Obesity in *db* and *ob* animals leads to impaired hepatic very low density lipoprotein secretion and differential secretion of apolipoprotein B-48 and B-100

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Abstract Dyslipidemia secondary to obesity is commonly observed in both animals and humans. As it has been hypothesized that obesity can result in overproduction of VLDL, leading to the subsequent dyslipidemia, we have examined the triglyceride and apoB secretion rates in vivo in obese C57BI/KsJ *db/db* and C57BI/6J *ob/ob* mice and their lean littermates. In *ob/ob* animals, obesity resulted in significantly lower, not higher, triglyceride secretion rates in both males (3.94 ± 0.49 mg/h per g liver vs. 5.45 ± 0.29 mg/h per g liver in lean littermates, $P < 0.001$) and females (4.29 ± 0.81 mg/h per g liver vs. 5.25 ± 0.59 mg/h/g liver, $P < 0.001$). For *db/db*, the obese females did not show a statistically significant triglyceride secretion rate compared to their lean littermates. Only the male *db/db* animals showed a significantly higher triglyceride secretion rate compared with lean littermates (5.50 ± 1.1 mg/h per g liver vs. 3.37 ± 0.36 mg/h/g liver, $P < 0.001$). Examination of the apolipoprotein B (apoB) secretion rates showed that for *ob/ob* animals and *db/db* obese females, apoB-48 secretion was significantly decreased compared to that of normal littermates, with a small increase in apoB-100 secretion. Total apoB secreted, however, was not increased. Our data further suggest that the predominant cause of the dyslipidemia under these conditions is a defect in removal of VLDL from the circulation.—**Li, X., S. M. Grundy, and S. B. Patel.** Obesity in *db* and *ob* animals leads to impaired hepatic very low density lipoprotein secretion and differential secretion of apolipoprotein B-48 and B-100. *J. Lipid Res.* 1997. **38:** 1277–1288.

Supplementary key words obesity • lipoproteins • dyslipidemia • apolipoprotein B • rodent • in vivo • RNA editing

Obesity is commonly associated with dyslipidemia (1), insulin resistance (2), and hypertension (2–4). In humans, it has been postulated that one component of the dyslipidemia, secondary to obesity, results from increased hepatic secretion of very low density lipoproteins (VLDL) (5–7). This explanation is based partly on estimates of transport rates of apoB-100 and VLDL-triglycerides derived from simulation analyses of endogenous and exogenous tracers, as direct VLDL secretion rates cannot be measured in humans. Other supportive evidence for this contention is based on the increased free fatty acid flux associated with obesity in humans (8). Obesity is known to supply increased amounts of free fatty acids (FFA) to the liver which could enhance the secretion of VLDL particles. For example, in cultured hepatocytes, the availability of fatty acids, in general, is correlated with amounts of apoB-containing lipoproteins released into the media (9–17). The relationship between free fatty acids and VLDL secretion is not simple, as the presence of insulin, as well as the species of rodent and species of apoB secreted, appear to add to the complexity of this pathway (15, 18, 19).

Recent investigations indicate that transfer of triglycerides into newly forming VLDL is integral to their maturation and secretion. The discovery that a congenital absence of microsomal triglyceride transfer protein (MTP) is responsible for abetalipoproteinemia documents the critical role of neutral lipid transfer for VLDL formation (20). This observation, combined with studies in cultured cells, strongly suggests that availability of triglycerides for incorporation into VLDL particles is a major determinant of amounts of mature VLDL particles formed and secreted into the circulation (9–14).

Thus it might be anticipated, as isotope studies in obese humans suggest, that the increased influx of fatty acids into the liver of obese animals will produce an

Abbreviations: apoB, apolipoprotein B; VLDL, very low density lipoprotein; RT-PCR, reverse transcription-polymerase chain reaction; MTP, microsomal triglyceride transfer protein; FFA, free fatty acids.

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increased secretion of VLDL particles. To test this hypothesis, hepatic secretion rates of VLDL-triglyceride and VLDL-apoB were measured in obese mice deficient in OB protein (*ob/ob*) or OB protein receptors (*db/db*), using a technique previously reported (21). These animals were chosen on a background such that the *ob/ob* animals were obese and hyperinsulinemic (C57Bl/6J), and the *db/db* animals were obese, hyperinsulinemic, and diabetic (C57Bl/KsJ).

METHODS

Materials

Tyloxapol (triton WR-1339), diethyl pyrocarbonate (DEPC), and reagents for triglyceride measurements were obtained from Sigma Chemical Co. (St. Louis, MO), phenyl methyl sulphonyl fluoride, leupeptin, and reagents for cholesterol determinations were obtained from Boehringer Mannheim (Indianapolis, IN). Trasylol was purchased from Calbiochem (La Jolla, CA). All other reagents were obtained from local suppliers and were of reagent grade or better. Radiochemicals were purchased from Amersham (Arlington Heights, IL) or DuPont-NEN (Boston, MA). Reverse transcriptase for cDNA synthesis was purchased from Gibco-BRL (Bethesda, MD), and for primer extension from Life Sciences (St. Petersburg, FL).

Animal experimentation

All animal experimental protocols were approved by the local Animal Advisory Board, following AAALAC guidelines. C57Bl/KsJ *db* and C57Bl/6J *ob* animals, and their control littermates, were obtained from Jackson Laboratory, Bar Harbor, ME, or bred in-house. Lean animals were not genotyped, and hence all lean animals were arbitrarily designated *ob/+* or *db/+*, respectively. The animals were fed a standard rodent diet (Teklad diet #7002, Teklad, Madison, Wl) containing 24% protein, 6% fat, and 5% fiber, and maintained on a 12-h light/dark cycle.

Measurement of hepatic triglyceride secretion rates

Tyloxapol injection was carried out as previously described (21). Tyloxapol (a triton) blocks all clearance of all VLDL particles, hence the rate of accumulation of these particles in the plasma reflects their secretion rates. Briefly, all animal experimentation was begun at 7 AM; animals were fasted for 2 h and injected with tyloxapol at a dose of 400–900 mg/kg body weight. At fixed time points, 50 μl of blood was drawn by tail-vein puncture for measurements of plasma triglycerides. The animals were killed at the end of 5/h post-injection, blood was obtained by cardiac puncture and VLDL was isolated as previously described (21). For every mouse, a time versus triglyceride graph was plotted and the triglyceride secretion rates were computed from the linear parts of the graph, to allow for the variable lag phase seen as previously described. Hepatic triglyceride secretion rates were normalized for plasma volume and expressed as per g 'normalized' liver weight (see below). The plasma volume was measured as previously described (21). Briefly, a dilutional technique, based on injection of radio-iodinated mouse albumin was used. Based on mean determinations in three animals in each group, the following formulae were derived; male *ob/+*, 0.064 ml/g body wt.; female *ob/+*, 0.068 ml/g body wt.; male *ob/ob*, 0.037 ml/g body wt.; female *ob/ob*, 0.037 ml/g body wt.; male *db/+*, 0.059 ml/g body wt.; female *db/+*, 0.067 ml/g body wt.; male *db/db*, 0.037 ml/g body wt.; female *db/db*, 0.037 ml/g body wt. Although there was variability in plasma volumes for each group, the variability in the most diverse group was within 6% of the mean shown. Calculation of triglyceride secretion rates, normalized to liver weights, was performed as previously described. However, as the liver weights in obese animals are elevated by fat deposits (see Fig. 1), the livers from obese animals were corrected by normalizing for DNA content using lean animal liver as standard, as determined below.

Apolipoprotein B determinations

Apolipoprotein B rates of secretion were determined as previously described (21). Briefly, the plasma obtained at 5 h post-injection of triton was subjected to density centrifugation, VLDL was isolated, and apoB-100 and apoB-48 concentrations were determined by SDS-PAGE and quantitative densitometry, after Coomassie staining. All rates were corrected to normalized liver weights. Baseline apoB concentrations were determined in pooled plasma from three animals per determination, after a 2-h fast, as previously described (21).

Measurement of apoB mRNA editing levels

Total liver RNA was isolated from 100-mg portions of liver and treated with DN'ase to remove any genomic DNA contamination (confirmed by performing PCR in the absence of reverse transcription) and cDNA was synthesized, as previously described (21–23). The apoB segment spanning the edited site was amplified by PCR and used for the primer extension analysis as previously described (21–23). The oligonucleotides used for the cDNA synthesis, PCR, and for radiolabeled primer extension are as previously described (21). Briefly, 5 μg of DN'ase-treated total RNA was annealed with 40 ng of MB11 oligonucleotide, and cDNA synthesis was per-

formed using 2 U of AMV Reverse Transcriptase (Life Sciences, St. Petersburg, FL.) in a final volume of 20 μl, using the buffer conditions as indicated by the enzyme supplier. A tenth of the reaction mix was used for amplification in final volume of 25 μl, containing 25 ng of MB10 oligonucleotide. Note that no reverse oligonucleotide (MB11) was added, except that contained in the cDNA aliquot (4 ng). As previously reported, this leads to asymmetric amplification which improves the sensitivity of the primer extension step (23). Five μl of the PCR products was analyzed by gel electrophoresis, to confirm the presence of correct amplification products, and the remaining products were purified over QIAquick PCR purification cartridges (Qiagen, Chatsworth, CA) as described by the manufacturer, except the wash step was increased to three times to ensure complete removal of the unincorporated nucleotides. The purified PCR products were eluted in a final volume of 30 μl, and 3 μl was used for primer extension analysis as previously described (21, 23). To eliminate DNA contamination, all RNA samples were also similarly analyzed, except that reverse transcriptase was omitted at the cDNA synthesis step. No PCR products, but more importantly, no primer extension products were seen under these circumstances, confirming the lack of genomic contamination. Two primer extension products are obtained after denaturing acrylamide gel electrophoresis, one that is short and contains the unedited 'C' for apoB-100 and one that is longer and contains a 'U', resulting in an in-frame stop translation codon in the message which thus leads to the production of apoB-48 (24). Hence, the proportions of the two primer extension products represent the abundance of the mRNAs for apoB-48 and apoB-100. These products are quantitated as previously described (21). All analysis was peformed in triplicate from each liver RNA sample, and livers from three animals in each group were analyzed and quantitation of the edited products was performed using the phosphorimager.

Liver DNA content determinations

To correct for the number of hepatocytes in the liver, as opposed to wet weight of the liver, DNA was extracted from several weighed liver portions from each group of animals, as previously described (21). Labeled DNA was used to correct for losses during extraction. At least 9 liver portions per animal strain were used to determine DNA content in liver. When expressed as a ratio of mean liver DNA per g wet liver, liver from obese *ob* strain contained 45.6% DNA as normal liver per g wet liver compared to normal liver; for *db* strain of animals, obese animals had 48.6% DNA per g wet liver compared to normal liver. Therefore, the weights of the livers were normalized using this factor, as a reflection of the hepatocyte content. For example, a 3 g obese liver is equivalent to 3 × 0.456 g lean liver, or 1.368 g lean liver. All data are therefore expressed as per g 'normalized' liver.

Liver histology

Livers were harvested at the time of killing, weighed, and portions were frozen immediately in liquid nitrogen for storage. For histological analyses, liver portions were sectioned, frozen at a thickness of 20 μm, stained with oil red O, and counter-stained with hematoxylin and eosin (H and E). Portions of liver were also fixed in paraformaldehyde, using standard histological techniques, stained with H and E, and viewed by direct light microscopy.

Statistical analysis

Statistical analyses were performed using the nonpaired Student's *t* test, with the help of computer software, MiniTab for Windows (Addison-Wesley Publishing Co., Reading, MA).

RESULTS

Baseline characteristics

Table 1 shows the baseline characteristics of the animals used in this study. Lean animals are designated as *ob/+* or *db/+*, although they were not genotyped. To date, no metabolic differences between heterozygotes (+/−) and normal animals (+/+) have been described. Obese (homozygous) animals consistently showed significantly higher liver weights, triglycerides, and cholesterol, except for male *db/db* mice (Table 1). The latter had significantly higher cholesterol levels, but not triglyceride levels. Measurements of glucose and insulin levels in selected animals showed that lean animals of either strain had insulin levels between 0.5–1.25 ng/ml, but obesity caused these levels to rise to between 3–5 ng/ml for *ob/ob* animals and more than 5 ng/ml for *db/db* animals (data not shown).

Hepatocyte content of the liver

Obesity leads to a considerable deposition of fat throughout the body of the animal, including fat stores and the liver, resulting in an increase of body weight. However, both the blood volume and the liver weight increases were not commensurate with the increase in body weight. For example, the conventional formula for calculating the plasma volume, based on the body weight (25), does not hold under these circumstances (see Methods and ref. 21). We measured the DNA content of obese and lean livers and have found that, whilst

TABLE 1. Baseline characteristics of lean and obese mice

Mice	Age	Body Weight	Liver Weight	Baseline Triglyceride	Baseline Cholesterol
	weeks	*g*	*g*	*mg/dl*	*mg/dl*
ob Male					
Lean	15.5 ± 0.5	26.4 ± 2.3	1.34 ± 0.14	54.2 ± 7.4	76 ± 12
Obese	15.3 ± 0.2	55.7 ± 2.0	3.59 ± 0.16	76.2 ± 7.4	151 ± 26
P value		<0.01	<0.01	<0.01	<0.01
ob Female					
Lean	12.6 ± 3.1	20.4 ± 1.8	0.97 ± 0.10	54.4 ± 16.5	82 ± 16
Obese	12.9 ± 3.8	52.8 ± 8.4	3.28 ± 0.89	74.7 ± 18.7	129 ± 23
P value	0.79	<0.01	<0.01	<0.01	<0.01
db Male					
Lean	11.0 ± 0.0	21.9 ± 0.5	1.08 ± 0.01	52.2 ± 17.8	94 ± 11
Obese	11.2 ± 6.7	40.0 ± 8.2	1.88 ± 0.44	65.4 ± 14.5	120 ± 16
P value	0.93	<0.01	<0.01	0.21	0.01
db Female					
Lean	11.9 ± 3.5	21.2 ± 2.3	1.06 ± 0.15	34 ± 12.9	83 ± 19
Obese	9.7 ± 2.9	39.9 ± 4.7	2.01 ± 0.17	84 ± 5.6	118 ± 11
P value	0.18	<0.01	<0.01	<0.01	<0.01

Values express baseline parameters of obese mice and their lean littermates used in this study. The cholesterol and triglyceride levels were obtained after a 2-h fast at 9 AM. All data are expressed as mean ± SD.

obesity causes the wet liver weight to increase by up to 3-fold for *ob/ob* animals, the liver DNA content increased only 1.5-fold. The majority of the weight increase was not in hepatocyte number, but in hypertrophy of the cells from increased fat deposits. **Figure 1** shows the differences in the fat deposits between lean and obese livers from both the *ob* and *db* mice. Although samples of the livers from female animals are shown, these changes were also noted in the livers from male animals. Fat accumulations in the liver were consistently larger in the obese *ob* strain of mice compared to the *db* strain, reflected in the wet liver weights (Table 1). Therefore, to better compare results between lean and obese animals, all data were normalized to 'lean liver' weights, corrected for DNA content, determined as described in the Methods. Hence, in all figure legends, the per g liver value takes into account this normalization for DNA content.

Hepatic secretion of triglyceride

Figure 2 shows triglyceride secretion rates in *ob* and *db* mice. For *ob* strain of animals, obesity was significantly associated with a reduced triglyceride secretion rate in both male (3.94 ± 0.49 mg/h per g liver, obese, n = 8, vs. 5.45 ± 0.29 mg/h per g liver, n = 5 in lean littermates, $P < 0.001$) and females (4.29 ± 0.81 mg/h per g liver, obese, n = 19 vs. 5.25 ± 0.59 mg/h per g liver, n = 14 in lean littermates, $P < 0.001$). For *db* animals, obesity in the male was associated with an increase in the triglyceride secretion rate (5.50 ± 1.1 mg/h per g liver, obese, n = 9 vs. 3.37 ± 0.36 mg/h per g liver, n = 5 in lean littermates, $P < 0.001$). No statistically significant difference was observed for female mice for the *db* strain (4.14 ± 0.61 mg/h per g liver, obese, n = 6 vs. 4.88 ± 0.56 mg/h per g liver, n = 11 in lean littermates, $P = 0.18$). If these rates are expressed in the conventional way, that is per body weight, obesity resulted in a decrease in the triglyceride rate for both strains and either sex at a highly significant *P* value ($P < 0.001$). Although we do not favor the expression of the data thus, for comparative purposes with previously published data, the figures are 277 ± 20 mg/h per kg body weight vs. 122 ± 10 mg/h per kg body weight for male lean and obese *ob* mice, 248 ± 28 mg/h per kg body weight vs. 123 ± 20 mg/h per kg body weight for female *ob* mice, 166 ± 13 mg/h per kg body weight vs. 124 ± 20 mg/h per kg body weight for male *db* mice, and 238 ± 23 mg/h per kg body weight vs. 108 ± 13 mg/h per kg body weight for female *db* mice.

Apolipoprotein B secretion rates

The mouse liver synthesizes and secretes both apoB-100 and apoB-48; the latter is the result of apoB mRNA editing in the liver (24, 26). VLDL particles contain only one molecule of apoB, whether it is apoB-48 or apoB-100 (26). Therefore, to compare the data, apoB secretion was expressed in molar terms. The contribution of apoB-48 by the gut was suppressed by our experimental protocol, hence post-triton, the apoB measured reflects that secreted by the liver. All the data were normalized for liver weights, after adjustment for DNA content (**Fig. 3**). In general, obesity did not lead to an over-production of total apoB. However, obesity was associated with a relative increase in apoB-100 secretion in all of the

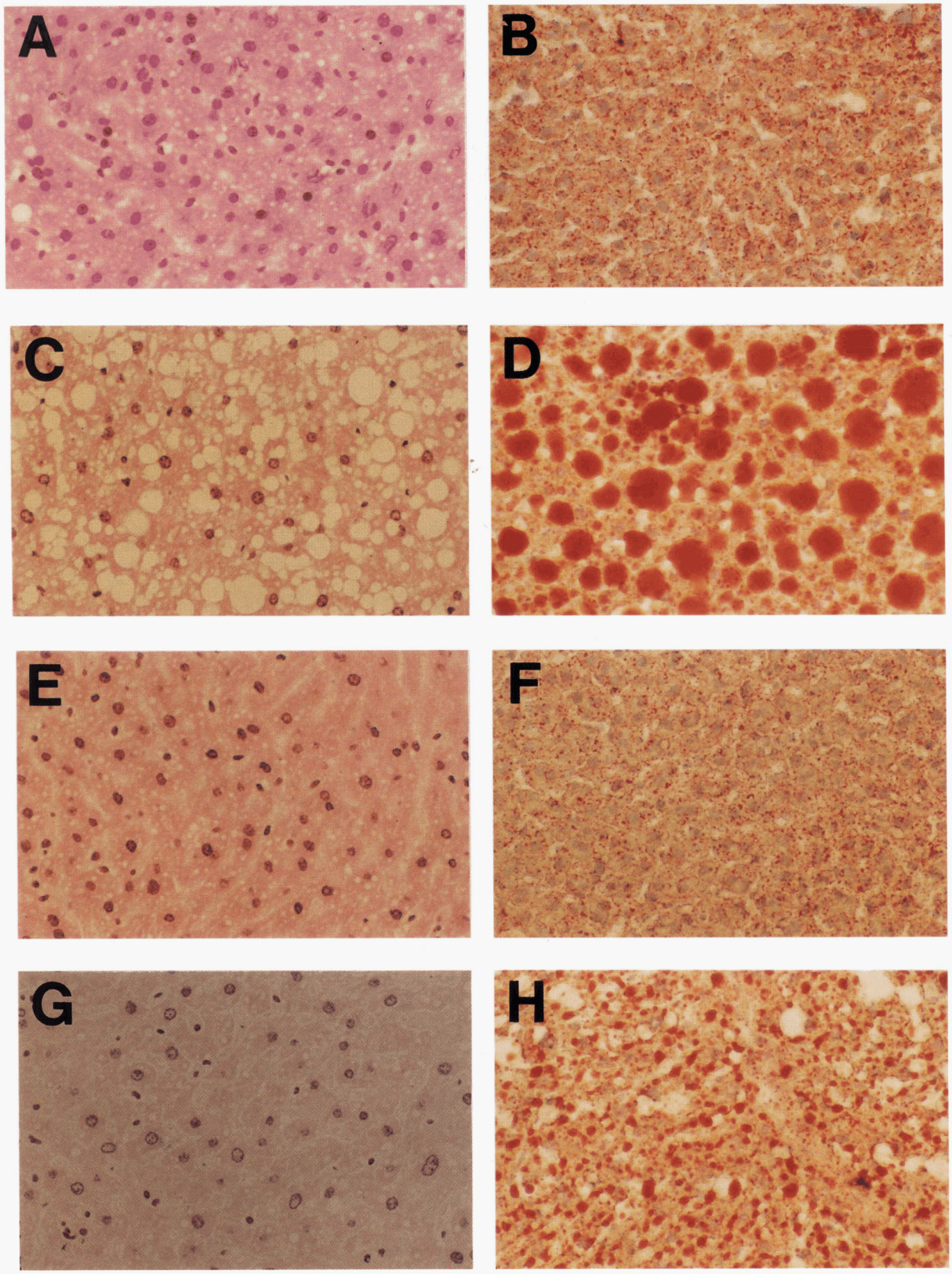

Fig. 1. Histological examination of liver from obese and lean animals. Liver portions were obtained from female *ob* or *db* strain animals, and either fixed and stained with haematoxyline and eosin (left hand panels), or frozen sections (right hand panels) were stained for fat with Oil Red O as described in Methods. Panels A and B are liver sections from a lean female *ob/+* animal, panels C and D from an obese *ob/ob* animal, panel E and F from a lean female *db/+* animal, and panels G and H from an obese *db/db* animal. The frozen sections were 20 μm thick and the magnification is ×1000. Similar findings were observed for male animals (data not shown).

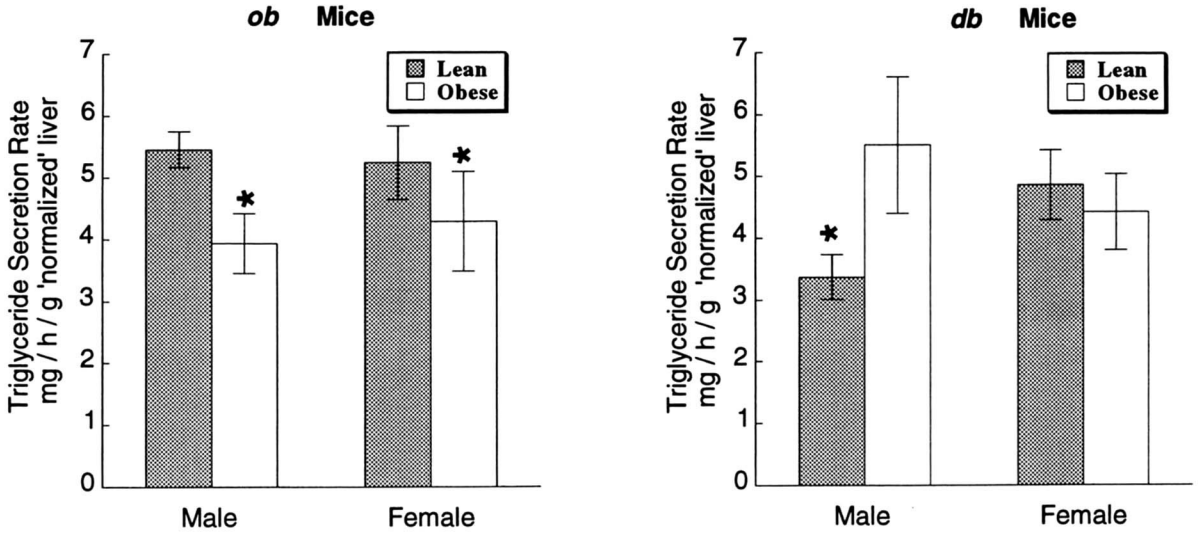

Fig. 2. Triglyceride secretion rates in lean and obese *ob* and *db* mice of either sex. Triglyceride secretion rates were determined as described in Methods. All data are expressed as per g 'normalized' liver, after correction for DNA content, as described in Methods. Lean animals are shown in the filled bars, and the obese animals in the open bars. Obesity resulted in a significant reduction in triglyceride secretion rate for *ob* strain of mice for both sexes. However, no significant differences were observed for *db* female mice. Male *db* mice showed a significant increase in triglyceride secretion rates compared to the lean animals. Note also that the lean male *db* animals have much lower triglyceride secretion rates compared with the other lean animals (see text for discussion). All data are expressed as mean ± SD. *, P value ≤ 0.05.

animals, with the exception of *db* female mice. Compared to lean littermates, *ob/ob* male animals showed a decrease in apoB-48 secretion and an increase in apoB-100 secretion. However, total apoB (apoB-48 + apoB-100) secretion rates were not significantly altered (290.3 ± 26.2 pmol/h per g liver, n = 3 for lean vs. 257.9 ± 15.9 pmol/h per g liver, n = 8 for obese, $P = 0.18$). For *ob/ob* female mice, apoB-48 secretion was significantly reduced and apoB-100 secretion rates were increased, although total apoB secretion rates remained unaltered (332.1 ± 60.2 pmol/h per g liver, n = 9 for lean vs. 303.5 ± 24.3 pmol/h per g liver, n = 5 for obese $P = 0.24$). For *db/db* female mice, apoB-48 secretion was decreased, but apoB-100 rates were essentially unchanged. However, total apoB rates were significantly reduced (262 ± 23.9 pmol/h per g liver, n = 4 for lean vs. 197.6 ± 28.6 pmol/h per g liver, n = 4 for obese, $P = 0.018$). Interestingly, despite the increase in the triglyceride secretion rates for *db/db* male mice, the output of apoB was unchanged overall (total apoB, 237.7 ± 25.3 pmol/h per g liver, n = 6 for lean vs. 259.8 ± 32.1 pmol/h per g liver, n = 4 for obese, $P = 0.30$), although a significant, but small increase in apoB-100 was also noted (Fig. 3). Except for the male *db/db* mice, there was a trend towards a reduction in apoB-48 secretion, with an increase in apoB-100 secretion in obese animals (see Fig. 3). Examination of the particle sizes by EM examination of negatively stained VLDL post-triton injection showed considerable size heterogeneity, with no apparent difference between obese or lean animals (data not shown). This was not pursued further.

To examine the effects of obesity on the relative proportions of apoB-48 and apoB-100 secreted, the molar ratios of both these species were measured prior to triton injection and after triton injection. This allowed us to compare the steady-state ratio of apoB-48 to B-100 in the plasma relative to de novo secreted apoBs. The data are shown in **Fig. 4.** Under baseline conditions, the steady-state ratio of apoB-48 to B-100 is close to unity, i.e., almost equal proportions of apoB-48- and apoB-100-containing lipoprotein particles are present in the plasma. However, the proportions of apoB-48 secreted relative to apoB-100 are much higher (as indicated by ratios greater than 2, and some cases up to 5, see Fig. 4). Two comparisons can be made. *1*) Relative to the baseline ratios (pre-injection, Fig. 4), because more apoB-48 is secreted compared to apoB-100 (post-injection, Fig. 4) than is present at steady-state, more of apoB-48 relative to apoB-100 must be cleared from the plasma. *2*) Comparing apoB-48 and apoB-100 secreted from the obese liver to the lean liver shows that the presence of obesity is associated with a decrease in the ratio of apoB-48 to apoB-100. As the production of apoB-48 is a direct result of the extent to which the mRNA for apoB is edited, we examined the extent of mRNA editing in the livers of these animals.

ApoB mRNA editing levels

Total liver RNA was extracted, and the extent of apoB mRNA editing was measured as described in Methods. **Table 2** shows the results of such analyses. Except for *ob* male mice, obesity did not result in a significant re-

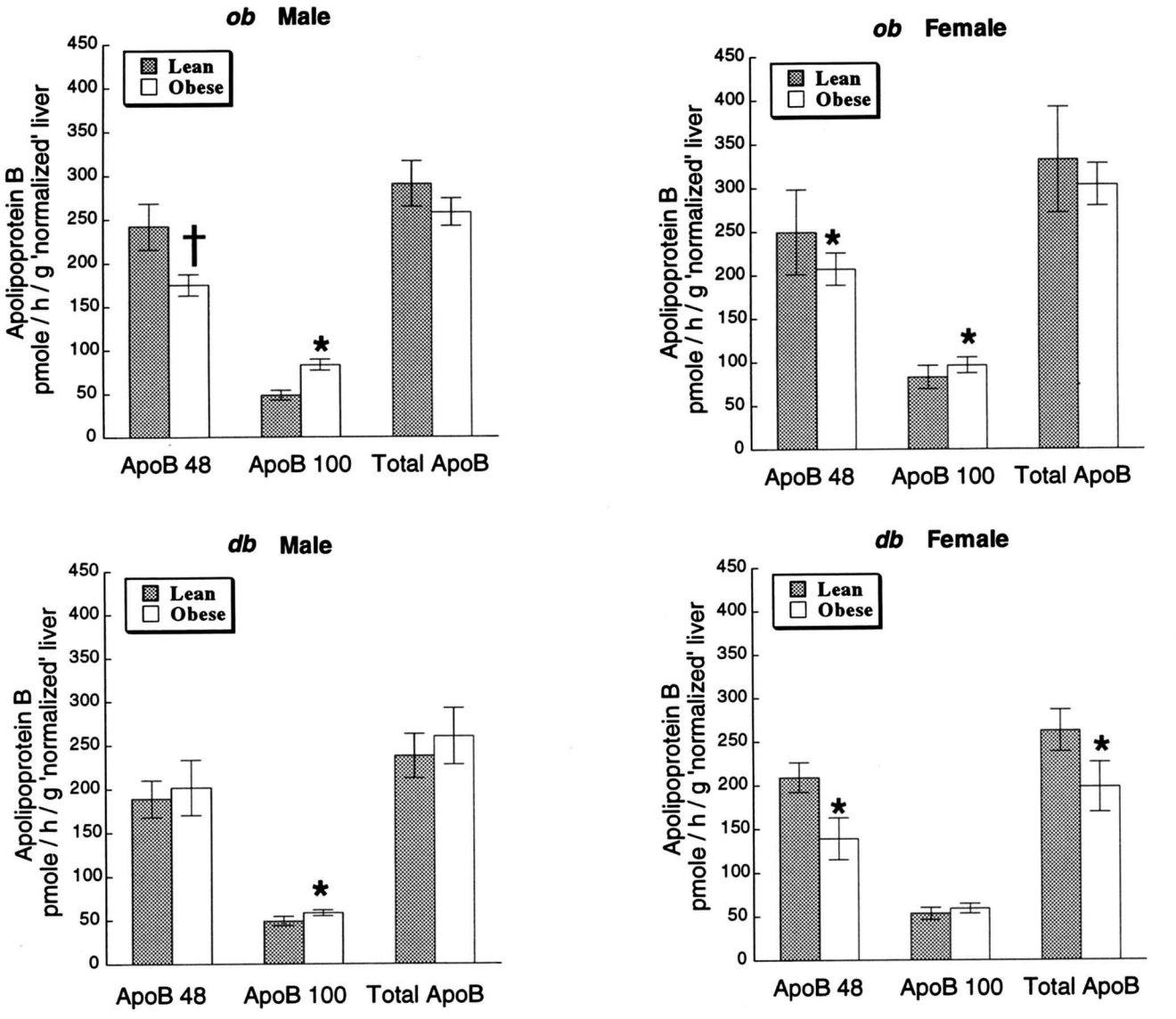

Fig. 3. Apolipoprotein B secretion rates in lean and obese *ob* and *db* mice of either sex. ApoB-48 and B-100 secretion rates were determined as described in Methods. All data are expressed as picomole secretion rate per g 'normalized' liver, after correction for DNA content. Lean animals are shown in filled bars and obese in open bars. Except for *db* male mice, obesity decreased the rate of apoB-48 secretion, and increased the rate of apoB-100 secretion. However, no significant differences were seen for total apoB secretion rates, except for female *db* mice (see bottom right panel; see text for discussion). All data are expressed as mean ± SD. *, P value ≤ 0.05; †, P value = 0.053.

duction in the extent of apoB mRNA editing. Assuming that the proportion of apoB mRNA editing reflects the proportions of the respective apoB proteins synthesized and secreted, the predicted molar ratios for apoB-48 and apoB-100 can be derived from the editing measurements and compared with the molar ratios found after triton injection. Lean animals have greater ratios than would be expected from the measurement of mRNA editing. The presence of obesity, with the exception of male *db* mice, reduces this discrepancy (see Table 2). These data provide evidence that apoB undergoes post-translational degradation in vivo, and that for lean animals, more apoB-48 is secreted than apoB-100, presumably by the selective processes in the ER (see Discussion). Obesity alters this selection such that the proportions of apoB-48 and apoB-100 secreted closely reflect the proportions of mRNA.

Effect of age on triglyceride and apoB secretion

To determine whether age has an effect on triglyceride secretion, we examined lean and obese animals. Lean littermates from *ob* or *db* strains did not show a change in triglyceride output with increasing age; for lean *ob* strain females the triglyceride secretions rates were 5.44 ± 0.23 mg/h per g liver at 9 weeks vs. 5.14 ± 0.71 mg/h per g liver at 15 weeks, $P = 0.28$; for lean

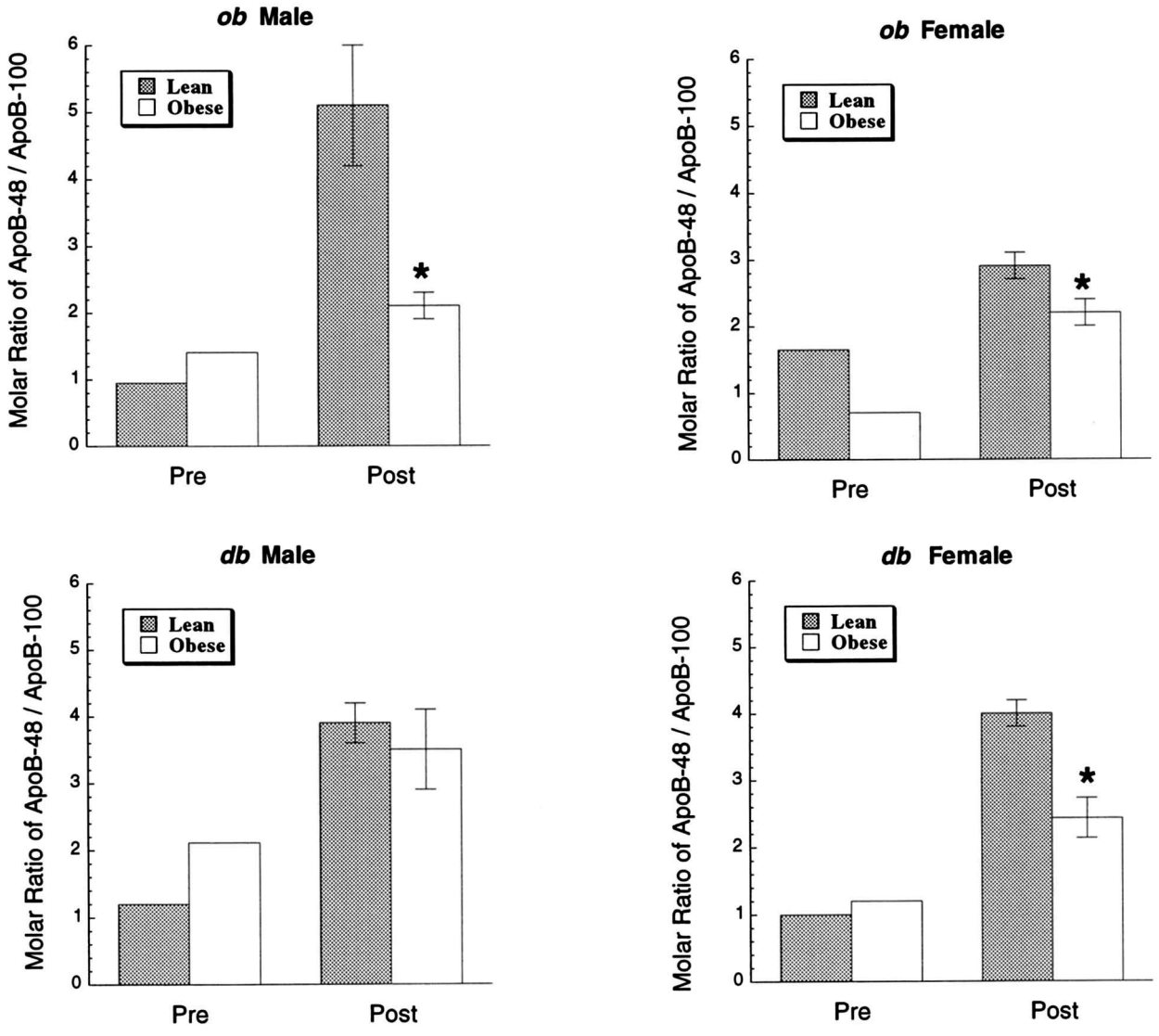

Fig. 4. Molar ratio of apoB-48 to apoB-100 at baseline and post-triton injection in lean and obese *ob* and *db* mice of either sex. The molar ratios of apoB-48 to apoB-100 were analyzed after triton injection; the numbers of animals in the triton-injected groups are as Fig. 3. For comparison, the fasting molar ratios of apoB are also shown. The latter were derived from pooled plasma samples of three mice per group, hence were not compared statistically. Statistic comparisons are therefore made between post-injection samples only. Lean animals are shown in filled bars and obese animals in open bars. Obesity resulted in a significant reduction in the ratio of apoB-48 to B-100 in all animals except male *db* mice (see text for discussion). No significant differences were seen for male *db* mice. Note that the baseline (pre-injection) ratios are close to unity, compared with the post-injection values, suggesting a much more rapid clearance of apoB-48-containing lipoprotein particles than apoB-100-containing lipoprotein particles. All data are expressed as mean ± SD. *, P value ≤ 0.01.

db strain females the triglyceride secretion rates were 4.88 ± 0.65 mg/h per g liver at 9 weeks vs. 4.82 ± 0.51 mg/h per g liver at 15 weeks, $P = 0.87$. Obese *ob/ob* females similarly showed no significant differences in triglyceride secretion rates; 4.35 ± 0.84 mg/h per g liver at 10 weeks vs. 4.22 ± 0.81 mg/h per g liver at 17 weeks, $P = 0.73$. However, total apoB rates were significantly different for female *ob/ob* female mice; 113.8 ± 0.23 mg/h per g liver at 9 weeks vs. 303.5 ± 24.3 mg/h per g liver, $P < 0.001$. The increases in apoB secretion were due to increases in both apoB-48 and apoB-100 (apoB-48, 70.6 ± 13.9 pmol/h per g liver, lean vs. 207.3 ± 18.5 pmol/h per g liver, obese; apoB-100, 43.2 ± 19.3 pmol/h per g liver, lean vs. 96.2 ± 9.1 pmol/h per g liver, obese, $P < 0.01$). ApoB rates were not examined in lean animals.

DISCUSSION

Obesity in humans is frequently associated with dyslipidemia (1). The mechanisms responsible for the dys-

TABLE 2. Relative proportions of RNA editing and apolipoprotein B-48 and B-100 secreted

Mice	Percentage RNA Editing	Predicted Apolipoprotein B-48/B-100 Molar Ratio	Secreted Apoliprotein B-48/B-100 Molar Ratio
ob Male			
Lean	70.0 ± 2.3	2.33 ± 0.02	5.05 ± 0.85
Obese	62.4 ± 2.5	1.66 ± 0.03	2.12 ± 0.18
P value	<0.05		<0.05
ob Female			
Lean	62.2 ± 2.9	1.64 ± 0.03	2.90 ± 0.19
Obese	60.7 ± 3.7	1.54 ± 0.04	2.16 ± 0.22
P value	0.46		<0.05
db Male			
Lean	67.0 ± 1.2	2.03 ± 0.01	3.87 ± 0.21
Obese	63.9 ± 3.4	1.77 ± 0.04	3.44 ± 0.58
P value	0.079		0.28
db Female			
Lean	64.8 ± 2.0	1.63 ± 0.02	3.97 ± 0.21
Obese	67.4 ± 4.6	2.06 ± 0.05	2.36 ± 0.26
P value	0.3		<0.05

Values express the percentage of RNA editing in hepatic RNA in lean and obese animals. Based on the percentage of editing of the apoB RNA, the predicted proportions of apoB-48 to apoB-100 are calculated and compared to the observed values, as determined post-triton injection. Except for the male *db/db* animals, obesity did not significantly alter the abundance of edited apoB RNA in the liver. Note, however, that for all the lean animals, the ratio of secreted apoB-48 to B-100 is considerably larger than would be expected, based on the relative abundance of the respective RNAs. Obesity appears to normalize this discrepancy for all animals except the male *db* strain (see text for discussion). All data are expressed as mean ± SD.

lipidemia are likely to be complex, but a common proposed feature for almost all of the mechanisms involves overproduction of VLDL-triglyceride and VLDL-apoB by the liver (5–7). Such a mechanism seems plausible and attractive, as it allows integration of some of the known metabolic derangements associated with obesity; hence the hyperinsulinemia of insulin resistance, glucose intolerance, and the increased free fatty acids could stimulate the liver to synthesiize and secret increased amount of triglycerides. Studies in tissue culture show that increasing the availability of free fatty acids enhances cellular secretion of apoB-containing lipoproteins at the expense of intracellular degradation of newly synthesized apoB (9–14). As excess adipose tissue in obesity provides increased amounts of fatty acids to the liver (8), an increased secretion of apoB-containing lipoproteins might be expected. Isotope kinetic studies in humans support the concept that hepatic secretion of VLDL particles is high in obese patients (5–7).

We have examined the hypothesis that obesity induces an overproduction of VLDL by the liver, utilizing two genetically obese mouse models, the *ob/ob* and the *db/db* strains. Phenotypically, both of these strains become obese from a very early age and manifest a dyslipidemia and, depending on the strain background, differ only in the presence of frank diabetes (27, 28). A surprising finding of the current study was that a striking increase in hepatic secretion of VLDL-triglyceride was not found in the obese animals. With the exception of male *db* animals (discussed below), the effects of obesity were concordant for both sexes for *ob* animals and for female *db* animals. For the following discussion, these latter three groups will be discussed together, the male *db* animals are considered separately.

In general, a significant increase in hepatic triglyceride secretion was not observed, whether expressed per body weight or normalized to DNA-corrected liver weights. The failure of obese mice to secrete more VLDL-triglyceride cannot be explained by a lack of excess triglyceride in the liver. Very large amounts of hepatic triglyceride storage pools were revealed by histology. Moreover, the data are quite clear that, contrary to proposed hypotheses, the availability of excess hepatic triglycerides was not sufficient to stimulate the secretion of increased amounts of VLDL-apoB (Fig. 3). Our methodology utilizes a 2-h fast, and we have previously discussed whether the gut could contribute to the measured lipoprotein secretion rates (21). Although the gut could contribute apoB-48 and chylomicrons to our estimations of hepatic secretion, a 2 h fast for a mouse is not a short fast, given its very rapid metabolic rate. Furthermore, the rate of triglyceride accumulation was linear over the ensuing 5-h period, after the tyloxapol injection, suggesting a single pool contributing to the plasma triglyceride under these circumstances.

In the mouse, newly-secreted VLDL-apoB is predominantly in the form of apoB-48. This excess secretion of apoB-48 relative to apoB-100 must be due, in part, to the finding that about two-thirds of apoB mRNA has been edited, allowing for translation of apoB-48 only from such messages (Table 2). In addition, however,

newly formed apoB-48 appears to be recruited preferentially for the formation of VLDL particles, as revealed by the higher than predicted molar ratios of apoB-48 to apoB-100 in newly secreted VLDL particles (Table 2). This change in ratio provides strong support for the notion that apoB degradation also takes place in vivo, a finding previously shown in tissue culture-based systems only (29). The only other explanation for the change in ratio would be selective translation of apoB-48 and apoB-100 mRNAs, for which no evidence exists to date. Hence, to account for the excess of apoB-48 secreted relative to apoB-100, either newly synthesized apoB-100 is degraded in preference to apoB-48 or both are degraded, but apoB-100 is degraded disproportionately.

In the obese mice, the ratio of apoB-48 to apoB-100 was lower than in lean animals, and the absolute secretion rate of apoB-48 was reduced. In addition, apoB-100 secretion rates were increased in the obese animals. Such a difference could be brought about by changing the extent of apoB mRNA editing, but our data show that this is unlikely to be the case (Table 2). No significant differences were measured for the extent of hepatic apoB mRNA editing. Note, however, that in the lean animals, more apoB-48 appears to be secreted than would be predicted on the basis of the abundance of its message relative to apoB-100. Obesity appears to normalize the ratio of secreted apoB-48 to apoB-100 towards that of their relative mRNA abundance (cf. observed ratios to expected ratios, Table 2), suggesting that obesity may inhibit the degradative pathway. One other possibility is that the relative recruitment rates of newly-synthesized apoB-48 and apoB-100 are different between obese and lean animals. These possibilities are not mutually exclusive. An important assumption, not tested here, is that the translational rates for edited and unedited apoB mRNAs are similar. If this is the case, then the conclusion may be valid that differential rates of post-translational mechanisms in the passage of apoB through the ER and Golgi are responsible for altering the proportions of apoBs secreted by the liver under lean and obese conditions. However, differential translational rates for apoB-48 mRNA and apoB-100 mRNA have been reported; in primary hepatocytes isolated from streptozotocin-treated rats, a decreased apoB-100 translational efficiency has been demonstrated (19). Our results, however, do not easily conform to such a model. Obesity, in the present study, led to an increase in apoB-100 secretion and a decrease in apoB-48 secretion. Whilst a differential translational rate may explain some of the discrepancy observed for apoB secretion in lean animals (Table 2), our data would suggest that post-translational mechanisms may also play a significant role. Of the latter, degradation of newly synthesized apoB is a likely candidate, thus supporting the model of in vivo degradation of apoB within the ER.

An important question is why the two sets of obese mice were not able to respond to the increased hepatic triglyceride availability with a greater output of VLDL-triglyceride, and yet their hepatic storage pools were greatly expanded (Fig. 1). The formation of fatty liver has been observed in a variety of conditions, such as obesity (30) and diabetes mellitus (31). One mechanism leading to the generation of fatty liver would be the failure to increase the output of VLDL secretion in the face of increased free fatty acid delivery to the liver. Exit of triglycerides from the liver is a defense against development of fatty liver. In obese mice the influx of fatty acids apparently exceeds the liver's availability to synthesize VLDL particles, hence the accumulation of excess fat in this organ. Similarly, development of fatty liver in obese humans also has been reported (30); this change, too, may represent a failure to remove excess triglycerides via the VLDL pathway. The fatty liver of obesity thus appears to result from a rate-limiting step in the recruitment of triglyceride into VLDL secretion in the face of increased lipid substrate delivery to the liver.

It may be that newly formed apoB is fully recruited in lean animals so that production of additional VLDL-apoB particles is not possible. For example, on a per kg basis, VLDL-triglyceride secretion in mice exceeds that of humans by 10- to 20-fold. Thus, one limiting factor for incremental VLDL formation in obese mice could be the lack of availability of unrecruited apoB for formation of new VLDL particles. This possibility also implies that apoB transcription is not increased in obese animals. The observation of an absolute increase in secretion of apoB-100 in obese mice is consistent with incremental recruitment of apoB-100. However, the increment was small and does not negate the possibility that most newly formed apoB is maximally recruited for VLDL in the lean animals.

Another limiting factor for VLDL formation in the obese animals could be the availability of the microsomal triglyceride transfer protein, MTP. This protein transfers triglyceride (and other neutral lipid) to apoB and is required for the formation of VLDL particles. If the triglyceride shuttle into VLDL is saturated in lean animals, transfer of additional triglyceride might not be possible in obese animals. Insulin has been shown to decrease transcription driven by MTP promoter elements (32). However, on a high fat diet, MTP mRNA levels were increased in the hamster (33). There are no data on the effects of hyperinsulinemia and obesity on MTP mRNA levels in rodents. The availability of apoB and MTP are two obvious rate-limiting steps in formation of VLDL particles, but the biosynthesis of triglyceride-rich lipoproteins is a multi-step process, and other limiting steps may also be important.

The current findings cause us to ask whether evi-

dence supporting elevated hepatic secretion rates of VLDL-triglyceride and VLDL- apoB in obese humans is therefore valid. Overproduction of VLDL particles is the usual explanation for elevated plasma triglycerides in humans. However, direct measurements of VLDL secretion rates are not possible in humans. Although it is possible that the human liver is able to respond to an increase in availability of triglycerides with the formation of more VLDL particles, as suggested by kinetic studies (5–7), the current study raises the need to reconsider the mechanisms involved in obesity and diabetes-induced dyslipidemia, with an important caveat; mouse lipoprotein physiology may not be an adequate surrogate for human lipoprotein physiology. Our findings also shift the focus to the process of removal of triglyceride-rich remnant lipoproteins.

Finally, a comment about the discordant results obtained for the male C57Bl/KsJ *db/db* mice. Whilst the effects of obesity for *ob* and female *db* mice are concordant, male *db* mice show changes in the opposite direction; obesity does not lead to a fasting hypertriglyceridemia, obesity increased the triglyceride secretion rates, and no change was observed for apo-B secretion rates. Note, however, that the lean animals have a much lower triglyceride secretion rate, compared with the lean animals in the other groups. The term 'diabesity' has been coined to describe the intertwining of obesity and diabetes (34), and the expression of diabesity is strain dependent. Hence, on a background of C57Bl/KsJ, the *db* locus alters the expression of sex steroid sulfotransferase genes, leading to a more hyperandrogenic profile (27, 35).

In conclusion, our data show that in genetically obese mice, despite the presence of hyperinsulinemia and increased free fatty acid delivery to the liver, there is no increased VLDL secretion relative to lean animals. The mechanism of the ensuing dyslipidemia in such obese mice is therefore caused by reduced clearance of the lipoprotein particles.

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