Monomethylethanolamine reduces plasma triacylglycerols and apolipoprotein B and increases apolipoprotein A-I in rats without induction of fatty liver

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Abstract Monomethylethanolamine (MME) inhibits very low density lipoprotein (VLDL) secretion from cultured rat hepatocytes by disruption of translocation of apolipoprotein (apo) B across the endoplasmic reticulum membrane (A. E. Rusiñol, E. Y. W. Chan and J. E. Vance. 1993. *J. Biol. Chem.* **268:** 25168–25175). We have now investigated whether or not plasma levels of lipids and apoB are reduced by dietary supplementation of rats with MME. In rats fed MME for 5 to 7 days, the levels of triacylglycerols and apoB in VLDL were reduced by 66% and 45%, respectively. At the same time, MME feeding also increased plasma apoA-I by 80%. No significant differences were found in body or liver weights between control and MME-fed rats, nor did the reduction of plasma VLDL in MME-fed rats result in accumulation of triacylglycerols in the liver. When the dietary period was extended to 15 weeks, essentially the same results were obtained except that plasma cholesterol was increased by 31% in MME-treated animals, apparently because of increased amounts of apoA-I and high density lipoproteins. According to post-mortem and microscopic examination, rats fed MME for 15 weeks were anatomically normal with no indication of any lipid accumulation in the liver. The ability of MME to reduce VLDL secretion and at the same time to increase the level of high density lipoproteins are attractive properties of a therapeutic agent for treatment of atherosclerosis in humans.—**Rusiñol, A. E., P. S. Lysak, G. T. Sigurdson, and J. E. Vance.** Monomethylethanolamine reduces plasma triacylglycerols and apolipoprotein B and increases apolipoprotein A-I in rats without induction of fatty liver. *J. Lipid Res.* 1966. **37:** 2296–2304.

Supplementary key words phospholipids • very low density lipoprotein • high density lipoprotein • cholesterol • atherosclerosis

A strong correlation exists between high plasma levels of apolipoprotein (apo) B and the development of atherosclerosis and coronary heart disease (1). The plasma concentration of apoB is determined by the balance between its rate of secretion from the liver and intestine and its rate of clearance from plasma by the liver and other tissues.

ApoB is one of the largest monomeric proteins known to be secreted. Mature human apoB-100 has a molecular mass of 512 kDa and computer-based structural analysis predicts a pentapartite structure consisting of a globular N-terminal domain followed by alternating amphipatic β-sheets (probably lipid binding regions) and α-helices (2). ApoB is essential for the assembly and secretion of hepatic very low density lipoproteins (VLDL), and is unique amongst secretory proteins because it is secreted in association with lipids in the form of lipoprotein particles. Modulation of the secretion of apoB appears to occur mainly post-translationally (3, 4). Not all newly synthesized apoB is secreted, but instead, some is rapidly degraded within the cell (5–8). Several lines of evidence indicate that two populations of apoB exist intracellularly. In one pool, apoB is fully translocated across the endoplasmic reticulum membrane and is present in the lumen in the form of lipoprotein particles. In the other pool, apoB is membrane-bound with portions of the molecule being exposed to the cytosol (5, 9–11). Many recent studies have suggested that translocation of apoB across the endoplasmic reticulum membrane, perhaps in conjunction with assembly of the protein with lipids, might be an important regulatory step in VLDL secretion (9–12).

We have recently developed a model for studying factors that regulate apoB secretion. Cultured hepatocytes were incubated with monomethylethanolamine (MME), a structural analog of choline and ethanolamine, that becomes incorporated into the phospho-

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; MME, monomethylethanolamine; PMME phosphatidylmonomethylethanolamine.

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lipid phosphatidylmonomethylethanolamine (PMME) in cellular membranes (13). When hepatocyte membranes are enriched in PMME the secretion of apoB-100 and apoB-48, but not other secretory proteins such as apoA-I and albumin, is inhibited by 50–70% (13). We also demonstrated that administration of MME to cultured rat hepatocytes does not alter the rate of synthesis of apoB or the principal lipids associated with VLDL (i.e., triacylglycerols, cholesteryl esters, phosphatidylcholine, and phosphatidylethanolamine) (13, 14). We subsequently demonstrated that the decreased secretion of apoB in response to PMME enrichment of membranes results in: *i*) a decreased amount of apoB being present in the microsomal lumen, *ii*) an increased exposure of apoB on the cystolic surface of microsomes, and *iii*) an increased intracellular degradation of apoB (11). The inhibitory effect of PMME enrichment on apoB secretion is also evident for the secretion of carboxy-terminal truncated apoB variants (e.g., the amino terminal 15, 18, 23, and 28% of human apoB-100) expressed in McArdle 7777 rat hepatoma cells (14). As apoB-15 and apoB-18 are not secreted as buoyant lipoprotein particles, but are secreted without a core of neutral lipids with a density of 1.2 g/ml, we conclude that PMME enrichment of endoplasmic reticulum membranes impairs the translocation or folding process of apoB per se and is not related to association of apoB with the bulk of neutral lipids such as triacylglycerols.

We have now extended the MME model to animals and report that the apoB- and triacylglycerol-lowering effects of dietary MME in rats are accompanied by a marked elevation of HDL. Moreover, our data show that the decreased secretion of triacylglycerol does not result in the development of fatty livers in rats.

METHODS

Materials

Monomethylethanolamine was purchased from Sigma. Polyclonal antibodies directed against rat apoproteins B and A-I were generated in rabbits and characterized in our laboratory by standard procedures (15). Antibodies directed against human apoB were purchased from Boehringer Mannheim (Germany). The reagents used for electrophoresis were supplied by Bio-Rad. Polyvinylidene difluoride membranes were from Millipore. All other chemicals were purchased from either Sigma or Fisher Scientific.

Animals

Male Wistar rats weighing 200–300 g were maintained under a 12-h light–dark cycle with free access to food and water. Control rats were fed laboratory Purina chow. MME-treated animals were fed the same chow supplemented with 1% (v/w) MME. After 5 to 7 days the unfasted animals were anesthetized with diethyl ether, then killed by exsanguination from the descending vena cava. Blood was collected into a test tube containing EDTA (4 mg/ml blood) and plasma was obtained by centrifugation. Livers were rapidly excised, rinsed in ice-cold, phosphate-buffered saline and frozen at −20°C for lipid analysis. In the long-term feeding experiments the animals were fed MME for 15 weeks according to the following regimen. For the first 11 weeks 0.5% (v:v) MME was provided in the drinking water, then drinking water was given without added MME, but at the same time 1% MME (v/w) was included in the diet for 4 additional weeks. At the end of the 15-week feeding period the amount of PMME in the livers was ~10% of total phospholipids. The PMME concentration of erythrocytes, as a percentage of the total amount of phospholipids, was 4.8% after 1 week, 5.1% after 8 weeks, and 5.3% after 15 weeks of MME feeding.

Separation and analysis of lipoproteins and apolipoproteins

VLDL ($d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml), and HDL ($1.063 < d < 1.18$) g/ml were isolated from plasma by sequential ultracentrifugation on a bench-top Beckman TL-100 ultracentrifuge as described by Brousseau et al. (16) which allows the complete separation of these lipoprotein classes in 1 day. The total plasma concentration and lipoprotein content of apoB and apoA-I were determined by immunoblotting or slot-blotting assays using polyclonal antibodies directed against rat apoB and rat apoA-I. For immunoblotting, proteins were separated on 7% polyacrylamide minigels containing 0.1% SDS, then transferred to polyvinylidene difluoride membranes for 12 h at 50 v. The membrane was blocked with 5% (w/v) dry non-fat milk in Tris-buffered saline solution (pH 7.5) containing 0.1% Tween, and probed with rabbit anti-rat apoB (dilution 1/10,000) or rabbit anti-ratA-I (dilution 1/5,000) as primary antibodies. Immunoreactive proteins were detected by incubation with goat anti-rabbit IgG (dilution 1/10,000) antibodies linked to horseradish peroxidase, as secondary antibody. Bound antibodies were visualized with enhanced chemiluminescence reagents according to the manufacturer's instructions. For the slot-blot assay, 25-µl aliquots of 1/1,500 diluted plasma samples were blotted directly onto the polyvinylidene difluoride membranes under a slight vacuum using a mini slot-blot apparatus from Bio-Rad. Immunoblotting was performed as described above. The assay was standardized using a series of dilutions of HDL_3 or VLDL containing only apoB, prepared from rats injected with Triton 1339 which causes

stripping of exchangeable apoproteins from VLDL (17). The amounts of apolipoproteins were selected so that the assay was performed in the linear portion of the standard curve.

Isolation and analysis of lipids from liver, plasma, and lipoproteins

Extraction of total lipids from liver samples was performed by Bligh and Dyer extraction (18). Individual lipid classes were separated by thin-layer chromatography using the following two-solvent system which allows separation of polar and neutral lipids on one plate. The samples were first developed to about two thirds of the length of the plate in chloroform–methanol–acetic acid–formic acid–water 65:35:12:4:2 (v/v). The solvents were allowed to evaporate and the plate was run to the top in hexane–isopropyl ether–acetic acid 65:35:10 (v/v). PMME was separated from other phospholipids by thin-layer chromatography as described previously (19). After separation, the lipids were quantitated either by charring and densitometric scanning or by scraping the individual bands from the plate after impregnation with iodine vapors, followed by chemical or enzymatic determination. The content of cholesterol (including both esterified and unesterified cholesterol) and triacylglycerols in plasma and lipoproteins was measured using commercial kits from Sigma and Wako Pure Chemical Industries Ltd. (Japan), respectively. The amounts of total phospholipids were determined by measurement of the phosphorus content of the lipid extract (20). The protein content of samples was measured by the BCA protein assay kit from Pierce Chemicals (Rockford, IL).

The statistical significance of differences between data from control and MME-fed animals was analyzed by Student's *t* test.

RESULTS

Dietary MME reduces plasma triacylglycerols and cholesterol

In rats fed a diet containing 1% (v/w) MME for 5–7 days no significant differences in body weights were observed compared to those of untreated animals (281 ± 74 g for control animals, 272 ± 58 g for animals fed MME). Liver weights were also similar in the two groups of animals (12.3 ± 4.3 g for control rats and 13.0 ± 5.1 g MME-fed rats). The effects of MME supplementation on plasma lipid (triacylglycerols, phospholipids, and cholesterol combined with cholesteryl esters) levels are shown in **Fig. 1.** When 1% MME was included in the diet for a period of 5–7 days, plasma cholesterol and

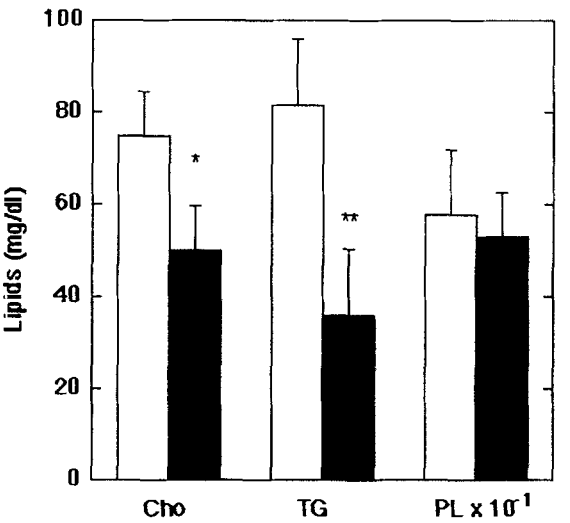

Fig. 1. Response of plasma lipids to dietary supplementation (5–7 days) with MME. Male rats were maintained on regular chow (open bars) or chow supplemented with 1% (v/w) MME (solid bars) for 5–7 days. The concentrations (mg/dl plasma) of triacylglycerols (TG), cholesterol (Cho) (including unesterified and esterified cholesterol), and phospholipids (PL) were determined. Values are averages of determinations from 12–18 individual rats ± SD. $^{*}P < 0.05$; $^{**}P < 0.001$ for MME-fed animals compared to controls.

triacylglycerols were significantly reduced by 27.3 ± 8.0% and 55.6 ± 9.3%, respectively. The concentration of phospholipids in plasma and the total protein concentration in plasma were not significantly different in control and MME-fed animals.

We next analyzed the lipid content of the individual plasma lipoproteins. Three lipoprotein fractions of densities corresponding to VLDL, LDL, and HDL were separated from plasma by ultracentrifugation. As shown in **Fig. 2A,** MME feeding reduced the amount of triacylglycerols in the VLDL fraction by 66%. This substantial reduction of triacylglycerols in VLDL was not compensated for by an increase in the triacylglycerol content of any of the other lipoprotein fractions. The cholesterol content of VLDL was also reduced (Fig. 2B), but to a smaller extent (by 38%) than for triacylglycerols. A small, but statistically significant, increase in HDL cholesterol content was also observed (Fig. 2B). Likewise, MME feeding caused a small, statistically insignificant, increase in the amount of HDL phospholipids (Fig. 2C). The PMME content of VLDL, LDL, and HDL in the MME-fed animals was 5.3%, 3.2%, and 4.3% of total phospholipids, respectively.

Dietary MME reduces plasma apoB and increases plasma apoA-I

As a reduction in the VLDL triacylglycerol level could be the result of either smaller-sized VLDL particles or a reduced number of particles secreted, we examined the effect of MME on the plasma concentration of apoB

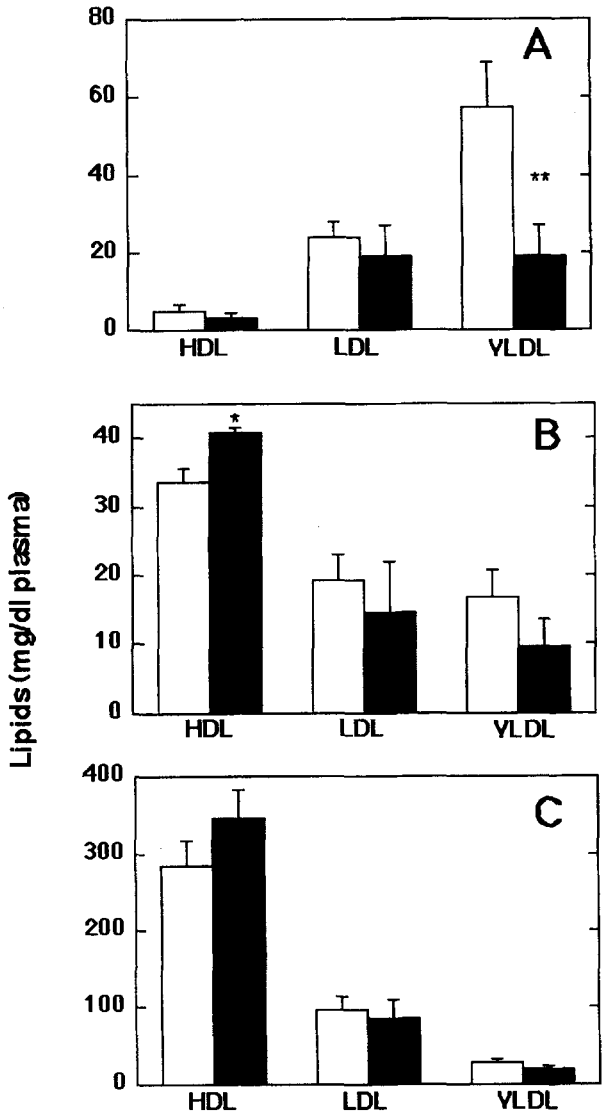

Fig. 2. Response of lipids of plasma lipoproteins to dietary supplementation (5–7 days) with MME. Male rats were maintained on regular rat chow (open bars) or rat chow supplemented with 1% (v/w) MME (solid bars) for 5–7 days. Three lipoprotein fractions, VLDL, LDL, and HDL, were isolated from plasma by sequential ultracentrifugation. Lipids wre extracted from the lipoprotein fractions and concentrations (mg/dl plasma) of triacylglycerols (panel A), cholesterol (panel B), and phospholipids (panel C) were determined in each lipoprotein class. Values are averages of 4 determinations ± SD. $^{**}P < 0.001$; $^{*}P < 0.01$ for MME-fed animals compared to control animals.

by a standardized slot-blotting assay (**Table 1**). In animals fed MME, the amount of plasma apoB was 45 ± 9% lower than in control animals, the majority of the reduction being in the VLDL fraction. The slot-blotting assay does not discriminate between apoB-100 and B-48, therefore plasma samples from MME-treated and control animals were subjected to electrophoresis and immunoblotting using polyclonal antibodies directed against rat apoB. MME feeding reduced the amounts of both apoB-100 and apoB-48 in plasma (**Fig. 3,** left panel). The intensities of the immunoreactive bands were quantitated by densitometric scanning of the film using a series of external standards to calibrate the scanner and software. In rats fed dietary MME, the reduction of plasma apoB-48 (49.8 ± 12%) was more pronounced than of apoB-100 (31.0 ± 7.5%) (Fig. 3, right panel).

TABLE 1. Dietary MME supplementation (5–7 days) reduces plasma apoB and increases plasma apoA-I concentrations

	Plasma	VLDL	LDL	HDL
ApoB	0.55 ± 0.09[b]	0.60 ± 0.11[a]	0.78 ± 0.13	ND
ApoA-I	1.18 ± 0.07[b]	ND	ND	1.49 ± 0.12[a]

Male rats were maintained on regular chow or chow supplemented with 1% (v/w) MME for 5–7 days. Three lipoprotein fractions (VLDL, LDL, and HDL) with densities defined in the legend to Fig. 2 were isolated. Aliquots of plasma or isolated lipoproteins were blotted onto polyvinylidene difluoride membranes and probed with anti-rat apoB or anti-rat apoA-I antibodies. The amounts of apoB and apoA-I were determined by slot-blotting assays. Values represent the ratio between the amounts of apolipoproteins in MME-treated rats versus control rats and are averages from 3–8 individual rats ± SD.

[a]$P < 0.05$; [b]$P < 0.001$; for MME-treated animals compared to untreated animals; ND, not determined.

The level of apoA-I in plasma and in the HDL fraction was also determined (Table 1). Surprisingly, the plasma level of apoA-I was almost doubled in the MME-fed animals. The increase was mainly in the HDL fraction (49% increase), the remainder of the increase in apoA-I presumably being in lipid-poor or in non-lipoprotein-associated apoA-I.

MME does not cause accumulation of triacylglycerols in liver

As the liver is an important source of plasma triacylglycerols, and as the plasma triacylglycerol level was decreased by 56% after MME treatment for 5–7 days (Fig. 1), one might anticipate that triacylglycerols would accumulate in the liver as a consequence of the blockage in VLDL secretion. Therefore, the amounts of the major lipids were analyzed in liver samples from control rats fed MME (**Fig. 4**). In contrast to our prediction, the triacylglycerol concentration was not higher but was 56.3 ± 7.3% lower in the livers of MME-treated rats than in livers from control rats. No significant change in the cholesterol level of the liver was detected as a result of MME feeding. Nor were the amounts of total liver phospholipids significantly affected, although the concentration of PMME in the liver increased from trace amounts (less than 1% of total phospholipid) in control animals to 15% of total phospholipids in MME-fed animals.

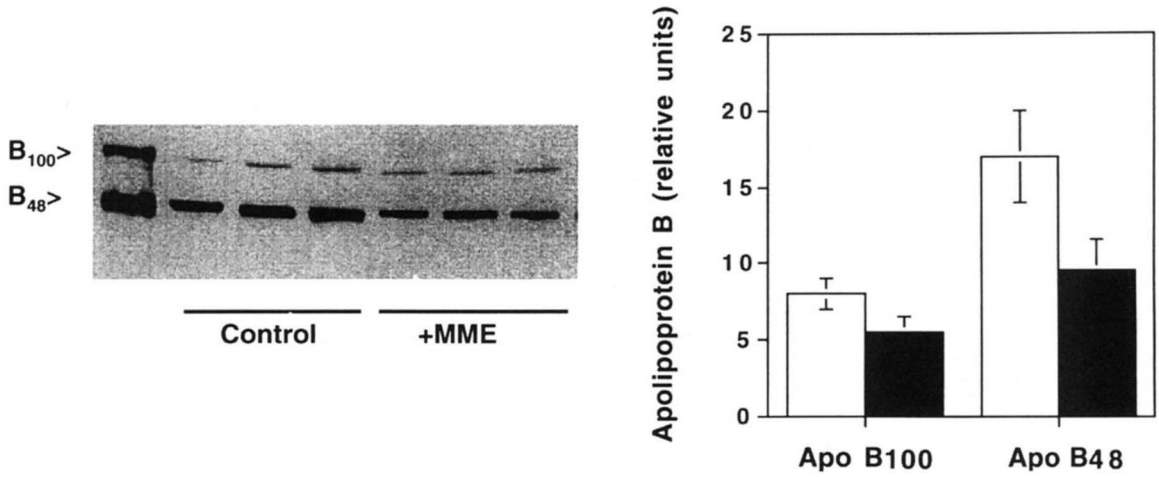

Fig. 3. Plasma content of apolipoproteins B-100 and B-48 is reduced upon dietary supplementation (5–7 days) of rats with MME. Male rats were maintained on regular chow (control and open bars) or chow supplemented with 1% (v/w) MME (+MME and solid bars) for 5–7 days. Left panel: equal amounts of plasma proteins were separated by electrophoresis on 7% polyacrylamide gels containing 0.1% SDS, then immunoblotted using an antibody directed against the rat apoB. Samples from 3 control animals and 3 MME-fed animals are shown as examples. The apoB-100 and apoB-48 bands on the blot were scanned by densitometry and their integated densities are represented as relative arbitrary units (right panel). In the right panel, values represent averages of data from 6 individual animals ± SD.

Long-term effects of MME supplementation

We next examined longer-term effects of MME feeding on plasma lipid and apoprotein levels and the health status of the animals. After 15 weeks of treatment with MME, all control and MME-treated rats appeared to be healthy and were anatomically normal, based upon a gross post-mortem examination and histological examination of the livers. Moreover, body weights and liver weights of MME-fed animals were normal. The average liver weight of control rats was 16.9 ± 3.3 g, and for MME-fed rats was 18.1 ± 5.3 g. The MME-fed rats did not develop fatty livers and showed no evidence of accumulation of lipid droplets in their livers. As shown in **Fig. 5B,** the triacylglycerol concentration in the livers of rats fed MME for 15 weeks was not increased compared to that of control animals but was decreased by 37.5 ± 6.1%.

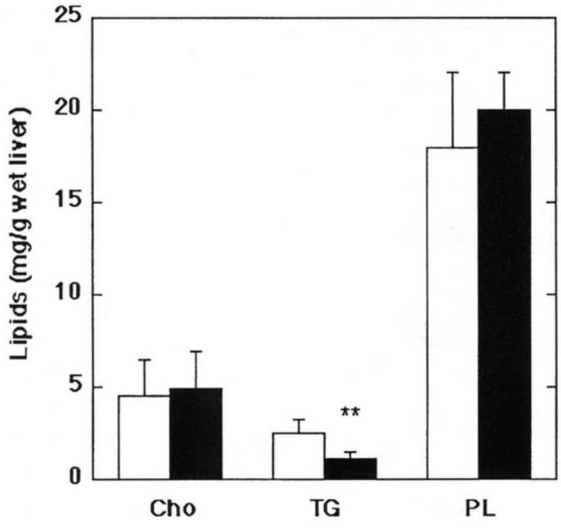

Fig. 4. The amount of triacylglycerols in the liver is reduced in response to dietary supplementation (5–7 days) with MME. Male rats were maintained on regular chow (open bars) or chow supplemented with 1% (v/w) MME (solid bars) for 5–7 days. Lipids were extracted from liver samples. The concentrations (mg/g wet weight of liver) of triacylglycerols (TG), cholesterol (including esterified and unesterified cholesterol) (Cho), and phospholipids (PL) were determined. Values are averages of data from 4–8 individual rats ± SD. $^{**}P < 0.001$ for animals fed MME versus control animals.

The lipid content of plasma of control rats and rats fed the MME-supplemented diet over a 15-week period is shown in Fig. 5A. With the exception of plasma cholesterol, the changes in lipid and apolipoprotein profiles upon long-term (15 weeks) MME feeding were similar to those in the short-term feeding experiments (5–7 days) (compare Fig. 5A with Fig. 1). After 15 weeks of dietary supplementation of rats with MME, the plasma content of apoB was reduced by 48 ± 22% compared to that in control animals, and the amount of plasma apoA-I was greatly increased, by 201 ± 45% (**Table 2**). Corresponding to the decrease in plasma apoB, the content of plasma triacylglycerols in the MME-fed rats was 45.8 ± 2.8% lower than that in control animals. This decrease was similar to that observed in the shorter term experiments (Fig. 1) and was mainly in the VLDL fraction (**Fig. 6A**).

In contrast to the reduction in plasma cholesterol observed after short-term dietary supplementation with MME, after 15 weeks of MME administration the plasma cholesterol level was significantly higher (by 31.1%, $P < 0.05$) than in control rats (Fig. 5A). This increment reflected the increase in the amount of cholesterol in

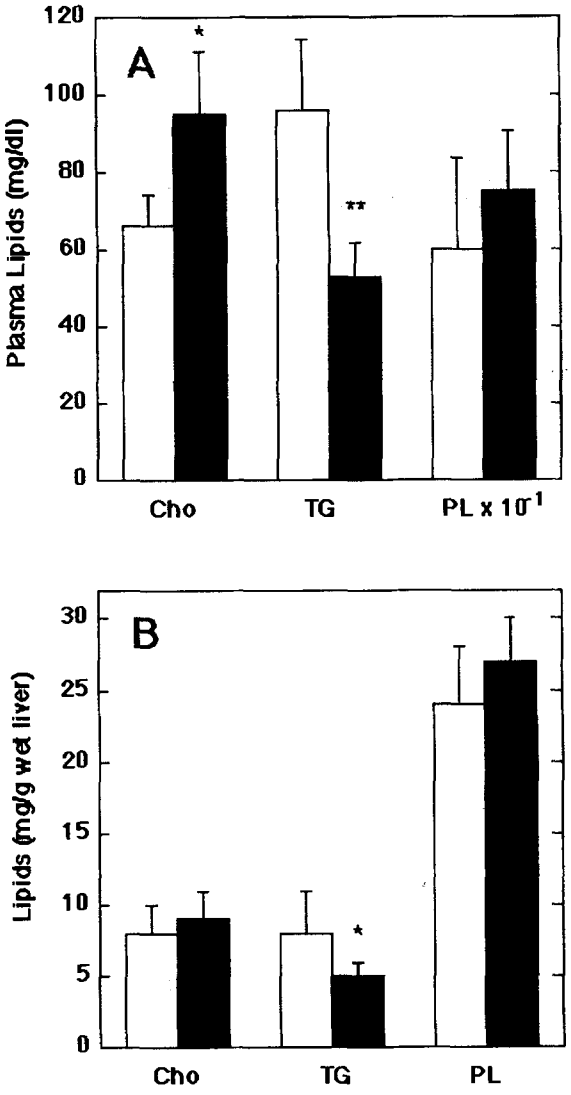

Fig. 5. Response of liver and plasma lipids to dietary supplementation with MME for 15 weeks. Male rats were maintained on regular chow (open bars). Other animals were maintained on a MME-supplemented diet for 15 weeks (solid bars) after which the concentrations of plasma (panel A) and liver (panel B) triacylglycerols (TG), cholesterol (Cho), and phospholipids (PL) were determined. Values are averages ± SD of 4 determinations. $*P < 0.05$; $**P < 0.001$ for MME-fed versus control animals.

the HDL fraction (37.7%) (Fig. 6B). The concentrations of phospholipids (Fig. 6C) and proteins in plasma were not significantly different in control rats and rats treated with MME for 15 weeks.

DISCUSSION

This study demonstrates that dietary supplementation of rats with MME for as little as 5 days markedly reduced plasma lipids, in particular VLDL triacylglycerols which were reduced by 66%. The reduction in the triacylglycerol content of plasma was accompanied by an almost parallel decrease (by 45%) in the amount of plasma apoB. These data indicate that the number of plasma VLDL particles was reduced in rats fed MME. These observations in intact rats support our previous finding that when cultured primary rat hepatocytes were incubated with MME the secretion of VLDL lipids and apoproteins was reduced by 50–70% (11, 13). The VLDL-lowering effect of MME did not appear to be detrimental to the growth or health of the animals as no significant differences in body or liver weight were detected between the two groups of rats. Complications sometimes observed after diet-induced reduction in serum lipoproteins, such as an increase in liver size or liver cell proliferation (21, 22), were not apparent in this study.

TABLE 2. Dietary supplementation with MME (15 weeks) reduces plasma apoB and increases plasma apoA-I concentrations

	Plasma	VLDL	LDL	HDL
ApoB	0.52 ± 0.22[b]	0.55 ± 0.21[a]	0.68 ± 0.22	ND
ApoA-I	2.01 ± 0.45[b]	ND	ND	1.80 ± 0.40[a]

Male rats were maintained on MME-supplemented diet for 15 weeks. Three lipoprotein fractions (VLDL, LDL, and HDL) were isolated. Aliquots of plasma or isolated lipoproteins were blotted onto polyvinylidene difluoride membranes and probed with anti-rat apoB or anti-rat apoA-I antibodies. The amounts of apoB and apoA-I were determined by slot-blotting assays. Values represent the ratio between the amounts of apolipoproteins in MME-treated rats versus control rats and are the averages from 4 individual rats ± SD.

[a] $P < 0.05$; [b] $P < 0.001$; for MME-treated animals compared to untreated animals; ND, not determined.

Although the plasma apoB and triacylglycerol concentrations were reduced by 45% and 66%, respectively, upon MME feeding, triacylglycerols did not accumulate in the liver as might have been anticipated to be the result of inhibition of VLDL secretion. The observation is, however, consistent with our earlier finding that when VLDL secretion was inhibited by MME in cultured rat hepatocytes, triacylglycerols did not accumulate within the cells (11, 13). These observations are in sharp contrast to those from other experimental manipulations that reduce VLDL secretion [e.g., choline deficiency (23, 24) or treatment with orotic acid (25, 26)] in which inhibition of apoB secretion was accompanied by a massive accumulation of triacylglycerols and other lipids in the liver and hepatocytes. We do not yet have an explanation for why fatty liver does not develop in MME-treated animals. One possible explanation is that MME inhibits the synthesis of triacylglycerols and/or increases the rate of degradation of triacylglycerols in the liver, although preliminary experiments in cultured rat hepatocytes suggested that triacylglycerol metabolism is unaffected by MME (13). Another hypothesis

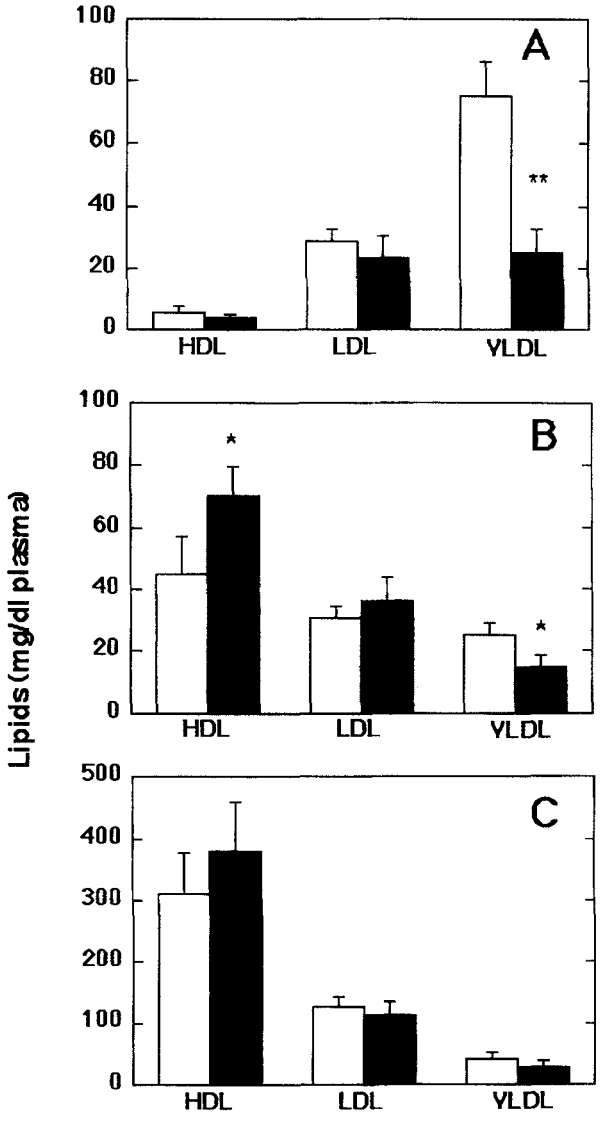

Fig. 6. Response of lipids of plasma lipoproteins to dietary supplementation (15 weeks) with MME. Male rats were maintained on regular chow (open bars) whereas another group of rats was maintained on a MME-supplemented diet for 15 weeks (solid bars). Three lipoprotein fractions (VLDL, LDL, and HDL) were isolated and lipids were extracted. Concentrations (mg/dl plasma) of triacylglycerols (panel A), cholesterol (panel B), and phospholipids (panel C) in each lipoprotein fraction were determined. Values are averages from 4 individual rats ± SD. * $P < 0.05$; ** $P < 0.001$ for animals fed MME compared to control animals.

currently being tested in our laboratory is that secretion of apoB-containing chylomicrons from the intestine is also blocked by MME treatment. A reduction in chylomicron secretion would be expected to decrease the influx of fatty acids into the liver and thereby might limit VLDL secretion as incubation of HepG2 human hepatoma cells with oleic acid is known to stimulate both lipid synthesis and secretion of apoB and its associated lipids (7). The availability of lipids such as triacylglycerols, phospholipids, and cholesteryl esters in the liver is generally believed to regulate how much apoB is secreted in the form of VLDL (3). In support of our hypothesis, we have shown in preliminary experiments that the secretion of apoB-100 and apoB-48, as well as their associated lipids, from intestinal adenocarcinoma CaCo2 cells was inhibited by 50–70% by MME treatment (A. E. Rusiñol and J. E. Vance, unpublished data). However, in the human disease abetalipoproteinemia, in which apoB secretion is impaired because of a defect in the microsomal triacylglycerol transfer protein (27), lipids accumulate in the liver, even though secretion of chylomicrons from the intestine is apparently also impaired (27, 28). Moreover, in MME-treated primary rat hepatocytes, although apoB secretion is decreased by 50–70%, triacylglycerols do not accumulate in the cells (13).

One unexpected finding from these experiments is that the amounts of plasma apoA-I and HDL are greatly elevated in rats supplemented with dietary MME. In the short- and long-term feeding experiments with MME, the amount of plasma apoA-I was increased by 80% and 200%, respectively. In addition, after 5–7 days, the amount of plasma cholesterol was slightly decreased, and plasma triacylglycerols (reflecting VLDL secretion) were reduced by 66%. When rats were maintained on the MME-containing diet for a longer time period (15 weeks), total plasma cholesterol (Fig. 5A) and HDL cholesterol (Fig. 6B) were significantly higher than in control animals, most likely reflecting the increased plasma levels of apoA-I and HDL. At the present time we have no satisfactory explanation for why plasma HDL and apoA-I levels are increased by MME feeding. We have previously shown, however, that incubation of rat hepatocytes with MME did not result in an increased secretion of apoA-I (11, 13). Thus, rather than MME increasing apoA-I secretion, it is likely that removal of apoA-I from plasma of MME-treated rats is impaired. Other examples are known in which a concomitant decrease in plasma apoB and an increase in HDL occurs, for example, when rats are fed cholestyramine, but the mechanism of these changes is not known (21).

The data presented in this study suggest a novel approach for the therapeutic reduction of serum levels of apoB and lipids, and thus for decreasing the risk of atherosclerosis in humans, particularly in those suffering from familial combined hyperlipidemia. This common disorder of lipid metabolism is characterized by an increased production of VLDL (29) although the molecular basis for the phenotype is not clear. One advantage of using MME to reduce VLDL secretion is that MME is apparently relatively nontoxic. Indeed, MME and the corresponding phospholipid PMME are com-

pounds that occur naturally at low levels in animal cells. Previously, the general consensus was that if apoB secretion from the liver were disrupted, triacylglycerols would accumulate in the liver and fatty liver would develop. Such a liver abnormality has previously been reported in individuals suffering from the inherited disorder abetalipoproteinemia in which apoB secretion is impaired (27, 28), and also in animal models in which VLDL secretion is reduced (23–26). In contrast to these situations, MME effectively reduces apoB secretion in rats without induction of fatty liver.

A second potential benefit of MME therapy is that, at least in rats, MME increases the plasma concentration of apoA-I, the principal apolipoprotein of HDL, by 80–200%. An inverse correlation has been established between HDL levels and the risk of developing coronary heart disease. High levels of serum HDL are thought to be beneficial due to the role of HDL in reverse cholesterol transport, the process by which cholesterol is believed to be removed from peripheral tissues and delivered to the liver for excretion.

Although we anticipate that the effects of MME in rats in reducing plasma concentrations of apoB and its associated lipids, and increasing circulating HDL levels, would be similar to those in humans, the usefulness of the rat as a model for human lipoprotein metabolism has been questioned. In rats the major cholesterol carrier is HDL, whereas in humans the majority of serum cholesterol is carried in LDL. Experiments are currently underway in our laboratory to examine the effect of MME on lipoprotein metabolism in hamsters whose serum lipoprotein profile more closely resembles that of humans.

Our previous studies have shown that MME inhibited the secretion of apoB-containing VLDL by rat hepatocytes (11, 13) and rat hepatoma cells (14) by disrupting the translocation of apoB across the endoplasmic reticulum membrane, thereby limiting the entry of apoB, and its associated lipids, into the secretory pathway. As a result, the intracellular degradation of apoB was increased. In these cultured cells, MME affected neither apoB synthesis nor the synthesis of VLDL lipids; nor was protein secretion in general inhibited by MME. In the present study we show that in rats MME reduces the serum content of VLDL-associated apoB and triacylglycerols in vivo. We therefore conclude that the most feasible mechanism by which MME reduces the amount of serum VLDL in vivo is the same as that deduced from the in vitro studies, i.e., PMME enrichment of endoplasmic reticulum membranes impairs the translocation of apoB across the membrane into the lumen. However, the effect of MME on translocation of apoB across the endoplasmic reticulum membranes of the liver in intact rats was not directly examined in the present study. Currently, the drugs most commonly used for reduction of serum levels of apoB-containing lipoproteins in humans are: *i*) 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (which function primarily by increasing LDL receptor number and thereby increasing LDL removal from serum; *ii*) "fibrates," whose mechanism of action is not clear; and *iii*) bile acid sequestrants that interrupt the enterohepatic circulation of bile. Data from our in vitro experiments with cultured hepatocytes suggest that the mechanism of action of MME in lowering serum apoB levels is distinct from that of these other agents.

This research was supported by an operating grant from the Heart and Stroke Foundation of Alberta. PSL and GTS were summer students supported by the Alberta Heritage Foundation for Medical Research. We would like to thank Dr. Nick Nation (University of Alberta) for performing the post-mortem examination of the animals.

Manuscript received 28 May 1996 and in revised form 13 August 1996.

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