Endotoxin promotes preferential periportal upregulation of VLDL secretion in the rat liver

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Abstract **Zonation affects liver parenchymal cell function and metabolism as well as nonparenchymal cell activation, but whether VLDL production is zonated has yet to be elucidated. Infection induces enhanced VLDL secretion by the liver. Ex vivo studies were undertaken to examine the liver heterogeneity for VLDL formation and secretion and their in vivo response to endotoxin. Highly pure periportal (PP) and perivenous (PV) hepatocytes were isolated from fasted lipopolysaccharide-treated, fasted, and fed rats. They were used to assess their capacity to release VLDL-apolipoprotein B (apoB) and lipid classes in relation to de novo lipid synthesis and the expression of genes crucial to VLDL production. Despite the common superior ability of PP hepatocytes for lipid release and zonal differences in lipid synthesis, zonated secretion of VLDL particles was observed in septic but not in normal fed or fasted livers. The endotoxin-induced apoB secretion was more accentuated in PP hepatocytes; this was accompanied by a preferential PP increase in apoB and microsomal triglyceride transfer protein mRNA levels, whereas lipogenesis indicators were, if anything, similarly modified in hepatocytes of either acinar origin. We conclude that PP and PV hepatocytes exhibited similar capabilities for VLDL formation/secretion in normal conditions; however, the endotoxic pressure did zonate periportally.**—Aspichueta, P., S. Pérez, B. Ochoa, and O. Fresnedo. **Endotoxin promotes preferential periportal upregulation of VLDL secretion in the rat liver. *J. Lipid Res.* 2005.** 46: **1017–1026.**

Supplementary key words Gram-negative bacterial sepsis • liver metabolic zonation • lipid metabolism • apolipoprotein expression • very low density lipoprotein

Septic hyperlipidemia and hyperlipoproteinemia have been postulated to be components of the innate defense against infection in humans and experimental models (1). The insertion of the lipid A component of Gram-negative bacterial lipopolysaccharide (LPS; or endotoxin) in the phospholipid layer of lipoproteins diminishes toxicity (2), as it enables endotoxin clearance and excretion by hepatic parenchymal cells (3). Septic hypertriglyceridemia is mainly caused by the accumulation of VLDL particles in the plasma (4) attributable to many changes in their metabolism (5, 6; see recent review in 7), including increased secretion by the liver (8).

VLDL particles are assembled at the endoplasmic reticulum (ER) of hepatocytes in a process that depends absolutely upon the cellular availability of lipids, such as triglycerides (TGs), phospholipids, cholesterol, and cholesteryl esters, to correctly translate and translocate apolipoprotein B (apoB) to the lumen. ApoB-100 and apoB-48 are the essential apolipoproteins for VLDL assembly in the rat (9). They are expressed in excess, and the protein that is not lipidated to a sufficient extent is targeted to and eventually degraded by proteasomes (10). Correct apoB lipidation and translocation are controlled by the microsomal triglyceride transfer protein (MTP), whose binding and lipid transfer activities are probably the major determinant in VLDL secretion (11–13). The assembly of VLDL is a complex process that includes two major lipidation steps. A relatively small, dense, TG-poor lipoprotein particle is formed in the ER by cotranslational loading of apoB chains with lipid. Then, bulk lipidation and final maturation of the VLDL precursor occurs, possibly in both the ER (14) and post-ER compartments (15).

VLDL secretion rate and composition can be modulated by a variety of factors strikingly related to liver parenchymal zonation, such as nutritional state (16, 17), diet (18), and endotoxin (19). Zonation refers to a phenotypic heterogeneity that is well established in many essential liver functions (20), but investigations into lipid and lipoprotein metabolism zonation are still insufficient to delineate a realistic panorama. It has been suggested that the parenchymal capacity for the secretion of VLDL particles is zonated. For some authors, the secretion of VLDL by perivenous (PV) hepatocytes might be higher (20) because of their higher rates of fatty acid synthesis (21). Oth-

Manuscript received 4 January 2005 and in revised form 2 February 2005.

Published, JLR Papers in Press, February 16, 2005.
DOI 10.1194/jlr.M500003-JLR200

Abbreviations: apoB, apolipoprotein B; APR, acute-phase reaction; ER, endoplasmic reticulum; IL, interleukin; LPS, lipopolysaccharide; MTP, microsomal triglyceride transfer protein; PP, periportal; PV, perivenous; TG, triglyceride.

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ers, however, have pointed out that it could be concentrated in the periportal (PP) area (22) because of the higher expression of the cholesterol synthesis rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (23). To our knowledge, direct estimates of the acinar differences in VLDL secretion have not been provided. Zonation is also suspected in nonparenchymal liver cells. Kupffer cells, the resident macrophages in the liver and an important source of cytokines, are localized periportally (24). Indeed, interleukin 6 (IL-6), the key signal for triggering the expression of acute-phase reaction (APR) genes in hepatocytes (25, 26), exhibits PP expression within the liver lobule (27). By contrast, the longer perimeter of the sinusoids, together with the higher porosity of the endothelial lining in the PV zone, suggest that exchange processes between the parenchymal cells and the blood may be easier at the distal end of the sinusoid (28). The host response to LPS, therefore, is a good model in which to study the in vivo regulation of the putative zonation of VLDL secretion.

To delineate the zonation of lipid metabolism in a more comprehensive way, we examined ex vivo the parenchymal heterogeneity for VLDL secretion, de novo lipid synthesis, and mRNA levels for proteins crucial to VLDL production in basal conditions and upon endotoxin administration in the rat. We used highly pure PP and PV hepatocyte subpopulations from the hepatocyte population isolated from the intact animal. Besides enabling the measurement of interdependent parameters in the same cell preparation, this approach allowed the comparison of compositional and functional parameters of high interindividual variability between two cell subpopulations derived from the same individual. We found that, although there were PP-to-PV differences in some VLDL biogenesis markers, zonated secretion of VLDL was observed in the septic but not in the normal fed or fasted liver. In addition, we found that the major endotoxin-induced response in VLDL-apoB secretion occurs in PP hepatocytes and seems to involve the provision of functional apoB but not of lipid.

MATERIALS AND METHODS

Reagents

[α-^{32}P]dCTP (3,000 Ci/mmol), [9,10(n)-^{3}H]oleic acid (57 Ci/mmol), and [^{3}H]acetic acid (10.4 Ci/mmol) were purchased from Amersham Pharmacia Biotech. Collagenase A and enzymes were from Roche Molecular Biochemicals. Essentially fatty acid-free BSA, Triton WR1339 (tyloxapol), and LPS from *Escherichia coli* strain 011:B4 were obtained from Sigma Chemical Co. (St. Louis, MO). The cDNAs for rat apoB and FAS were provided by Dr. M. P. Sowden (University of Rochester Medical Center, Rochester, NY) and Dr. M. Scheiwer (University of Heriot-Watt, UK), respectively.

Animals

Female Sprague-Dawley rats (~200 g) were housed as described previously (29) and used in compliance with institutional guidelines. Animals were administered intraperitoneally 1 mg/kg body weight LPS in 0.5 ml of saline or saline alone, and were simultaneously food-deprived, as sepsis is known to alter food intake. They were used for blood sampling and hepatocyte isolation 18 h later and are referred to as the LPS group and the fasted group, respectively. To further investigate the effect of fasting, we used a third group of animals, the fed group, which received food ad libitum.

Isolation and characterization of PP and PV hepatocytes

Parenchymal hepatocytes were isolated by a collagenase perfusion technique (30), washed, and fractionated into subpopulations by centrifugal elutriation as described (29). Briefly, 120 × 10^6 hepatocytes suspended in 6 ml of the incubation medium were subjected to centrifugal elutriation. Four subpopulations of hepatocytes were eluted from the rotor using Krebs-Henseleit buffer with 1 mM $CaCl_2$, 5 mM glucose, 10 mM pyruvate, 6.5 mM HEPES, and 0.05% gelatin by changes in rotor speed and buffer flow rate: 860 rpm, 52 ml/min; 860 rpm, 76 ml/min (PP hepatocytes); 860 rpm, 85 ml/min; 830–810 rpm, 95 ml/min (PV hepatocytes). The enrichment of PP and PV hepatocytes was monitored by flow cytometry on an EPICS 752 system (Coulter, Hialeh, FL) by following the distribution of four cell parameters that are known to reflect the asymmetry (20): size (forward angle light scatter); cytoplasmic complexity (side light scatter); ploidy (propidium iodide fluorescence); and mitochondrial content (nonylacrydine orange fluorescence). Because of the elutriation sensitivity to putative changes in cell volume and density associated with feeding status and sepsis, flow cytometric characterization was performed in cells from the three treatment groups. A representative sample of fed rat hepatocyte subsets is illustrated in **Fig. 1**. Most PP hepatocytes were 2n (Fig. 1C) and, compared with the PV hepatocytes (many of which were binucleated, tetraploid), were smaller (diameter = 17–19 μm for PP cells and 21–23 μm for PV cells) (Fig. 1A), had lower cytoplasmic complexity (PP/PV ratio of 0.5–0.7) (Fig. 1B), and had higher mitochondria content (PP/PV ratio of 1.6–2) (Fig. 1D). According to differences in these markers, in good agreement with published values (20), and to dual-parameter statistical analyses (shown in Fig. 1E for size and cytoplasmic complexity), the separation of PP and PV hepatocytes was excellent for all of the experiments reported here. Routine PP hepatocyte populations were 90–95% pure and accounted for 75% of the parenchymal cells within the acinus. Cross-contamination of the PV hepatocyte populations was higher, averaging 20%. It should be noted that the distribution of cell parameters varied little between hepatocyte preparations of the same group or of different treatment groups. The final viability of cells, as determined by trypan blue exclusion, was always >85%.

Finally, the hepatocytes were suspended in Krebs-Henseleit solution, pH 7.4, containing 20 mM glucose, 2.5 mM $CaCl_2$, 6.5 mM HEPES, 10 mM pyruvate, and 2% fatty acid-free BSA and incubated as described previously (31).

RNA isolation and analysis

Total RNA was extracted from hepatocytes using TRIzol (Invitrogen Life Technologies, Barcelona, Spain) according to the manufacturer's recommendations. The concentration and integrity of RNA were determined by measuring the absorbance at 260 and 280 nm followed by electrophoresis on a 1% agarose gel.

mRNA levels for apoB and FAS were estimated by Northern blot analysis. RNA blots were hybridized with the corresponding probe labeled with [α-^{32}P]dCTP (3,000 Ci/mmol) by random priming (Amersham Pharmacia Biotech, Buckinghamshire, UK). The blots were exposed to an Imaging Screen-K (Kodak, Rochester, NY), and the intensities of the bands were quantified after laser densitometric scanning (Molecular Imager FX and Quantity One software; Bio-Rad, Hercules, CA). The mRNA level for MTP was estimated by RT-PCR using primers for a cDNA fragment between 784 and 1,138 bp of the hamster MTP large subunit gene

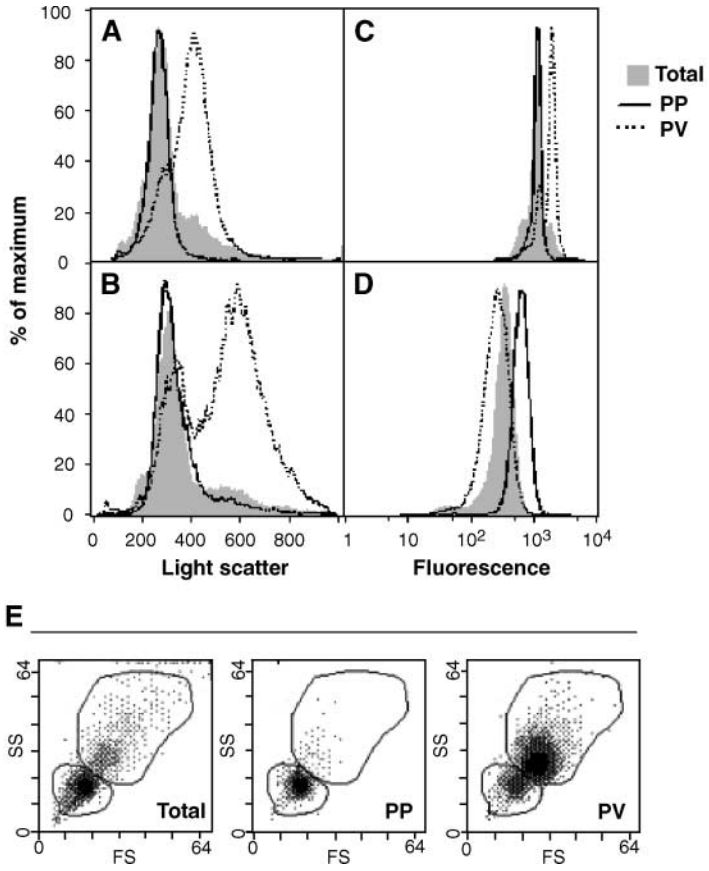

Fig. 1. Routine flow cytometric monitoring of hepatocyte populations. The total population of hepatocytes isolated from one ad libitum-fed rat before centrifugal elutriation and the resulting periportal (PP) and perivenous (PV) subpopulations were characterized on a flow cytometry system for the distribution of size [forward angle light scatter (A)], cytoplasmic complexity [side light scatter (B)], content in double-stranded nucleic acids [propidium iodide fluorescence (C)], and content in mitochondrial membranes [nonyl-acrydine orange fluorescence (D)]. The results are expressed as percentages of the maximum intensity, set to 100 arbitrary units in each cell set. E: Biparametric (side light scatter vs. forward angle light scatter) representation of the purity of PP and PV subpopulations: 90.3% and 81.8%, respectively, for this example.

(32) (GenBank accession number U14995). The PCR mixture contained 0.144 μM sense (5′-GCCAATTCGAAGCCGGCCCAAGAATGGTAC-3′) and antisense (5′-GCCCAAGCTTCCAAAAAGTCCAGGATGGCTTCC-3′) primers and 2.5 units of *Taq* DNA polymerase (Qiagen, Hilden, Germany). The amplification conditions were as follows: 6 min at 95°C, followed by 35 cycles of 30 s at 95°C, 60 s at 60°C, and 90 s at 72°C, followed by 10 min at 72°C. The products were analyzed by agarose gel electrophoresis, and the bands were quantified using optical density volume analysis. As demonstrated by others (33, 34), we found that LPS modifies the mRNA levels of common housekeeping genes in the liver (data not shown). Hence, we used nonnormalized data sets for comparisons between groups and data sets normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA signal for comparisons between the PP and PV cells of each group. Extreme care was taken in RNA quantification and loading. The load was further checked by the fluorescence level of the rRNA bands in the gel.

Isolation of the VLDL secreted by primary hepatocytes and assessment of VLDL-lipid and -apoB content

At the end of the 2 h incubation period of 5×10^6 cells/ml, the hepatocytes were pelleted and VLDL was isolated by ultracentrifugation after adjusting the density of the medium to 1.006 g/ml, as described by Vance, Weinstein, and Steinberg (35). Aliquots of VLDL were used for the quantification of lipid and apoB classes, which was performed exactly as detailed previously (31).

De novo synthesis and secretion of lipids in primary hepatocytes

The amounts of the labeled lipogenic precursors, sodium [^{3}H]acetate (20 μM, 20 μCi/ml) and potassium [^{3}H]oleate (20 μM, 2 μCi/ml), incorporated into the cellular lipids and the lipids released to the medium were quantified at 15, 30, 60, and 120 min in 2×10^6 cells/ml incubations, as described previously (36). The incorporation of [^{3}H]acetate into TGs and cholesterol was considered to be a relative measure of lipogenesis and an absolute measure of cholesterogenesis, respectively, and that of [^{3}H]oleate into CE and TG was considered to be a measure of lipid esterification.

Assessment of hepatic VLDL-apoB and lipid production and serum lipoprotein lipids

Triton WR1339 inhibits lipolysis and clearance of the TG-rich lipoproteins from the circulation (37). Thus, the hepatic production of VLDL-lipid and -apoB was defined as the post-Triton accumulation of the lipoprotein components in the serum. For each group, it was calculated by the subtraction of the 2 h accumulation in vehicle-injected rats from the 2 h accumulation in Triton-injected rats. Accumulation of lipids in other serum lipoproteins was calculated similarly. Control and fasted LPS-treated rats were injected into the tail vein with Triton WR1339 (600 mg/kg) using 30% (w/v) detergent solution in 0.9% NaCl or vehicle alone. Blood samples were withdrawn from the tail vein before, and from the inferior cava vein 2 h after, the injection of animals. The serum was separated and processed immediately. VLDL was isolated by ultracentrifugation at a density of 1.006 g/ml using a TLA 100.4 rotor (Optima TL; Beckman, Palo Alto, CA) at 100,000 rpm and 16°C for 2.5 h and characterized for lipid and apoB classes. One aliquot of the collected infranatant was used for lipid analysis. ApoB lipoproteins (mostly LDL, as Triton WR1339 inhibits intermediate density lipoprotein formation) in the remaining infranatant were precipitated by phosphotungstic acid treatment and further centrifugation; the resulting supernatant was assayed for cholesterol and TGs. This enables lipid characterization of both HDL, in the second supernatant, and LDL by dif-

ferences in the values of the two estimates. Enzymatic analyses of TG and total cholesterol levels in each lipoprotein fraction were conducted in a Hitachi autoanalyzer by routine hospital procedures (Galdakao Hospital, Vizcaya, Spain). The quantification of apoB-48 and apoB-100 in serum VLDL was performed as described previously (31).

Protein and statistical analysis

Cell protein was measured by the dye-binding technique of Bradford (38) using BSA as a standard. Multiple comparisons among groups were statistically evaluated by two-way ANOVA followed by the Bonferroni test. Comparisons between the PP and PV cells in each group were statistically evaluated by the paired Student's *t*-test. The level of significance was set at $P \leq 0.05$.

RESULTS

Eighteen hours after treatment with 1 mg/kg LPS and simultaneous food deprivation, animals displayed the expected APR-associated serum phenotypical changes (4): increased levels of TG (+80%; $P \leq 0.05$, n = 4) and glucose (+70%; $P \leq 0.001$, n = 4) and decreased levels of albumin (−10%; $P \leq 0.05$, n = 4) and HDL-cholesterol (−19%; $P \leq 0.01$, n = 4) with respect to control, fasted rats (data not shown).

We first addressed the functional heterogeneity of liver PP and PV parenchymal cells for VLDL secretion in basal conditions and upon endotoxin administration (**Table 1**). PP and PV hepatocytes from ad libitum-fed rats secreted, during the 2 h of incubation, a similar particle number (according to apoB quantities) and type, with no difference in the amount of apolipoprotein and lipid classes analyzed. In experiments not shown, using the whole population of hepatocytes before elutriation, VLDL secretion was found to be ∼30% suppressed ($P \leq 0.01$, n = 4) by fasting and ∼70% increased ($P \leq 0.05$, n = 4) by endotoxin administration. A similar decrease in VLDL-apoB secretion caused by fasting was seen in PP hepatocytes, affecting both the apoB-48- and apoB-100-bearing VLDL particles, whereas the reduction in PV secretion was marginal and did not reach statistical significance. Lipid output from fasted rat PP and PV cells together also tended to be lower (ANOVA, $P \leq 0.05$), but the acinar differences were not significant. Hence, although hepatocytes of different acinar origin did not respond equally to fasting, zonated secretion of VLDL did not occur. Administration of endotoxin to animals and simultaneous food deprivation led to PP zonation of VLDL particle release. The PP hepatocyte response to LPS was much more accentuated than that of PV hepatocytes, with the former secreting ∼90% more apoB (both apoB-48 and apoB-100) and the latter secreting only ∼38% more VLDL particles in 2 h than the corresponding control, fasted cells. The LPS-induced increase in lipid output by PP cells, which was statistically significant for cholesterol and phospholipid but not for TGs, was lower than the apoB mass output. Hence, the endotoxic hepatocytes of the PP zone appear to be primed for nearly double the secretion of apoB molecules in lipoprotein particles containing somewhat less TG, whereas more VLDLs of control-like lipid composition were secreted by septic PV cells. It is interesting that, taking the three treatment groups together, the net amount of lipid secreted as VLDL by PP cells was significantly greater (ANOVA, $P \leq 0.05$) than that released by PV hepatocytes.

The mRNA mass for apoB, MTP, and FAS was analyzed in PP and PV hepatocytes freshly isolated from fed, fasted, and septic rats. The data presented in **Fig. 2** as 0 h levels demonstrate that fasting and endotoxin had a greater ef-

TABLE 1. ApoB and lipid composition of the VLDL particles secreted for 2 h by PP and PV hepatocytes of fed, fasted, and endotoxin-treated rats

	PP Hepatocytes			PV Hepatocytes		
Variable	Fed	Fasted	LPS	Fed	Fasted	LPS
ApoB (fmol/mg cell protein)	570 ± 120	400 ± 70[a]	750 ± 110[a,b,c]	520 ± 100	470 ± 140	650 ± 100[a]
ApoB-48	480 ± 90	330 ± 50[a]	620 ± 90[a,b,c]	440 ± 90	380 ± 100	540 ± 80
ApoB-100	90 ± 10	70 ± 10[d]	130 ± 10[b,c,e]	80 ± 10	90 ± 20	110 ± 10[a]
Lipid (nmol/mg cell protein)	15.7 ± 1.5	11.6 ± 2.3	14.8 ± 2.6	13.3 ± 2.1	7.9 ± 1.4	11.8 ± 2.3
Cholesteryl ester	0.14 ± 0.02	0.11 ± 0.03	0.11 ± 0.02	0.19 ± 0.07	0.09 ± 0.02	0.09 ± 0.04
Triglyceride	12.7 ± 0.7	9.1 ± 1.5	12.7 ± 1.7	10.4 ± 2.2	7.3 ± 1.7	10.0 ± 1.1
Cholesterol	0.49 ± 0.12	0.34 ± 0.12	0.55 ± 0.14[f]	0.53 ± 0.13	0.27 ± 0.08	0.49 ± 0.16
Phospholipid	1.61 ± 0.32	1.29 ± 0.23	2.41 ± 1.02[f]	1.68 ± 0.47	1.11 ± 0.31	2.04 ± 0.64
Lipid/VLDL particle (nmol/pmol apoB)	31.3 ± 5.6	31.3 ± 5.5	22.3 ± 4.9[a,f]	28.8 ± 6.4	22.8 ± 7.5	20.4 ± 3.7[a]
Cholesteryl ester	0.23 ± 0.08	0.32 ± 0.13	0.16 ± 0.04	0.48 ± 0.27	0.24 ± 0.08	0.15 ± 0.03
Triglyceride	25.7 ± 4.7	25.1 ± 4.6	18.7 ± 3.8[f]	23.1 ± 5.2	19.7 ± 5.8	16.9 ± 2.7
Cholesterol	0.92 ± 0.26	0.81 ± 0.2	0.73 ± 0.18	1.06 ± 0.2	0.60 ± 0.16	0.75 ± 0.18
Phospholipid	3.45 ± 1.20	3.65 ± 0.82	3.63 ± 0.84	3.65 ± 1.36	3.12 ± 1.16	3.41 ± 1.16

ApoB, apolipoprotein B; LPS, lipopolysaccharide; PP, periportal; PV, perivenous. PP and PV hepatocytes isolated from ad libitum-fed, 18 h-fasted, and 18 h-fasted and LPS-treated (1 mg/kg) rats were incubated (5×10^6 cells/ml) for 2 h as described in Materials and Methods. VLDLs were isolated from the d < 1.006 g/ml fraction of medium, and the content of apoB-48, apoB-100, and each lipid class in VLDL was determined. The results are expressed as means ± SEM of six independent experiments performed in duplicate.

[a] $P \leq 0.05$ versus the corresponding acinar origin of the fed group.
[b] $P \leq 0.01$ versus the corresponding acinar origin of the fasted group.
[c] $P \leq 0.05$ versus the PV cells of the same treatment group.
[d] $P \leq 0.001$ versus the corresponding acinar origin of the fed group.
[e] $P \leq 0.01$ versus the corresponding acinar origin of the fed group.
[f] $P \leq 0.05$ versus the corresponding acinar origin of the fasted group.

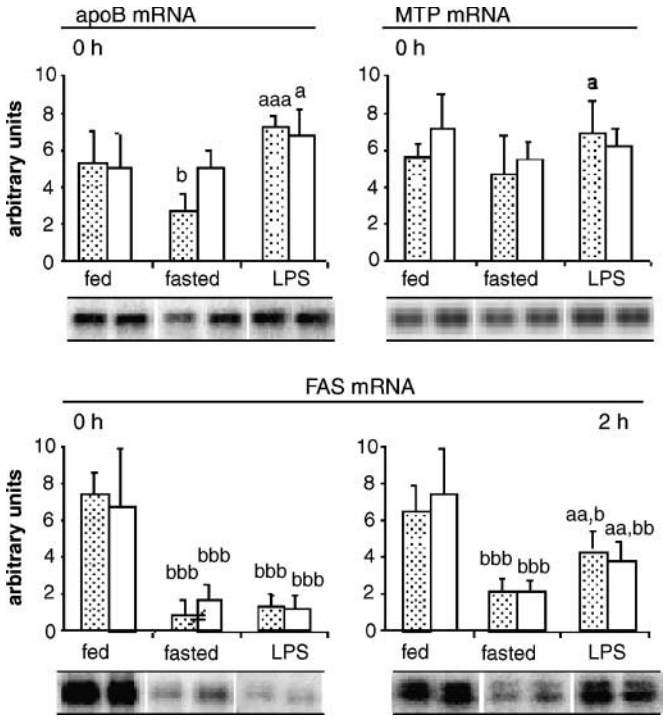

Fig. 2. Transcript levels for apolipoprotein B (apoB), microsomal triglyceride transfer protein (MTP), and FAS in PP and PV hepatocytes of fed, fasted, and endotoxin-treated rats. PP (dotted bars) and PV (open bars) hepatocytes isolated from ad libitum-fed, 18 h-fasted, and 18 h-fasted and lipopolysaccharide (LPS)-treated (1 mg/kg) rats were incubated (5×10^6 cells/ml) for 2 h as described in Materials and Methods. Total RNA was extracted from the cells before (0 h) and after (2 h) incubation. The mRNA levels for apoB and FAS were determined by Northern blotting, and that for MTP was determined by RT-PCR. Representative images are shown at the bottom of the corresponding histogram. The results are expressed as means ± SD from four animals assayed in triplicate. Statistical differences are denoted by ^{a} $P \leq 0.05$, ^{aa} $P \leq 0.01$, and ^{aaa} $P \leq 0.001$ versus the corresponding zone of the fasted group and by ^{b} $P \leq 0.05$, ^{bb} $P \leq 0.01$, and ^{bbb} $P \leq 0.001$ versus the corresponding zone of the fed group.

fect on PP compared with PV mRNA expression of apoB and MTP. Thus, a fasting-promoted decrease in apoB mRNA was recorded only in PP hepatocytes, and compared with fasted cells of the same acinar origin, endotoxin doubled the PP apoB mRNA mass and increased by 50% that of MTP mRNA; minor changes, significant for apoB mRNA, were seen in PV hepatocytes. Regarding the mRNA expression of FAS, there was a dramatic decrease caused by fasting that was more marked in PP cells and was not modified significantly by LPS administration. To determine putative temporal changes in cell transcript levels inherent to this ex vivo approach, we also quantified mRNAs in hepatocytes upon 2 h of incubation. The mRNA levels for apoB and MTP in all groups were found not to differ substantially from those seen before incubation (data not shown). However, as shown in Fig. 2, an acinar origin-independent increase of ~80% in the FAS mRNA level over that of fasted cells was observed in septic hepatocytes incubated for 2 h.

To elucidate whether the capacity of hepatocytes for de novo synthesis and secretion of lipids was zonated and affected by *E. coli* sepsis, the incorporation of labeled substrates into cellular and medium lipids was determined. We measured the incorporation of [^{3}H]acetate into TGs and cholesterol (**Fig. 3A**), a measure of de novo lipid synthesis, and the incorporation of [^{3}H]oleate into TGs and cholesteryl esters (Fig. 3B), a measure of lipid esterification. Labeling was determined at 15, 30, 60, and 120 min, although only the final trend-representative data are shown. A consistent zonation pattern was observed in de novo synthesis of TG and cholesterol, which dominated periportally in the fed state and perivenously in the fasting condition, without any further effect caused by endotoxin (Fig. 3A). This zonal distribution was followed closely by the cellular newly formed lipid, whereas the pattern of zonation for de novo lipid output was less consistent. Of particular interest is that the proportion of de novo synthesized TGs directed to secretion was higher in PP than in PV hepatocytes of fed rats (39% vs. 28%, respectively; $P \leq 0.05$), and this preferential PP efficiency of secretion was unaffected by fasting or endotoxin. The data presented in Fig. 3B show that the overall esterification of glycerol-3-phosphate and cholesterol did not change substantially along the acinus, as the sum of the label incorporated into cellular and medium TGs and cholesteryl esters was similar in PP and PV hepatocytes. However, both the net amount and the proportion of secreted [^{3}H]oleate-labeled lipids were, if anything, zonated periportally, reinforcing the idea that PV hepatocytes have a relative tendency to retain de novo lipid intracellularly.

The results presented in Table 1 suggested that endotoxin treatment led PP hepatocytes, which accounted for 75% of total hepatocytes (see Materials and Methods) (20), to a marked increase in TG-poor VLDL secretion. To test whether the hepatocytes' behavior was comparable in vivo under similar conditions, hepatic VLDL production was determined in septic and control, fasted rats that had been injected with Triton WR1339, a blocker of LPL-mediated VLDL-TG clearance (37), or saline. Before and 2 h after injection, the serum was obtained, the VLDL was isolated, and the LDL and HDL lipids were measured. This allowed us to estimate the amount of apoB-48 and apoB-100 (**Fig. 4B**) and lipids (Fig. 4A) produced by the liver in VLDL and the lipids accumulated in the circulating LDL and HDL during the time period selected (Fig. 4A). We noted that septic rats had serum VLDL-apoB levels approximately eight times the control levels and that differences were either maintained or accentuated at 2 h after Triton injection. The inset in Fig. 4B shows that the amount of VLDL-apoB produced in 2 h was 26% higher in the septic rats compared with fasted rats, the relative increment of apoB-100 being more pronounced than that of apoB-48. However, there were no differences in the amounts of VLDL-TGs and total cholesterol produced between the two groups (Fig. 4A). Figure 4A shows that the changes in LDL lipid levels were also similar in both treatment groups. Additionally, the alteration in the lipid composition of HDL shown in Fig. 4A supports previous reports of increased levels of TGs and cholesterol in acute-phase HDL particles (39, 40).

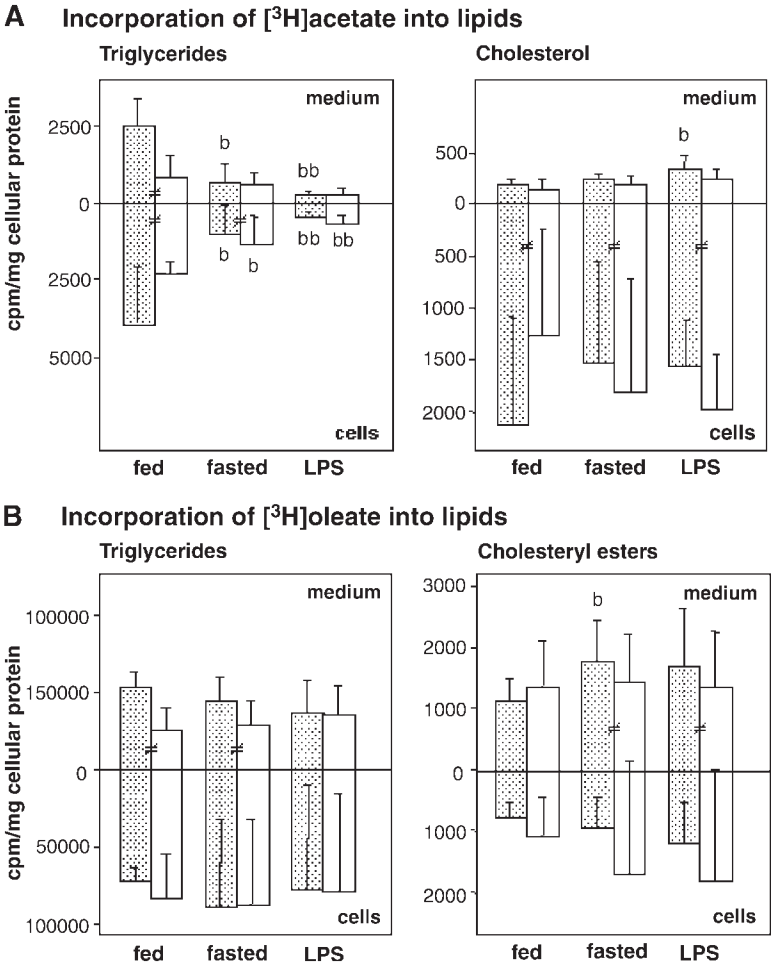

Fig. 3. De novo synthesis and secretion of lipids in PP and PV hepatocytes of fed, fasted, and endotoxin-treated rats. PP (dotted bars) and PV (open bars) hepatocytes isolated from ad libitum-fed, 18 h-fasted, and 18 h-fasted and LPS-treated (1 mg/kg) rats were incubated (2×10^6 cells/ml) for 2 h with [^{3}H]acetate [20 μCi/ml (A)] or [^{3}H]oleate [2 μCi/ml (B)] as described in Materials and Methods. The label incorporated into triglycerides (TG) and cholesterol (A) or TGs and cholesteryl esters (B) of the medium and cells was measured and expressed relative to the cell protein. The results are expressed as means ± SD from four independent experiments performed in duplicate. Statistical differences are denoted by $^{\neq}$ $P \leq 0.05$ versus the PV cells of the same treatment group and ^{b} $P \leq 0.05$ and ^{bb} $P \leq 0.01$ versus the corresponding zone of the fed group.

DISCUSSION

Hypertriglyceridemia is an early hallmark of sepsis caused mainly by an increase in VLDL levels. This increase has been ascribed to both enhanced hepatic production of VLDL (5–8) and decreased peripheral ability to clear TG-rich lipoproteins efficiently (5, 7). We now show that, in fasted rats, the major endotoxin-promoted increase in VLDL secretion occurs in PP hepatocytes and is accompanied by a preferential upregulation of apoB and MTP mRNA levels, whereas endotoxin has modest effects on cellular de novo lipid synthesis and its zonation. We also show that, although the PP hepatocytes tend to release more lipids as VLDL, the number of VLDL particles secreted is not zonated in the ad libitum-fed and the 18 h-fasted rat liver.

The potential for modulation of the VLDL particle assembly leading to quantitative and/or qualitative changes in VLDL secretion exists at various levels, including apoB synthesis, efficiency of apoB lipidation and translocation, and efficiency of the cell secretory pathway machinery. A role for the cellular availability of TG (41, 42), cholesterol (36, 42, 43), and phospholipid (44, 45) in determining how much apoB enters the VLDL assembly/secretion or the ubiquitin-proteasome/degradation pathway is firmly established. However, we present evidence strongly suggesting that the differences in cellular capacities for de novo lipid synthesis, esterification, and secretion are not of sufficient magnitude to dictate per se a zonated apoB secretion in the normal fed and fasted rat liver. Neither can they account for the endotoxin-induced increase in the number of VLDL particles assembled and secreted by rat hepatocytes. Consistent with previous reports (20, 21), we observed a feeding condition-driven zonation pattern of de novo lipogenesis in hepatocytes, which was accompanied by changes of the same tendency in apoB secretion, although in most cases these did not reach statistical significance. Thus, de novo synthesis of TG and cholesterol dominated in PP hepatocytes in the fed state and, conversely, in cells of the PV area upon fasting or LPS treatment. PP hepatocytes, however, secreted both more lipid as VLDL and a higher proportion of all newly made lipids than PV hepatocytes in the fed, fasted, and endotoxic states. Moreover, what we have come to call septic hepatocytes released more VLDL particles to the medium while showing patterns of de novo lipid synthesis and FAS mRNA levels that differed little from those of fasted animals.

When looking deeply into the regulation of VLDL secretion under the fasting condition, we found reported zonated actions of insulin (46) and glucagon (47) in the liver. Thus, whereas the insulin receptor protein is expressed predominantly in PV cells (46), the glucagon receptor

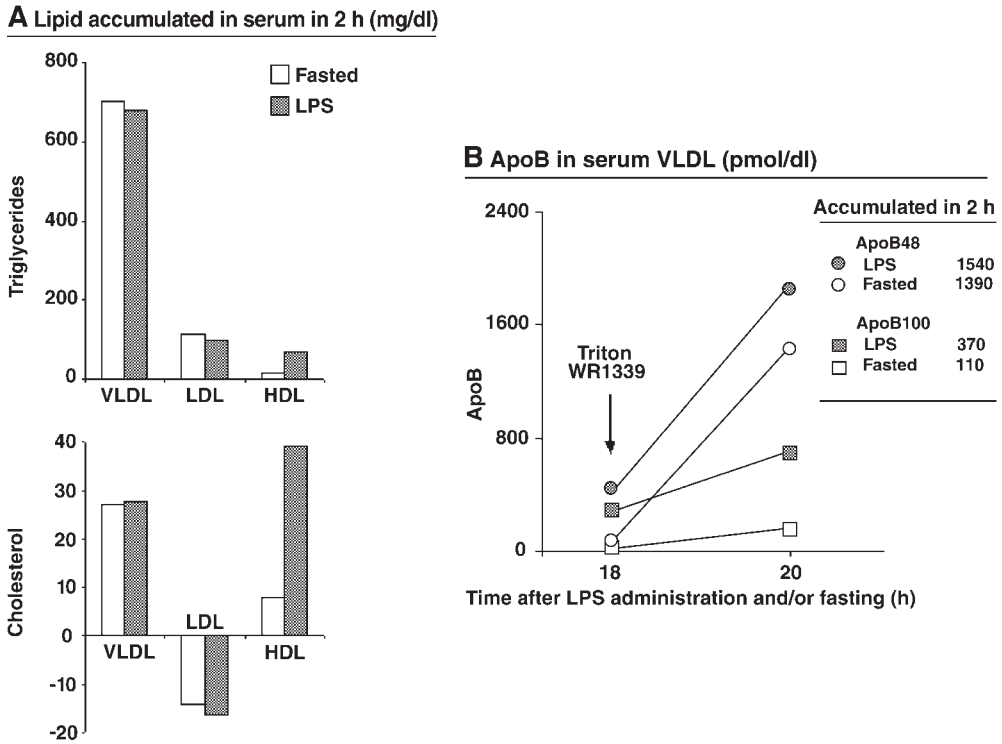

Fig. 4. Endotoxin effects on hepatic VLDL production and serum LDL and HDL lipids. Eighteen hour-fasted and 18 h-fasted and LPS-treated (1 mg/kg) rats were injected into the tail vein with 600 mg/kg Triton WR1339 in 0.9% NaCl or saline as described in Materials and Methods. Blood samples were drawn before and 2 h after injection, VLDL was isolated from the serum and characterized for apoB and lipid classes, and HDL and LDL were assayed for TG and total cholesterol levels. The results are expressed as means from four separate experiments; the SD (not shown) averaged 25%. Production of VLDL-lipid and -apoB was defined as the post-Triton accumulation of the lipoprotein components in 2 h, which was calculated by subtracting the accumulation in vehicle-injected rats from the accumulation in Triton-injected rats. Accumulation of serum lipoprotein lipids was calculated similarly. Therefore, differences in these variables cannot be analyzed statistically. Differences in VLDL-apoB content between fasted and LPS groups were statistically significant at $P \leq 0.05$.

mRNA is expressed periportally (47). Were this difference in mRNA levels reflected by the functionality of the glucagon receptor protein, the PP hepatocytes would be expected to be able to switch their fuel metabolism rapidly from the well-nourished to the fasted state and decrease de novo lipid synthesis more markedly than the hepatocytes of the PV zone. This hypothesis is supported by our own data for FAS mRNA level (Fig. 2), TG and cholesterol synthesis (Fig. 3A), and apoB secretion (Table 1) and by other data for de novo lipogenesis (21). Despite this, however, there were no substantial differences in the liver parenchymal capacity for VLDL production.

Evidence described below, combined with the overall superior ability of the PP hepatocytes for lipid output (Table 1), supports the notion that the PP-to-PV concentration gradient of a specific cytokine or, most likely, a certain combination of mediators might account for the LPS-promoted zonated VLDL-apoB secretion. Both the liver macrophages, an important source of cytokines (24), and the LPS-stimulated mRNA expression of IL-6 (27) are concentrated periportally. Endotoxin (8, 48) and specific cytokines (49) can dose-dependently enhance hepatic apoB secretion. Transcriptional regulation of the apoB gene is a controversial issue. Although in most studied conditions apoB secretion regulation is a posttranscriptional event, several studies have demonstrated increases in apoB mRNA levels stimulated by cytokines and other treatments. Dose-dependent upregulation of apoB mRNA has been reported to occur in human hepatoma HepG2 cells as early as 1 h after IL-6 or IL-1β stimulation and persisted for up to 18 h (49). ApoB secretion, however, was low, as apoB lipidation failed in this cell type, with severe abnormalities in TG recruitment (50) and enhanced intracellular degradation (49). A differential display analysis of the transcriptome profile of IL-1β-stimulated HepG2 cells also documented a rapid, sustained response of the apoB gene (51). By contrast, others have found no change in apoB mRNA levels during sepsis (8). In our studies, a direct correlation ($r = 0.95$, $P \leq 0.01$) between the initial apoB transcript levels in hepatocytes and the apoB mass secreted in 2 h was observed when individuals of the three experimental groups were analyzed collectively (data not shown). In particular, our findings support the view that apoB overproduction by septic hepatocytes is to some extent steered by overexpression of apoB mRNA.

A major finding in the current study is that PP hepato-

cytes are the principal source of the LPS-induced production of VLDL particles. Endotoxin almost doubled the secretion of relatively TG-poor VLDL particles by PP hepatocytes, whereas it modestly increased the PV secretion of normal particles, in terms of lipid composition (Table 1). The mechanism for the former effect was not pursued, but limited lipidation of the abundant apoB might occur in PP hepatocytes. This is substantiated by the 50% upregulation of the MTP mRNA mass in these cells (Fig. 2), with double the level of apoB mRNA (Fig. 2) and, presumably, apoB protein mass. Cotranslational and posttranslational degradation of apoB are prevented by MTP (10–14). Therefore, our findings, which would also explain the decrease in liver apoB degradation found in *E. coli* sepsis (8), suggest that primordial lipoprotein particles probably abounded at the PP hepatocyte ER upon endotoxin administration. Septic VLDLs were normal in terms of apoB composition. Rat hepatocytes edit the transcript of the apoB gene (9) so that they will secrete apoB-48- and apoB-100-bearing VLDL particles. PP and PV hepatocytes from either group secreted approximately five times more apoB-48 than apoB-100 (Table 1), suggesting that apoB transcript editing was not zonated or affected by sepsis, as reported previously (8). Although we did not perform a compositional analysis of those secreted particles containing truncated or complete forms of apoB, our findings basically agree with previous work showing that endotoxic perfused rat livers secreted two VLDL populations, one with decreased mean particle size (and hence TG) and the other with normal size containing mainly apoB-100 that contributed less to the apolipoprotein pool (17).

Experiments addressing LPS target identification revealed that lipid transcriptional regulation in sepsis is, to a great extent, species-specific and dependent on the feeding regimen of the animal. Thus, whereas endotoxin, tumor necrosis factor-α, and IL-1 have been reported to induce de novo fatty acid synthesis and hepatic TG synthesis in vivo and in vitro in rats (52–57; recently reviewed in 7), the transcript levels of a number of fatty acid synthetic enzymes decreased in response to LPS in the mouse (58). In freshly isolated hepatocytes from fasted LPS-treated rats, we did not observe enhanced expression of FAS mRNA (Fig. 2) or de novo lipid synthesis over 2 h (Fig. 3A); both of these findings support the idea that the provision of de novo lipid might be limited. It is known that an important part of the TG secreted by hepatocytes derives from a cytosolic storage pool after the lipolysis and reesterification of the lipolytic products to TGs at the ER before their incorporation into the VLDL particle (59–61). Sepsis seems to lead to a substantial recruitment of preformed TGs for apoB lipidation. Rough evidence for this is provided by the observation that the specific radioactivity of the TG secreted in VLDL, calculated from the data values in Table 1 and Fig. 3A, by septic hepatocytes, both PP and PV, was only ~35% of that of their corresponding controls. Subtle differences in the specific activity of precursors might partially account for such an effect. Nevertheless, as infection is usually accompanied by increased plasma fatty acid levels, as a result of activated adipose tissue lipolysis and decreased fatty acid oxidation in peripheral tissues (reviewed in 7), the TGs formed using those peripherally derived fatty acids are likely to be a major contributor to VLDL-TG secretion during the APR.

The time sequence and the mechanisms leading to the development of hypertriglyceridemia of bacteremic sepsis are not fully understood. Also relevant to this study is the extent to which the quantitative changes at the hepatocyte level may explain the endotoxin-associated increases in serum VLDL-apoB and -TGs. Analyses of the 2 h post-Triton VLDL production revealed that, as found in 2 h incubations of isolated PP hepatocytes (Table 1), TG and total cholesterol secretion levels were not increased by sepsis to the same extent as the apoB production (Fig. 4). The capture of newly secreted lipoproteins by receptor would not be expected to occur under the in vivo and ex vivo conditions of the current work. Hence, collectively, our findings add evidence that, during the APR, a population of apoB molecules, probably of PP origin, is secreted in TG-poor VLDL particles, suggesting that, in periods of food restriction, increased hepatic TG secretion does not contribute much to the sepsis-associated hypertriglyceridemia.

Taken together, our results demonstrate that the PP, compared with the PV, hepatocytes isolated from fed or fasted rats, despite their differences in subcellular structure abundance and lipid metabolism indicators, exhibited similar capabilities for VLDL assembly and secretion; only under the endotoxic pressure that stimulates the host immune system and VLDL production did both the VLDL-forming gene expression and the VLDL secretion zonate periportally. If cells regulate their gene expression to meet changing needs, these findings would further support liver zonation for host requirements to be accomplished in vivo and would offer an interesting model for the study of the significance of zonation in lipid metabolism.

The authors thank Montse Busto for her excellent technical assistance, M. P. Sowden and M. Scheiwer for providing the rat apoB and FAS cDNA, respectively, and M. Esteban (Galdakao Hospital, Vizcaya, Spain) for performing the serum biochemical and lipoprotein lipid analyses. This work was supported by grants from the Spanish Ministerio de Ciencia y Tecnología (BMC2001-0067) and Ministerio de Sanidad y Consumo (G03/015) and the Basque Government (PE02UN04).

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