rabbits.

Figure 1 shows MTP activity and mRNA expression in intestine and liver. Intestinal MTP activity was significantly higher in diabetic compared with control rabbits (0.07 $\pm$ 0.01 *v* 0.045 $\pm$ 0.015 flourescent units/microgram microsomal protein, $P < .05$). MTP mRNA was also significantly higher (66 $\pm$ 21 *v* 37 $\pm$ 11 amol MTP mRNA/microgram total RNA, $P < .005$). In the liver, there was no difference in MTP activity (0.09 $\pm$ 0.03 *v* 0.09 $\pm$ 0.02 flourescent units/microgram microsomal protein) or mRNA expression (23 $\pm$ 14 *v* 27 $\pm$ 9 amol MTP mRNA/microgram total RNA) between diabetic and control animals.

Combining the diabetic and control groups, there was a positive correlation between intestinal MTP mRNA and both lymph chylomicron apo B48 ($r = .65$, $P < .005$) and B100 ($r = .75$, $P < .0005$). There was also a positive correlation between intestinal MTP mRNA and lymph chylomicron cholesterol ($r = .55$, $P < .02$), but no correlation between intestinal MTP mRNA and blood glucose.

DISCUSSION

The cholesterol-fed alloxan diabetic rabbit is a model of diabetes and atherosclerosis. In this study, the lymphatic duct was isolated and cannulated to collect pure intestinally-derived chylomicron particles. The rabbit, unlike the human, secretes both apo B48 and B100 in the intestine.[33] We found an increase in both apo B48 and B100 in the lymph of the diabetic animals indicating an increased number of intestinally-derived particles, since there is only 1 apo B molecule/particle. The particles from diabetic rabbits were significantly smaller as calculated by

Table 2. Lymph Chylomicrons From Diabetic and Control Rabbits

	Diabetic n = 9	Control n = 9
Total cholesterol (μmol/h)	15.8 ± 10.6*	4.9 ± 2.7
Free cholesterol (μmol/h)	6.6 ± 3.5	3.4 ± 3.3
Esterified cholesterol (μmol/h)	10.15 ± 9.0†	2.4 ± 1.3
Triglyceride (μmol/h)	58.9 ± 42.1	34.9 ± 21.0
Phospholipid (μmol/h)	38 ± 36	13.5 ± 13
Apo B48 (nmol/h)	0.54 ± 0.47†	0.15 ± 0.08
Apo B100 (nmol/h)	0.32 ± 0.26‡	0.11 ± 0.11
Lipid/apo B	301 ± 194‡	505 ± 195

NOTE. Values are means ± SD.
*$P < .01$, †$P < .02$, ‡$P < .05$ different from control rabbits.

the ratio of lipid/apo B than those from control animals. This increase in the number of small chylomicron particles is in keeping with previously reported findings in diabetic rabbits[34] and type 2 diabetic patients.[7]

The diabetic animals had hypertriglyceridemia, but lymph chylomicron triglyceride was not significantly different from control animals, suggesting that the increased plasma triglycerides were either due to increased hepatic synthesis or slower chylomicron clearance. We have shown in a previous study that lymph chylomicrons from diabetic rabbits, which also contained less lipid/particle compared with nondiabetic chylomicrons, were cleared more slowly even when injected into nondiabetic animals, suggesting a structural abnormality of the particle in diabetes.[34] Martins et al[35] have demonstrated that particle number is more important than size in relationship to clearance. Although they showed that triglyceride in small particles was cleared more quickly than large particles, they also showed that an increase in the number of particles delayed overall clearance. The increased number of small particles in the diabetic rabbits might be another explanation for the delayed clearance of chylomicrons in diabetes that we described in the above study.[34]

Diabetic rabbits had similar serum cholesterol to control animals, but their lymph chylomicron cholesterol was significantly higher. Since both groups had the same amount of dietary cholesterol, it is likely that the increase in chylomicron cholesterol is due to endogenous cholesterol synthesis. We have previously shown an increase in HMGCoA reductase, the rate-limiting enzyme for cholesterol synthesis, in the intestine of diabetic rabbits.[13,14] The increased lymph chylomicron cholesterol found in diabetic rabbits in the present study was therefore to be expected, whereas in nondiabetic rabbits, we found that cholesterol feeding suppressed intestinal cholesterol synthesis.[20] It is possible that biliary cholesterol was increased and may have contributed to the increase in lymph cholesterol in this animal model.

MTP is necessary for the successful assembly of the chylomicron particle.[22,23,36,37] A negative insulin response element within the MTP promoter has been identified, and MTP expression was shown to be downregulated by insulin experimentally in hepatocytes.[24] We found a significant increase in intestinal MTP activity, which reflected a 2-fold increase in intestinal MTP mRNA, in our diabetic rabbits. There was no increase in hepatic MTP activity or mRNA in the present study, which is in agreement with the findings of Brett et al,[26] who showed no change in MTP protein in the liver of diabetic rats. The hepatic response of MTP mRNA to a high-fat diet has been shown to be much slower than that of the intestine,[28] and it is possible that had we examined the rabbits after a longer period of diabetes, the liver MTP mRNA would also have been upregulated.

Hepatocytes from fructose-fed Syrian Golden hamsters, an animal model of insulin resistance characterized by VLDL apo B overproduction, have been found to have increased MTP mass. However, hepatocyte cell lysates were only examined in 2 control and 2 fructose-fed hamsters, the experiment being repeated only once with similar results according to the investigators.[38] In a recent study in the Zucker obese fatty rat, a model of nondiabetic insulin resistance, we demonstrated an increase in MTP in both liver and intestine with a significant correlation between MTP mRNA and the glucose/insulin ratio.[39] In a previous study, we showed a 4-fold increase in MTP mRNA expression in the intestine of streptozotocin diabetic rats, but in that study, we did not examine the liver.[29]

The significantly higher intestinal MTP mRNA and activity found in the diabetic animals suggests a role for MTP in chylomicron formation in the intestine similar to that for VLDL in the liver. It has been shown in HepG2 cells that sterols positively regulate MTP gene expression,[40] and we have shown that dietary cholesterol increased hepatic MTP mRNA in hamsters.[41,42] Heterozygous MTP knockout mice, expressing half normal levels of MTP, showed a 25% to 35% reduction in plasma apo B100 levels,[43] and it has been shown that adenoviral overexpression of MTP in the liver of a mouse resulted in increased secretion of both VLDL apo B and triglycerides.[44] Apo B secretion is regulated at the posttranscriptional level, and lipidation of newly synthesized apo B appears to prevent its degradation.[45] We have previously demonstrated that both cholesterol and fat upregulate hepatic MTP in hamsters and that the VLDL triglyceride, as well as cholesterol, correlates significantly with hepatic MTP mRNA.[41,42] In the current study, control and diabetic animals were fed a similar diet and, since the intestine depends on dietary fatty acids for the production of triglyceride, it is perhaps not surprising that in our study triglyceride in the chylomicron particle did not correlate with intestinal MTP.

We have recently demonstrated an increase in apo B48 and B100 in intestinally-derived chylomicrons collected from the lymph of alloxan diabetic rabbits,[34] and in type 2 diabetes, we have shown an increase in chylomicron apo B48 in both the early and late phase postprandially.[7] These findings suggest that diabetes, in both rabbit and humans, is associated with an increase in chylomicron particle production. The present study

Table 3. Lymph Chylomicrons From Diabetic and Control Rabbits (mg/mg apoB)

	Diabetic n = 9	Control n = 9
Cholesterol	63 ± 72	39 ± 22
Free cholesterol	17 ± 21	18 ± 22
Esterified cholesterol	46 ± 60	21 ± 17
Triglyceride	299 ± 350	397 ± 214
Phospholipid	110 ± 58	108 ± 55

NOTE. Values are means ± SD.

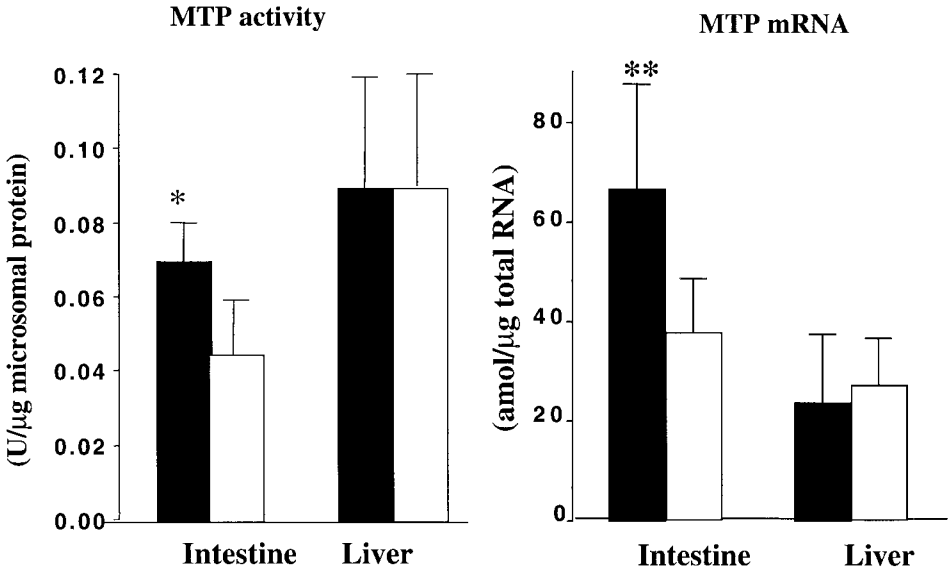

Fig 1. (A) MTP activity (fluorescent units/microgram microsomal protein) and (B) mRNA (amol MTP mRNA/microgram total RNA) in the intestine (n = 9) and liver (n = 8) of alloxan diabetic (■) and control (□) rabbit pairs. *$P < .05$, **$P < .005$.

demonstrates an increase in MTP expression and activity in the intestine in diabetes with no change in the liver, suggesting that intestinal MTP upregulation may be responsible for the production of an increased number of small chylomicron particles. Our previous study in the streptozotocin diabetic rat model demonstrated an association of increased intestinal MTP with lymph cholesterol and triglyceride, but no relationship to apo B48 or B100.[29] The animal models, however, are not comparable since they were fed different diets, the rats having a high fatty acid diet and rabbits high cholesterol. The relationship between the regulation of MTP in the intestine and liver needs further exploration. The higher levels of MTP that we find in the intestine compared with the liver suggest that insulin deficiency and cholesterol may have an additive effect in the intestine, but not in the liver.

Interest in MTP has been stimulated by reports of the effect of MTP inhibitors on lipoproteins. Wetterau et al[46] have described an MTP inhibitor that was able to normalize atherogenic lipoprotein levels in the Watanabe-heritable hyperlipidemic rabbit, a model for homozygous familial hypercholesterolemia. Unfortunately, in humans, MTP inhibitors cause severe hepatic lipid accumulation. The results in our report suggest that MTP inhibitors designed to act only in the intestine may find a valuable place in the treatment of postprandial dyslipidemia of diabetes.

In conclusion, the diabetic rabbits secreted a significantly greater number of small chylomicron particles. The association between increased MTP in the intestine and abnormal chylomicron particles suggests that MTP may play an important role in the postprandial dyslipidemia of diabetes.

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