The Role of microsomal triglyceride transfer protein and dietary cholesterol in chylomicron production in diabetes

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Abstract

Aims/hypothesis. The aim of this study was to examine factors involved in chylomicron production in the streptozotocin diabetic rat, our hypothesis being that the synthesis of the chylomicron is abnormal in diabetes.

Methods. Diabetic rats ($n = 20$) were paired with control rats ($n = 20$). Cholesterol emulsion was given by gavage and the lymph duct was cannulated. Lymph was collected for 4 h. Chylomicrons were prepared from the lymph by ultracentrifugation. Lymph apolipoprotein B48 was isolated by gradient gel electrophoresis and quantified by densitometric scanning. Intestinal microsomal triglycerol transfer protein mRNA was measured by solution hybridisation nuclease protection, using a rat specific [³²P]-labelled cRNA probe.

Results. Serum triglyceride and cholesterol were greatly increased in diabetic compared with control animals (258 ± 77 and 8.9 ± 6.4 mg/ml vs 1.04 ± 0.37

and 0.54 ± 0.03 mg/ml, $p < 0.0001$). Lymph chylomicron triglyceride and cholesterol were also higher in diabetic rats (29.4 ± 27.3 and 0.28 ± 0.3 mg/h vs 16.8 ± 10.6 and 0.18 ± 0.09 mg/h, $p < 0.05$). Lymph chylomicron apo B48 was similar in the two groups. Intestinal microsomal triglycerol transfer protein mRNA was higher in the diabetic rats (12.6 ± 3.2 vs 3.8 ± 3.0 amol/ μ g RNA, $p < 0.0001$) and there was a positive correlation between lymph triglyceride and microsomal triglycerol transfer protein mRNA in the whole group ($r = 0.65$, $p < 0.01$).

Conclusion/interpretation. The study shows that microsomal triglycerol transfer protein mRNA is raised in diabetes without an increase in apolipoprotein B48 in the lymph suggesting that microsomal triglycerol transfer protein regulates chylomicron triglyceride content but not particle number. [Diabetologia (1999) 42: 944–948]

Keywords Chylomicron composition, apolipoprotein B48, microsomal triglyceride transfer protein.

Cardiovascular disease is the major cause of death in diabetes. It is up to five times more common than in the general population [1]. Disturbance in lipoprotein metabolism and its relation to hyperglycaemia and hyperinsulinaemia appear to be the most impor-

tant abnormalities that might account for the increase in atherosclerosis [2, 3]. Although hypercholesterolaemia is not particularly associated with diabetes the Scandinavian Simvastatin Secondary Prevention Study [4] has recently shown that lowering cholesterol by 30 % in diabetic patients with myocardial infarction reduced total mortality by 43 %.

Hypertriglyceridaemia is the commonest abnormality found in poorly controlled diabetes and the chylomicron remnant may be particularly atherogenic [5, 6] and is abnormal in diabetes [7]. Cholesterol synthesis in the intestine is increased in diabetes [8–10]. Apolipoprotein (apo) B48 is the lipid carrying protein for the chylomicron and there is a single copy

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Abbreviations: MTP, Microsomal triglyceride transfer protein; apo, apolipoprotein; ACAT, AcylcoenzymeA cholesterol:acyl-transferase.

of apo B48 on the chylomicron particle in humans [11] and rats [12]. In humans apo B48 is only formed in the intestine [13] but in some animals including the rat it is also formed in the liver. Chylomicron cholesterol composition depends on cholesterol absorption and de novo cholesterol synthesis in the intestinal mucosa. AcylcoenzymeA cholesterol:acyltransferase (ACAT) is involved in chylomicron formation and decreased apo B48 in the lymph of rats treated with an ACAT inhibitor has been reported [14]. We have shown an increase in post-prandial apo B48 in poorly controlled diabetes [15–17] and that dietary cholesterol was responsible for an increase in production of small chylomicron particles in diabetic patients [7]. Improvement in diabetic control resulted in reduction in post-prandial apo B48-containing chylomicron particles [18] but we were unable to clearly define the effect of diabetes on chylomicron assembly as opposed to clearance.

Microsomal triglyceride transfer protein (MTP) catalyses the transfer of triglycerides, cholesterol esters and phospholipids. It is found in the endoplasmic reticulum of hepatocytes and enterocytes and is involved in the assembly of apolipoprotein B-containing particles [19]. It has been shown to regulate apo B secretion in rat hepatoma cells and primary rat hepatocytes [20] and in HepG2 cells. Insulin and high concentrations of glucose have been shown to decrease MTP mRNA expression [21]. Little is known of the effect of insulin and glucose on MTP production in the enterocyte although reference has been made [22] to MTP mRNA being unchanged in the intestine of insulin-deficient rats. Our study was to examine the relation between MTP and apo B48 in the synthesis of the chylomicron in the diabetic rat model.

Materials and methods

Animals. Male Sprague Dawley rats ($n = 40$) weighing between 350 and 500 g were fed a commercial rat pelleted diet. Of these, 20 rats were made diabetic by subcutaneous injection with streptozotocin (Sigma, Poole, UK) (60 mg/kg in 0.2 mmol/l sodium citrate buffer, pH 4). Each diabetic rat was paired with a control rat and the control rat was injected with buffer alone. Urinary glucose concentrations were monitored to confirm diabetic status. Rats with a urine glucose greater than 24 mmol/l were considered to be diabetic and diabetes was confirmed by blood glucose determination at death. The weight and food intake of each rat was monitored daily. The principles of laboratory animal care (NIH publication number 85.23) revised 1985 were followed. Animals were housed under license from the Department of Health and experiments were carried out according to Irish law as administered by the Department of Health.

Lymph duct cannulation. On the 5th day after diabetes had been induced the diabetic and control rat pairs were switched to a diet of sunflower seeds. After 2 days on this diet, control and insulin-deficient rats were given by gavage 1.5 ml of a

freshly prepared cholesterol emulsion containing 110 mg cholesterol with 90 mg phosphatidyl choline (Sigma) dissolved in chloroform. The solvent was evaporated off over nitrogen and the mixture sonicated with warm (55°C) saline for 20 min using a Branson Sonifier 250 probe at 50% duty cycle, output 5 with a flat tip (Branson Ultrasonics, Danbury, Conn., USA).

One hour after gavage rats were anaesthetised and a 2 mm portex tube inserted in the thoracic lymph duct through a transverse incision in the abdominal wall. The tubing was allowed to exit from the left-hand side of the incision which was then closed with a 4.0 nylon suture. A small section of the wound remained open to expose the stomach. Rats were hydrated hourly by gastric injection of 1.5 ml normal saline and lymph was collected for 4 h. Rats remained anaesthetised for the entire procedure. After 4 h rats were exsanguinated by cardiac puncture and the intestine removed and weighed. In 7 of the diabetic and 7 control rats, the intestine was washed with PBS and the intestinal mucosa scraped off and snap frozen in liquid nitrogen for MTP mRNA determination. Samples were stored at -70°C and analysed within 6 months.

Preparation of chylomicrons. Lymph was collected for 4 h immediately after cannulation. Aprotinin (0.05 TIU) NaN_3 (0.02%, w/v) EDTA (0.1%, w/v), PPACK II (1 mmol/l), and PMSF (0.1 mmol/l) were added to protect the lymph from oxidation and degradation and the volume of lymph collected was measured. Lymph was overlaid with 1.006 density solution and centrifuged at 10°C in a Sorval TFT 45.6 fixed angle rotor in a Sorval Combi Plus centrifuge (Dupont, Wilmington, Del., USA) for 30 min at 20 000 rev/min. The opaque layer at the top of the tube containing lymph chylomicrons was removed using a fine glass pipette, the volume was measured chylomicrons were stored at 4°C . ApoB48 and lipoproteins were determined within 1 week of lymph collection.

Quantification of apo B48. Lymph apo B48 was measured using a modification of the method previously described for human plasma apo B48 [7]. Lymph chylomicrons were partially delipidated by mixing 62.5 μl of sample with 375 μl of diethyl ether. Samples were centrifuged for 15 min in microfuge (International Equipment Company (IEC), Needham Heights, Mass., USA), at 14 000 rev/min and room temperature and the organic phase discarded. The protein was dried and re-dissolved in 24 μl of denaturing buffer (0.1 mol/l TRIS HCl, pH 6.8, 4% (w/v) SDS, 2% (v/v) β -mercaptoethanol, 0.01% (w/v) bromophenol blue, 20% (v/v) glycerol), heated for 5 min at 96°C , and loaded on a 4–15% polyacrylamide gel. Electrophoresis was for 1 h at 60 mA constant current in 0.019 mol/l TRIS, 0.192 mol/l glycine.

We used LDL, isolated from human plasma by sequential ultracentrifugation as an apo B100 protein standard. Apo B100 protein standards (0.4 μg , 0.6 μg and 1.0 μg) were applied to each gel. We calculated LDL protein concentration from the mean value of ten determinations measured by a modified Lowry procedure [23].

Gels were stained for 1 h with Coomassie Blue R250 0.1% (w/v) in methanol:water:acetic acid, (4:5:1 v/v) and destained with the same solvent. The bands were quantified by densitometry using Vilber Lourmat equipment (Vilber Lourmat Biotechnology, Marne le Vallee, France). Video images of the gels were generated and imported into Bio1D v6.32 software (Vilber) 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 0.95 accepted. The concentration of apoB48 was determined from this standard. Previous researchers [24] showed equal chromogenicity for apo

Table 1. Serum and lymph lipoproteins in diabetic and control rats

Serum		triglyceride (mg/ml)	cholesterol (mg/ml)	protein (mg/ml)	
Diabetic (<i>n</i> = 20)	mean	258 ± 77 ^b	8.9 ± 6.4 ^b	36.0 ± 5.8	
	median	266			
	range	118–383			
Control (<i>n</i> = 20)	mean	1.04 ± 0.4	0.54 ± 0.3	34.7 ± 0.3	
	median	1.03			
	range	0.24–1.63			
Lymph chylomicrons		triglyceride (mg/h)	cholesterol (mg/h)	protein (mg/h)	apo B48 (µg/h) (<i>n</i> = 10)
Diabetic (<i>n</i> = 20)	mean	29.4 ± 27.3 ^a	0.28 ± 0.26 ^a	2.6 ± 2.6	9.1 ± 4.0
	median	21.1			
	range	9.2–109.1			
Control (<i>n</i> = 20)	mean	16.8 ± 10.6	0.18 ± 0.09	2.0 ± 1.3	11.3 ± 4.4
	median	13.8			
	range	0.7–24.8			

Means ± SD

^a *p* < 0.05, ^b *p* < 0.0001 different from control

B100 and apo B48 bands and we have shown that apoB100 staining is linear within the range 0.1–2.0 µg of protein.

Lipoprotein analysis. Triglyceride and cholesterol in lymph chylomicrons and serum samples were determined by enzymatic colorimetric methods using kits from Boehringer Mannheim (Mannheim, Germany). Protein determination was by the Bradford method using Coomassie Protein Reagent (Pierce, Chester, UK).

Microsomal triglyceride transfer protein (MTP) mRNA. For determination of MTP mRNA, total RNA was isolated at 4°C using the ultraspec RNA isolation system (Biotecx, Houston, Tex., USA) [25]. Intestinal mucosa was homogenised in a potter homogeniser in Ultraspec and chloroform extracted. RNA was precipitated with isopropanol and washed with 75 % ethanol. Total RNA was quantified by absorbance at 260 nm assuming 1 OD unit = 39 mg RNA/ml. Reverse transcription PCR was used to amplify a 223 base pair MTP cDNA fragment corresponding to nucleotides 88 to 311 of the human cDNA sequence as previously described [26]. This PCR product was used to construct a cDNA probe which was rat specific. A [³²P]-labelled cRNA probe and a synthetic mRNA standard were synthesised using a Maxiscript T7/T3 transcription kit (AMS Biotechnology). The amount of MTP mRNA was quantified by solution hybridisation assay using an RPA11 RNase protection Kit (AMS Biotechnology). Increasing amounts of synthetic mRNA were used to construct standard curves from which the amount of MTP mRNA were calculated and expressed in amol MTP mRNA/µg total RNA normalised to 20 ng poly A/µg total RNA.

Statistical analysis. Results were expressed as means ± SD. Statistical analysis was done using the Student paired *t* test and ANOVA analysis of variance. Correlation coefficients were determined by linear and curvilinear regression analysis using Statworks software (Computer Associates International Inc., N.Y., USA) on an Apple Macintosh computer. Values of *p* < 0.05 were considered to be statistically significant.

Results

Mean weight of control rats at the beginning of the study was similar to the diabetic rats (404 ± 64 vs 422 ± 48 g *n* = 18). At the end of the study the weight of control rats was 395 ± 64 g and diabetic rats was 347 ± 86 g (*n* = 20) (*p* < 0.05) showing the usual weight loss of the diabetic animal model. Blood glucose for the control rats was 4.4 ± 1.5 compared with 20.1 ± 5.3 mmol/l for the diabetic animals (*p* < 0.0001).

Serum triglycerides in the control animals were 1.04 ± 0.37 mg/ml and in the diabetic animals 258 ± 77 mg/ml (*p* < 0.0001). Serum cholesterol was 0.54 ± 0.03 mg/ml compared with 8.9 ± 6.4 mg/ml (*p* < 0.0001). There was no difference in serum protein between the two groups (34.7 ± 0.33 vs 36.0 ± 5.8 mg/ml) (Table 1).

Lymph chylomicron triglyceride was higher in the diabetic rats (29.4 ± 27.3 vs 16.8 ± 10.6 mg/h *p* < 0.05) (Table 1) Lymph chylomicron cholesterol was also higher in the diabetic rats (0.28 ± 0.3 vs 0.18 ± 0.09 mg/h, *p* < 0.05). Lymph chylomicron protein was not significantly different between the control and diabetic rats and lymph chylomicron apo B48 was similar in the 2 groups (11.3 ± 4.4 vs 9.1 ± 4.0 µg/h). There was, however, a significant difference in the ratio of cholesterol + triglyceride to apo B48 between diabetic and control rats (*p* < 0.02).

There was a positive correlation between serum triglyceride and lymph triglyceride in the control rats (*r* = 0.69, *p* < 0.02) but no correlation in the diabetic rats. There was no correlation between serum and lymph cholesterol in either group. There was a positive correlation between lymph cholesterol and lymph triglyceride in the diabetic and control rats (*r* = 0.67, *p* < 0.020 and *r* = 0.55, *p* < 0.05 respectively)

Fig. 1. Microsomal triglyceride transfer protein mRNA in the intestine of 7 diabetic and control rat pairs ($n = 7$ in each group) MTP mRNA was higher in the diabetic animals $*p < 0.0001$

Fig. 3. Correlation between lymph chylomicron cholesterol and intestinal MTP in 7 diabetic ■ and control rat pairs □ ($r = 0.49$, $p < 0.05$)

Fig. 2. Relation between lymph chylomicron triglyceride and intestinal MTP in 7 diabetic ■ and control □ rat pairs. ($r = 0.65$, $p < 0.01$)

and a positive correlation between lymph protein and lymph cholesterol in the diabetic rats ($r = 0.78$, $p < 0.003$) but no correlation in the control rats.

Rat intestinal MTP mRNA concentration was higher in the diabetic than the control rats 12.64 ± 3.20 compared with 3.76 ± 3.03 amol MTP mRNA/μg total mRNA ($p < 0.0001$) (Fig. 1). There was a positive curvilinear correlation between lymph triglyceride and MTP ($r = 0.65$, $p < 0.01$) when diabetic and control rats were included in the analysis (Fig. 2) and a positive correlation between lymph cholesterol and MTP mRNA ($r = 0.49$, $p < 0.05$) (Fig. 3)

Discussion

Diabetes in the rats was associated with weight loss and massive hypertriglyceridaemia and hypercholesterolaemia showing severe insulinopaenia. We and others have shown that the intestinal hypertrophy which occurs in diabetes is associated with an increase in 3hydroxy3methylglutaryl coenzyme A (HMG-CoA) reductase the rate limiting enzyme for cholesterol synthesis [8–10]. The cannulation of the lymphatic duct allowed us to examine the chylomicron before it entered the circulation and we could therefore dissociate synthesis from clearance. Post-prandial lymph chylomicron triglyceride and cholesterol were significantly higher in the diabetic animals with no difference in apo B48 compared with the control rats. Thus the chylomicron particle being delivered to the circulation was both cholesterol and triglyceride enriched.

Microsomal triglyceride transfer protein regulates triglyceride transfer between membranes and is essential for chylomicron and VLDL production [19]. It has been shown to stimulate apo B100 secretion in rat hepatoma cells and primary rat hepatocytes [20] and it appears to be regulated by insulin and cholesterol both in the human and the hamster [27]. The results in the HepG2 cells which showed that insulin suppressed MTP mRNA suggests that in diabetes, lack of insulin would result in raised MTP and increased apo B secretion. We found a statistically significant threefold increase in MTP mRNA in the intestine of diabetic compared with control rats. Our results suggest that if MTP mRNA is translated into MTP protein, MTP does not stimulate apo B48 production. This finding is supported by a recent study [28] that found, in Caco-2 cells (an intestinally derived cell line that secretes both apo B100 and apo

B48) that MTP inhibition reduced apo B100 secretion but had no effect on apo B48. Thus in the intestine MTP appears to be important in regulating the triglyceride content of the chylomicron particle without increasing the number of particles. In diabetes the increase in MTP mRNA was associated with a triglyceride-enriched particle. The study suggests that a drug which could either interfere with MTP action or down regulate MTP mRNA might be of value in reducing the triglyceride-enriched chylomicron particle found in diabetes.

The effect of particle size on plasma clearance of chylomicrons could be of great importance in our quest to understand the atherogenicity of the chylomicron particle. The clearance from plasma of chylomicron emulsion particles has been examined [29]. When equal numbers of either small or large particles were injected into rats the large particles were slower to clear than the small particles ($p < 0.001$) and larger particles were found to be lipolysed less than small ones ($p < 0.001$). It is possible that the delay in clearance of these large particles may impede the clearance of VLDL particles since apo B48-containing particles are preferentially taken up by the BE receptor in the liver [30].

In conclusion our study has shown that in diabetes MTP mRNA is increased in the intestine and is associated with a triglyceride-enriched chylomicron particle.

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References

- Stamler J, Vaccaro O, Neaton JD, Wentworth D (1993) Diabetes and other risk factors and 12-year cardiovascular mortality for men screened in the multiple risk factor intervention trial. *Diabetes Care* 16: 434–444
- Tomkin GH (1994) Dyslipidaemia and diabetes. *Diabetes Annual* 8: 407–436
- Griffin M, Frazer A, Collins P, Johnson A, Owens D, Tomkin GH (1998) Cellular cholesterol synthesis – the relationship to post-prandial glucose and insulin. *Atherosclerosis* 138: 313–318
- Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G (1997) The Scandinavian Simvastatin Survival Study (4S) Group. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. *Diabetes Care* 20: 614–620
- Zilversmit DB (1979) Atherogenesis: a post-prandial phenomenon. *Circulation* 60: 473–485
- Koren E, Corder C, Mueller G et al. (1996) Triglyceride-enriched lipoprotein particles correlate with the severity of coronary artery disease. *Atherosclerosis* 122: 105–117
- Taggart C, Gibney J, Owens D, Collins P, Johnson A, Tomkin GH (1997) The role of dietary cholesterol in the regulation of post-prandial apolipoprotein B48 levels in diabetes. *Diabet Med* 14: 1051–1058
- O'Meara N, Devery R, Owens D, Collins P, Johnson A, Tomkin GH (1990) Cholesterol metabolism in the alloxan-induced diabetic rabbit. *Diabetes* 39: 626–633
- O'Meara N, Devery R, Owens D, Collins P, Johnson A, Tomkin GH (1991) Serum lipoproteins and cholesterol metabolism in two hypercholesterolaemic rabbit models. *Diabetologia* 34: 139–143
- Feingold KR, Wilson DE, Wood LC, Kwong LK, Moser AH, Grunfeld C (1994) Diabetes increases hepatic hydroxymethyl glutaryl coenzyme A reductase protein and mRNA levels in the small intestine. *Metabolism* 43: 450–454
- Phillips ML, Pullinger C, Kroes I et al. (1997) A single copy of apolipoprotein B-48 is present on the human chylomicron remnant. *J Lipid Res* 38: 1170–1177
- Martins IJ, Sainsbury AJ, Mamo JC, Redgrave TG (1994) Lipid and apoprotein B48 transport in mesenteric lymph and the effect of hyperphagia on clearance of chylomicron-like emulsions in insulin-deficient rats. *Diabetologia* 37: 238–246
- Hoeg JM, Sviridov DD, Tennyson GE et al. (1990) Both apolipoproteins B-48 and B-100 are synthesized and secreted by the human intestine. *J Lipid Res* 31: 1761–1769
- Martins IJ, Mortimer B-C, Redgrave TG (1997) Effect of the ACAT inhibitor CL277,082 on apolipoprotein B48 transport in mesenteric lymph and on plasma clearance of chylomicrons and remnants. *Arterioscler Thromb Vasc Biol* 17: 211–216
- Curtin A, Deegan P, Owens D, Collins P, Johnson A, Tomkin GH (1994) Alterations in apolipoprotein B-48 in the post-prandial state. *Diabetologia* 37: 1259–1262
- Curtin A, Lenahin K, Deegan P et al. (1994) Intestinal abnormality as the cause of abnormal cholesterol metabolism in diabetes. *Diabetologia* 37: 66
- Curtin A, Deegan P, Owens D, Johnson A, Collins P, Tomkin GH (1996) Elevated triglyceride-rich lipoproteins in diabetes: a study of apo B48. *Acta Diabetol* 33: 205–210
- Muragasu G, Phillips C, Owens D, Collins P, Johnson A, Tomkin GH (1998) Effect of improvement in glycaemic control on apo B48 and post-prandial lipoproteins in non-insulin-dependent diabetes. *Diabet Med* 15:S3
- White D, Bennett A, Billett M, Salter AM (1998) The assembly of triacylglycerol-rich lipoproteins: an essential role for the microsomal triacylglycerol transfer protein. *Br J Nutr* 80: 219–229
- Jamil H, Chu C-H, Dickson JK Jr et al. (1998) Evidence that microsomal triglyceride transfer protein is limiting in the production of apolipoprotein B-containing lipoproteins in hepatic cells. *J Lipid Res* 39: 1448–1454
- Linn MC, Gordon D, Wetterau JR (1995) Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. *J Lipid Res* 36: 1073–1081
- Wetterau JR, Lin M, Jamil H (1997) Microsomal triglyceride transfer protein. *Biochim Biophys Acta* 1345: 136–150
- Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* 87: 206–210
- Karpe F, Steiner G, Uffelman K, Olivercrona T, Hamsten A (1994) Postprandial lipoproteins and the progression of coronary atherosclerosis. *Atherosclerosis* 106: 83–97
- Chomczynski P, Sacchi N (1987) Single step method of RNA isolation by guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159
- Bennett A, Billett MA, Salter AM, White DA (1995) Regulation of hamster hepatic microsomal triglyceride transfer protein mRNA levels by dietary fats. *Biochem Biophys Res Commun* 212: 473–478
- Hagan DL, Kienzle B, Jamil H, Hariharan N (1994) Transcriptional regulation of human and hamster microsomal triglyceride transfer protein genes. *J Biol Chem* 269: 28737–28744
- Van Greevenbroek MM, Robertus-Teunissen MG, Erkelens DW, de Bruin WA (1998) Participation of the microsomal triglyceride transfer protein in lipoprotein assembly in Caco-2 cells: interaction with dietary saturated and unsaturated fatty acids. *J Lipid Res* 39: 173–185
- Martins IJ, Mortimer B-C, Miller J, Redgrave TG (1996) Effects of particle size and number on the plasma clearance of chylomicrons and remnants. *J Lipid Res* 37: 2696–2705
- Bjorkegren J, Karpe F, Milne RW, Hamsten A (1998) Differences in apolipoprotein and lipid composition between human chylomicron remnants and very low density lipoproteins isolated from fasting and postprandial plasma. *J Lipid Res* 39: 1412–1420