Hepatic microsomal triglyceride transfer protein messenger RNA concentrations are increased by dietary cholesterol in hamsters

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Abstract In hamsters fed high fat diets enriched in trimyristin, tripalmitin or tristearin, increased dietary cholesterol content was associated with increased plasma concentrations of very low density lipoprotein (VLDL) cholesterol and triacylglycerol ($p < 0.0001$ and $p = 0.0017$, respectively). Hepatic microsomal triglyceride transfer protein (MTP) mRNA concentration also increased ($p < 0.0001$), independent of the nature of dietary fat, and was significantly correlated with the plasma VLDL lipid concentrations ($p = 0.0002$ and $p = 0.0106$ for cholesterol and triacylglycerol, respectively) and hepatic cholesterol concentrations. Increased expression of the MTP gene may be part of a coordinated response to hepatic cholesterol accumulation leading to increased VLDL lipid secretion.

Key words: Hepatic microsomal triglyceride transfer protein; mRNA; Dietary cholesterol; Very low density lipoprotein secretion; Golden Syrian hamster liver

1. Introduction

The microsomal triglyceride transfer protein (MTP) is a heterodimeric protein thought to play an essential role in the synthesis of apoB-containing lipoproteins: chylomicrons in the intestine and very low density lipoprotein (VLDL) in the liver. The larger of its two subunits catalyses the transfer of triacylglycerols (TAGs), cholesteryl esters and phospholipids between phospholipid surfaces [1] and in enterocytes and hepatocytes has been postulated to ferry TAGs from their site of synthesis on the smooth endoplasmic reticulum to the site of VLDL assembly. Support for a critical role for the protein in vivo is provided by patients with abetalipoproteinaemia. Such patients lack functional MTP, due to defects in the MTP gene and are unable to assemble or secrete chylomicrons and VLDL [2]. The human and hamster MTP genes have been cloned and the promoter sequences shown to contain a negative insulin response element and modified sterol response element [3]. Transient transfection analysis of MTP promoter-driven luciferase gene expression in HepG2 cells indicated that insulin down regulates and cholesterol up regulates MTP promoter activity. More recent studies on HepG2 cells confirmed the negative regulation of MTP gene expression by insulin [4]. Studies in vivo in the hamster have implied that dietary factors may affect hepatic MTP mRNA concentrations. The feeding of high fat [5,6] or high sucrose [5] diets caused an increase in hepatic mRNA concentrations that correlated with increased plasma cholesterol and the cholesterol content of individual lipoprotein fractions. Interestingly, in these experiments [5,6], there were no significant correlations between hepatic MTP mRNA levels and either plasma total or VLDL TAG. Furthermore, in diabetic and suckling rats, both conditions in which there is a reduced output of VLDL, no decrease in hepatic MTP activity or protein was seen [7].

In hamsters fed modest amounts of cholesterol, VLDL chol and TAG concentrations were significantly increased and these increases were associated with increased activity of phosphatidate phosphohydrolase-1 (PAP-1), an enzyme thought to be important in regulating TAG synthesis [8]. There have been no reports to date of in vivo effects of dietary cholesterol upon MTP gene expression. In the present study we investigated the effects of diets enriched in both fat and increasing amounts of cholesterol on the concentrations of plasma VLDL chol and TAG and concentrations of hepatic MTP mRNA.

2. Materials and methods

2.1. Animals and diets

60 male DSNI Golden Syrian hamsters were obtained from a closed colony in the Biomedical Services Unit, University of Nottingham. They were housed individually, under environmentally controlled conditions and allowed free access to food and tap water. After a 2 week acclimatization period they were transferred for a further 2 weeks to a standard control diet containing: 235 g/kg casein, 106 g/kg sucrose, 503 g/kg cornstarch, 35 g/kg cellulose, 50 g/kg triolein and 5 g/kg linseed oil. The diet was also supplemented with standard rodent vitamin and mineral mixes (ICN Flow, Bucks., UK). Initially this diet was mixed with normal rodent chow at a ratio of 25:75 (Special Diet Supplies, Essex, UK) with the proportion of chow being decreased to 50% and finally to 20%. Animals were then allocated at random to groups of 6 (TM and TP diets) or 8 (TS diets) and were transferred to a high fat diet (20% w/w) containing 0.005% (diet A), 0.12% (diet B) or 0.24% (diet C) cholesterol. These high fat diets were isocaloric to the control diet and contained the same proportions of protein, sucrose, linseed oil, vitamin and minerals. The fat consisted of 100 g/kg triolein (TO, Fluka, Derbys., UK) and a further 100 g of either trimyristin (TM, Fluka), tripalmitin (TP, Fluka) or tristearin (TS, Sigma Chemical Co., Dorset, UK). Energy content was maintained by reducing starch to 156 g/kg and increasing cellulose to 232 g/kg. Diets were again fed in combination with 20% chow to enhance palatability. Thus, animals were fed one of 3 different types of high fat diets enriched in TO and a saturated fat. In each case, cholesterol was included at one of 3 different levels, giving a total of 9 different diets.

Animals were fed these diets for 28 days with food being completely replaced, every 2 days. On day 28 of the study period, the animals were fasted overnight prior to exsanguination the next day. Animals

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Abbreviations: VLDL, very low density lipoprotein; apoB, apolipoprotein B; MTP. microsomal triglyceride transfer protein; chol, cholesterol; TAG, triacylglycerol; PAP-1, phosphatidate phosphohydrolase-1; LDL, low density lipoprotein; HDL, high density lipoprotein

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were anaesthetized with Sagatal and blood was collected by cardiac puncture. The liver was flushed with saline, divided into two portions, snap frozen and maintained at −40°C until required.

2.2. Separation of lipoproteins

VLDL, low density lipoprotein (LDL) and high density lipoprotein (HDL) were separated by preparative ultracentrifugation at density ranges of < 1.006, 1.02–1.06 g/ml and > 1.06 g/ml, respectively, as previously described [9,10]. Cholesterol and triacylglycerol concentrations were determined automatically using the Olympus system reagent 5000 cholesterol and GPO Trinder triacylglycerol kits, respectively.

2.3. Determination of hepatic free and esterified cholesterol

Hepatic free and esterified cholesterol were extracted, separated by thin-layer chromatography and assayed as previously described [9]

2.4. Isolation of hepatic total RNA and determination of MTP and apoB mRNA concentrations

Total hepatic RNA was isolated by the guanidinium thiocyanate method essentially according to Chomczynski and Sacchi [11]. The mRNA concentrations for hepatic MTP and apoB genes were determined by a solution hybridization/RNAse protection assay as described previously [6,9]. Results were corrected for variation in the mRNA content of total RNA samples by quantitation of poly(A) RNA using oligo-dT_{18} hybridization [12]. All mRNA values are expressed as amol mRNA/μg total RNA.

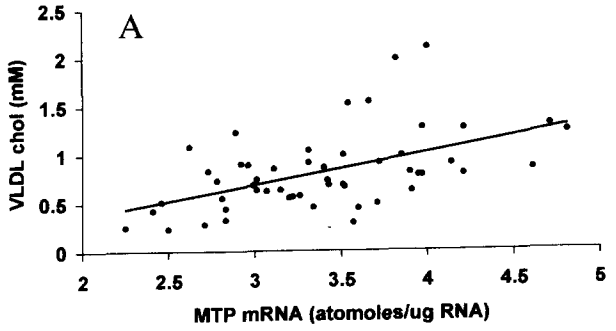
2.5. Statistical analysis

MTP mRNA concentration data were analyzed by two-way analysis of variance (ANOVA) using Genstat-5 statistical software (Lawes Agricultural Trust, Rothamsted, Herts.). If this produced a $p < 0.05$ groups were compared using the Tukey-Kramer multiple comparisons test. Linear regression was performed using C-Stat for Windows software (Cherwell Scientific Publishing Ltd., Oxford, UK).

3. Results and discussion

Diets were well tolerated by all but six animals (one in each TM group, one each in TP groups B and C, and one in TS group B) which were removed from the trial, having lost more than 10% of their initial body weight. No significant differences between the various groups were seen in starting or final body weight or food intake in the remaining animals.

When animals were fed increasing amounts of cholesterol as part of a diet enriched in 3 different saturated fats, the concentration of hepatic MTP mRNA increased (Table 1). Analysis of these data by two-way ANOVA indicated that the concentration of MTP mRNA is not significantly affected by the nature of the fat, with no significant interaction between type of fat and amount of dietary cholesterol. This is in contrast to our recent work on hepatic apoB, LDL receptor and hydroxymethylglutaryl-CoA reductase mRNA levels which appear to be differentially regulated by different saturated fats ([9]; unpublished observations). Thus, MTP mRNA concentration is primarily dependent on the amount of cholesterol in the diet. Indeed, a highly significant correlation between MTP mRNA and dietary cholesterol was evident (Table 2, $p < 0.0001$). Both VLDL TAG and cholesterol were also markedly increased in response to dietary cholesterol, each showing strong linear correlations (Table 2, $p = 0.0017$ and $p < 0.0001$, respectively) and were also highly correlated with each other (Table 3, $p < 0.0001$), consistent with increased VLDL secretion. This confirms our previous findings with a separate strain of Golden Syrian hamster which exhibited an even greater triacylglycerol response to dietary choles-



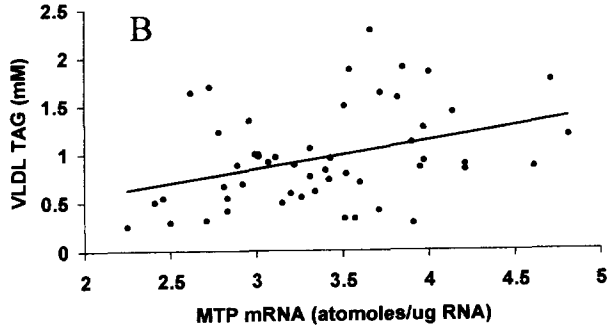


Fig. 1. Correlation of VLDL cholesterol (A) and VLDL triacylglycerol (B) concentrations with hepatic MTP mRNA concentrations. Hamsters were fed high fat (TM, TP or TS) diets containing 0.005, 0.12 or 0.24% cholesterol for 28 days. After an overnight fast, blood was collected and the liver removed. Plasma VLDL was isolated by preparative ultracentrifugation and cholesterol and triacylglycerol concentrations determined as indicated in Section 2. Hepatic mRNA concentrations for the MTP gene were determined by nuclease protection assay as described in Section 2. Linear correlation coefficients were: (A) $r = 0.4943$, $p = 0.0002$; (B) $r = 0.3515$, $p = 0.0106$.

Table 1
Hepatic MTP mRNA concentrations in hamsters fed different saturated fats and different amounts of cholesterol

Diet	Dietary chol (w/w %)	TM	TP	TS	All fats
(A)	0.005	3.14 ± 0.389	2.81 ± 0.302	2.88 ± 0.478	2.93 ± 0.403
		$n = 5$	$n = 6$	$n = 7$	$n = 18$
(B)	0.12	3.54 ± 0.393	3.07 ± 0.343	3.60 ± 0.575	3.45 ± 0.513
		$n = 4$	$n = 4$	$n = 8$	$n = 16$
(C)	0.24	3.86 ± 0.245	3.83 ± 0.557	3.73 ± 0.658	3.79 ± 0.519
		$n = 5$	$n = 5$	$n = 8$	$n = 18$ A^b, B^a

Values are in atomoles/μg RNA and represent mean ± standard deviation with the number of animals indicated. Data were analysed by 2-way analysis of variance with dietary fat and cholesterol as the variables. No significant effect of the type of fat and no significant interaction between type of fat and amount of dietary cholesterol were observed. Thus, data from each fat were pooled to give an overall effect of cholesterol. Significant differences between cholesterol concentrations are indicated: $^a p < 0.05$; $^b p < 0.001$.

Table 2
Linear correlation coefficients for relationships with dietary cholesterol

Parameter	r value	p value
VLDL chol	0.6904	< 0.0001
VLDL TAG	0.4171	0.0017
MTP mRNA	0.6079	< 0.0001
apoB mRNA	0.1842	0.2003

Hamsters were fed and analyses performed as described in Table 1 and Section 2. Data from all animals were pooled.

terol. In this previous trial we showed that cholesterol feeding was associated with an increase in the activity of PAP-1, a rate-limiting enzyme in TAG synthesis [8]. In the present study, while the concentrations of both lipid components of VLDL increased, the rise was greater for cholesterol and resulted in a fall in the VLDL TAG/chol ratio to produce a potentially more atherogenic particle (data not shown). There was a dramatic increase in hepatic cholesteryl ester with increasing dietary cholesterol and also a smaller, but highly significant, increase in free cholesterol, with significant linear correlations with dietary cholesterol ($p < 0.001$ and $p < 0.002$, respectively) for all three saturated fat diets. Furthermore, plasma VLDL cholesterol concentrations were strongly correlated with hepatic cholesterol ester concentrations (Table 3, $p < 0.0001$). These correlations support the suggestion that the increase in VLDL output from the liver may be an attempt to mobilize increasing hepatic cholesterol stores [8,13].

The hepatic TAG produced by increased PAP-1 activity has to be incorporated, together with apoB and cholesteryl ester into the VLDL particles. Both MTP activity and apoB are required for this process. However, there was no consistent increase in hepatic apoB mRNA concentrations on cholesterol feeding (Table 2) and no correlation with the plasma concentrations of either VLDL chol or TAG, suggesting that, at least in this situation, apoB mRNA levels are not rate limiting for VLDL synthesis and secretion. By contrast, not only were MTP mRNA concentrations increased (Table 1), but they also strongly correlated with VLDL chol and TAG concentrations ($r = 0.4943$, $p < 0.0002$ and $r = 0.3515$, $p < 0.0106$, respectively; Fig. 1). In contrast, no correlations were seen between MTP mRNA and apoB mRNA, or low density lipoprotein (LDL) chol, whereas a significant positive correlation was observed between MTP mRNA and high density lipoprotein (HDL) chol (Table 3). It is noteworthy that we also found stronger correlations for hepatic MTP mRNA concentrations with HDL chol than LDL chol, when hamsters were fed increasing dietary fat at low cholesterol concentrations [6]. It remains to be established that the increases in MTP mRNA concentration observed here result in increased protein levels and transfer activity. It is possible that increased amounts of MTP are required to transfer the increased amount of lipid to the maturing lipoprotein particle. Functional MTP appears to be required for VLDL production [2], facilitating both translocation of apoB across the endoplasmic reticulum and its association with lipid [14–16] and MTP appears to be rate limiting for these processes in cultured cells [17,18]. However, reduced VLDL output in vivo does not appear to be associated with reduced MTP activity [7]. Thus, MTP activity may be only one of a number of factors involved in regulating VLDL synthesis and secretion [19].

It appears likely that the increased VLDL lipid output is in response to the increased accumulation of cholesteryl ester in the liver. There is conflicting evidence in the literature on the effects of increased hepatic cholesteryl ester concentration on the secretion of VLDL. Wu et al. [20] showed that a variety of manipulations which altered cholesteryl ester concentration in HepG2 cells had no effect on VLDL secretion, while Fungwe et al. [13,21] have described a stimulation of VLDL formation in the livers of rats and hamsters fed cholesterol-enriched diets. Recent data from human subjects also emphasise the importance of available cholesterol in regulating VLDL secretion [22]. The current data, together with our previous results [8], support the findings of Fungwe et al. [13,21]. It could be speculated that the need to mobilize increased amounts of cholesteryl ester elicits a coordinated response in the liver, whereby TAG synthesis, via PAP-1, MTP mRNA concentrations and incorporation of chol and TAG into VLDL are all up regulated. The strong positive correlations between MTP mRNA and hepatic free and esterified cholesterol concentrations (Table 3, $p = 0.0017$ and $p < 0.0001$, respectively), and between VLDL chol and hepatic cholesterol ester (Table 3, $p < 0.0001$) support this notion. Approx. 50% of hamster VLDL chol is esterified (Salter, unpublished observations). It is entirely possible that MTP is involved in the transport of cholesteryl ester as well as triacylglycerol [23]. Interestingly, in the hamster, hepatic MTP mRNA concentrations tend to correlate better with lipoprotein cholesterol than with triacylglycerol concentrations ([5,6] and this work).

The promoter region of the MTP gene contains a modified sterol regulatory element and the expression of the gene may be up regulated by cholesterol [3]. However, addition of cholesterol to HepG2 cells had only a minimal effect on MTP gene expression and the authors suggest that this is probably due to the inability of their Northern blot assay system to detect any moderate increase in MTP mRNA [3]. The increase in MTP mRNA resulting from increased dietary cholesterol in the present study may be a direct result of increased transcription mediated by the modified sterol regulatory element in the MTP promoter. Such speculation is supported by (i) the fact that expression of the 7α-hydroxylase gene, which contains a similar modified sterol regulatory element in its promoter, is also moderately regulated by cholesterol [24] and (ii) the positive correlation of both hepatic free and esterified cholesterol concentrations with MTP mRNA concentrations (Table 3). Although an effect on mRNA stability cannot be ruled out at this stage, previous studies have demonstrated that increases in MTP mRNA concentrations are due to transcriptional activation [3].

To our knowledge these results represent the first demon-

Table 3
Linear correlation coefficients for relationships of hepatic MTP mRNA and lipoprotein or hepatic lipid concentrations

Parameter A	Parameter B	r value	p value
MTP mRNA	LDL chol	0.1166	0.4103
MTP mRNA	HDL chol	0.3828	0.0051
MTP mRNA	apoB mRNA	0.1508	0.2958
MTP mRNA	hep chol	0.4245	0.0017
MTP mRNA	hep chol ester	0.5220	< 0.0001
VLDL chol	hep chol ester	0.5333	< 0.0001
VLDL chol	VLDL TAG	0.6615	< 0.0001

Hamsters were fed and analyses performed as described in Table 1 and Section 2. Data from all animals were pooled. Hep chol and hep chol ester refer to hepatic free and esterified cholesterol, respectively.

stration of an increase in MTP mRNA in vivo in response to dietary cholesterol and differs from the high fat diet effects which have been ascribed to the induction of hepatic insulin insensitivity alleviating the down regulation of MTP gene expression via the insulin response element [4].

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