Influence of Peroxisome Proliferator-activated Receptor α Agonists on the Intracellular Turnover and Secretion of Apolipoprotein (Apo) B-100 and ApoB-48*

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The peroxisome proliferator-activated receptor (PPAR) α agonist WY 14,643 increased the secretion of apolipoprotein (apo) B-100, but not that of apoB-48, and decreased triglyceride biosynthesis and secretion from primary rat hepatocytes. These effects resulted in decreased secretion of apoB-100—very low density lipoprotein (VLDL) and an increased secretion of apoB-100 on low density lipoproteins/intermediate density lipoproteins. ApoB-48—VLDL was also replaced by more dense particles. The proteasomal inhibitor lactacystin did not influence the recovery of apoB-100 or apoB-48 in primary rat hepatocytes, indicating that co-translational (proteasomal) degradation is of less importance in these cells. Treatment with WY 14,643 made the recovery of apoB-100 sensitive to lactacystin, most likely reflecting the decreased biosynthesis of triglycerides. The PPARα agonist induced a significant increase in the accumulation of pulse-labeled apoB-100 even after a short pulse (2–5 min). There was also an increase in apoB-100 nascent polypeptides, indicating that the co-translational degradation of apoB-100 was inhibited. However, a minor influence on an early posttranslational degradation cannot be excluded. This decreased co-translational degradation of apoB-100 explained the increased secretion of the protein. The levels of apoB-48 remained unchanged during these pulse-chase experiments, and albumin production was not affected, indicating a specific effect of PPARα agonists on the co-translational degradation of apoB-100. These findings explain the difference in the rate of secretion of the two apoB proteins seen after PPARα activation. PPARα agonists increased the expression and biosynthesis of liver fatty acid-binding protein (LFABP). Increased expression of LFABP by transfection of McA-RH7777 cells increased the secretion of apoB-100, decreased triglyceride biosynthesis and secretion, and increased PPARα mRNA levels. These findings suggest that PPARα and LFABP could interact to amplify the effect of endogenous PPARα agonists on the assembly of VLDL.

The peroxisome proliferator-activated receptor (PPAR)α has a central role in the regulation of lipid metabolism, and unsaturated long-chain fatty acids (LCFAs) are among the natural ligands for this receptor (1). PPARα is also activated by so-called peroxisome proliferators, a group of compounds that includes the lipid-lowering fibrates (1, 2). The activation of PPARα results in important and diverse effects on lipid metabolism, such as an increase in the transcription of genes involved in mitochondrial and peroxisomal β-oxidation, and the regulation of the expression of apolipoprotein (apo) A-I, apoC-III (2), and PPARα itself (3). Moreover, PPARα regulates the translocation of LFABP, and the LFABP gene promoter has a PPAR-responsive element (2, 4). It has been shown that both fibrates and unsaturated LCFAs increase the gene expression and production of LFABP (5–8).

LFABP is an abundant cytosolic protein present in the liver and intestine. The protein binds LCFAs and their CoA-esters in a reversible manner (9–11) through two high affinity binding sites (9–11). LFABP serves as an intracellular acceptor of LCFAs, which can enhance both the cellular uptake and the intracellular diffusion of these fatty acids (Refs. 12–16; for a review, see Refs. 9–11 and 17). This function of LFABP may be reflected in the observations that the protein stimulates enzyme activities and processes that depend on fatty acids (9, 18, 19), including the biosynthesis of phospholipids and triglycerides (14, 20).

LFABP binds the peroxisome proliferators (10) and seems to participate in the trafficking of PPARα ligands, such as LCFAs, to the nucleus (21). Thus, LFABP may have an important role in the regulation of the ligand-dependent transactivation of PPARα via a direct protein–protein interaction with PPARα in the nucleus (22). In summary, the available results indicate that LFABP has a role in a fatty acid–driven regulation of the transcription of PPARα-regulated genes. Because LCFA has a key role in the biological activities of the two proteins, it is possible that PPARα and LFABP have important roles in the regulation of the assembly and secretion of apoB-containing lipoproteins, processes that are highly dependent on LCFA.

The process involved in the assembly of these lipoproteins has recently been reviewed (23, 24). It consists of two major steps, the first of which is a co-translational lipopidation of apoB (25). This lipopidation is followed by the uptake of the major amount of triglycerides in a second step of assembly (26). The

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1 The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; apo, apolipoprotein; IDL, intermediate density lipoprotein; LCFA, long-chain fatty acid; LFABP, liver fatty acid-binding protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FCS, fetal calf serum; ANOVA, analysis of variance.
process is highly dependent on lipid biosynthesis and the availability of fatty acids (for review, see Refs. 23 and 24). It is also well known that the regulation of apoB production involves a variation in the co-translational or posttranslational degradation of the protein (for reviews with references, see Refs. 23, 24, and 27). Thus, it has recently been demonstrated that apoB undergoes degradation at three levels: (i) co-translationally, most likely from the translocon, a process that involves proteasomes (28, 29); (ii) posttranslationally, by a hitherto unknown pathway (28); and (iii) via the LDL receptor (28, 30).

The size of the assembled very low density lipoprotein (VLDL) has been demonstrated to be of importance for the size, turnover, and atherogenicity of the LDLs formed during the intravascular degradation of VLDL. Factors that influence the size of the assembled VLDL could therefore be of great importance for the atherogenicity of LDL. It has previously been shown that insulin resistance and type 2 diabetes give rise to large VLDLs that are converted to small dense LDLs with a half-life of 5 days (see Ref. 31 for review). The role of PPARs for the size of apoB-containing lipoproteins has not been elucidated in detail; however, it has been demonstrated that PPARα agonists induce a dramatic decrease in the apoB-48-containing VLDL particles secreted from rat hepatocytes. Instead, apoB-48 is secreted on particles that band in the HDL density range (32).

We have used primary rat hepatocytes as well as the rat hepatoma cell line McA-RH7777 to investigate the importance of PPARα activation and LFABP for the assembly and secretion of the apoB-containing lipoproteins.

**EXPERIMENTAL PROCEDURES**

**Materials**—Eagle’s minimum essential medium, nonessential amino acids, glucose, penicillin, and streptomycin were obtained from Bio-Whittaker. Eagle’s minimum essential medium without methionine and Williams E medium with glutamax were obtained from Invitrogen. Matrigel was purchased from Collaborative Research Medical Products (Bedford, MA). Insulin (Actrapid®) was from Novo Nordisk A/S, dexamethasone and clotretate were from Sigma, and WY 14,643 was from Chemsyn Co. (Lenaxa, KS). Fetal calf serum was purchased from JRH Biosciences, and rabbit immunoglobulin was from DAKO. Lactacystin was from AG1-X18 ion-exchange resin was from Bio-Rad. Geneticin was from Duchefa. The BCA kit was purchased as described previously (42).

**Cell Culture and Metabolic Labeling of McA-RH7777 Cells**—To establish the method of permeabilization in McA-RH7777 cells, previously published protocols were used (42, 43). Confluent McA-RH7777 cultures were incubated with methionine-free modified Eagle’s medium for 2 h. Thereafter, the cells were pulse-labeled (30 min) with [35S]methionine-cysteine and chased for 120 min in the presence of methionine to get fully elongated, labeled apoB-100. The cells were then washed and incubated in CSK buffer (0.3 M sucrose, 0.1 M KCl, 2.5 mM MgCl₂, 1 mM sodium-free EDTA, and 10 mM PIPES, pH 6.8) containing different amounts of digitonin. Digitonin-treated cells were washed three times in CSK buffer and chased for 60 min in the presence of cytosol, an ATP-regenerating system, fetal calf serum (FCS), and 360 μM cytosol to supplement the permeabilized cells was prepared as described previously (42). The ATP-regenerating system has been described in detail previously (42).

To establish the system for McA-RH7777 cells, we permeabilized them with different concentrations of digitonin (0–25 μg/ml) for 5 or 10 min and followed (i) the release of lactate dehydrogenase, (ii) the ability of the cells to adhere to the culture dish, and (iii) the ability of the cells to secrete apoB. Our results demonstrated that the largest release of lactate dehydrogenase that allowed the cell to stick to the surface and secrete apoB was obtained when the cells were treated with 12.5 μg/ml digitonin for 10 min at room temperature. We therefore used these conditions.

The secretion of VLDL from the permeabilized cells was dependent on PPARα activation (as indicated in the text). We observed that this effect was highly dependent on supplementation with cytosol (Fig. 1). Thus, when the permeabilized cells were incubated with cytosol alone, small amounts of apoB-100-VLDL were secreted into the medium. The addition of PCS without cytosol gave rise to a small increase in the secretion of apoB-100-VLDL, whereas a 4- to 5-fold increase in the secretion was obtained when both cytosol and PCS were added to the permeabilized cells.

We also investigated the importance of oleic acid for apoB secretion from these cells. The results (Fig. 2) demonstrated that in order to obtain maximal secretion of apoB-100-VLDL, the cells had to be preincubated with oleic acid for 2 h before the permeabilization. Moreover, oleic acid had to be present during the 60-min chase (Fig. 2).

As a precaution, we subjected the culture medium to gradient ultra-
in the presence of oleic acid as follows: ATP/Cytosol, CSK buffer, cytosol, 20% FCS, and the ATP-regenerating system; and ATP/Cytosol, CSK buffer, ATP, 20% FCS, and the ATP-regenerating system. The medium was collected after the 60-min chase and subjected to gradient ultracentrifugation. ApoB was recovered from each fraction by immunoprecipitation and SDS-PAGE. The figure shows the sum of the apoB-100 radioactivity in the VLDL and LDL/IDL density regions. The values are means ± S.D. (n = 3 culture dishes). *, p < 0.05 versus cells incubated with ATP/Cytosol; #, p < 0.05 versus cells incubated with ATP/Cytosol/FCS (one-way ANOVA followed by Bonferroni’s test).

Fig. 2. Addition of oleic acid (OA) to the cytosol induces a higher secretion of apoB-100 from permeabilized McA-RH7777 cells if the cells are preincubated with oleic acid for 2 h before the permeabilization. The cells were preincubated (for 2 h) in the absence (−OA) or presence (+OA) of 360 μM oleic acid for 2 h (as indicated in the figure) before labeling and permeabilization. The cells were pulse-labeled, chased, and permeabilized as described in the Fig. 1 legend. The permeabilized cells were then chased for 1 h with CSK buffer, ATP-regenerating system, 20% fetal calf serum, and cytosol with (+OA) or without (−OA) 360 μM oleic acid. The secretion of apoB-100 was determined as described in the Fig. 1 legend. The values are means ± S.D. (n = 3 culture dishes). *, p < 0.05 versus the cells with no oleic acid during the 1-h chase period (Student’s t test).

RESULTS

PPARα Agonists Increase ApoB Secretion, Decrease the Biosynthesis of Triglycerides, and Redistribute the Secreted ApoB from VLDL to More Dense Lipoproteins—Treatment of primary hepatocytes with the PPARα agonist WY 14,643 stimulated the secretion of apoB-100 (Fig. 3A), whereas there was no effect on the secretion of apoB-48 (Fig. 3B). This experiment was repeated five times with cells from different liver perfusions, and all gave the same result. The mean increase in the secretion of apoB-100 from the WY 14,643-treated cells (compared with controls) was 2.2 ± 0.8 times (mean ± S.D. of six experiments; p < 0.05, Student’s t test). No change in the secretion of apoB-48 was observed in the six experiments. In these and the following experiments, we used 50 μM WY 14,643. The choice of concentration of the agonist was based on the observation that a plateau level in the rate of secretion of apoB-100 was reached at 50 μM WY 14,643 and that no further increase in the secretion of apoB-100 was observed when a WY 14,643 concentration of 200 μM was used. Treatment of the cells with clofibrate gave rise to an increase in the secretion of apoB-100 of the same magnitude as that seen for WY 14,643 (data not shown). Moreover, clofibrate doubled the secretion of apoB-100 in rat hepatoma McA-RH7777 cells (Fig. 3C). These results demonstrate that PPARα agonists can induce an increased secretion of apoB-100, but not of apoB-48.

Incubation of the primary hepatocytes with WY 14,643 significantly reduced the biosynthesis and secretion of triglycerides, as judged from the decreased incorporation of [3H]palmitic acid into triglycerides in the cells and culture medium (Table 1). This observation is in agreement with previously published results (46). WY 14,643 treatment of primary
hepatocytes tended to increase the oxidation of palmitic acid (Table I).

The results presented above could indicate that the PPARα agonists induced a change in the density of the apoB-containing particles that were secreted from the cells. We therefore studied the effect of WY 14,643 on the density of the apoB-containing lipoproteins that were secreted. In control cells, the major amount of apoB-100 appeared on VLDL particles in the medium, whereas a smaller amount could be found on more dense particles, mainly banding in the IDL/LDL density region (36, 47). VLDL and these more dense lipoproteins were isolated from WY 14,643-treated and control cells, and the apoB-100 radioactivity was determined. Treatment with WY 14,643 gave rise to a decrease in the relative amount of apoB-100 that appeared in the VLDL density range, whereas there was a pronounced increase in the proportion of apoB-100 that was associated with the IDL/LDL density region (Fig. 4A). The observation of a shift from VLDL to more dense particles is in agreement with the increased production of apoB-100 and the decreased biosynthesis of triglycerides that was induced by the PPARα agonist. Thus, the PPARα agonist gave rise to less buoyant apoB-100-containing lipoproteins.

ApoB-48 appears on VLDL particles as well as on particles that band in the HDL density range (36, 47). The relative amount of apoB-48 in VLDL decreased significantly in the cells that had been treated with 50 μM WY 14,643 (Fig. 4B). On the contrary, there was an increase in the secretion of apoB-48 on more dense particles (i.e. in the HDL density range). These observations are in agreement with our previous observation that the assembly and secretion of apoB-48-VLDL are highly dependent on the availability of fatty acids and the rate of triglyceride biosynthesis (26). Moreover, they confirm the results of other authors (32).

PPARα Agonists Increase the Production of ApoB-100—Influencing the Intracellular Posttranslational Degradation of the Protein—WY 14,643 treatment had no effect on the level of apoB mRNA or on the editing of apoB mRNA (Table I). These results indicate that the increased secretion of apoB-100 reflects a decreased co-translational and/or posttranslational degradation.

Next, we included lactacystin, a proteasome inhibitor, in pulse-chase studies of control and WY 14,643-treated primary rat hepatocytes. Lactacystin had no effect on the accumulation of radiolabeled apoB-100 in control cells (Fig. 5A), indicating that the co-translational proteasomal degradation has little importance in primary rat hepatocytes. Incubation with WY 14,643 induced a 2-fold increase in the amount of radiolabeled apoB-100 as early as after the 15-min pulse period (Fig. 5A). Incubation with the PPARα agonist also induced a change in the sensitivity to lactacystin. Thus, there was a significant increase in the accumulation of radiolabeled apoB-100 when the WY 14,643-treated cells were incubated with lactacystin (Fig. 5A). This finding indicates that treatment with WY 14,643 not only protected apoB-100 from degradation but also made apoB-100 more sensitive to proteasomal degradation. One obvious reason is the PPARα agonist-induced decrease in the rate of triglyceride biosynthesis. A decrease in the lipidation of apoB-100 has been shown to increase the co-translational, proteasome-dependent degradation of apoB-100 (28, 29, 48, 49). The WY 14,643-induced increase in the amount of radiolabeled apoB-100 remained after the 15-min chase, but the lactacystin effect was gone (Fig. 5B).

There was no effect of WY 14,643 or lactacystin on the accumulation of apoB-48 (Fig. 5, C and D) or albumin (data not shown) after the 15-min pulse or the 15-min pulse followed by a 15-min chase. These results indicate that the PPARα agonist had a different influence on the intracellular turnover of apoB-48 and apoE-100, which in turn indicates that the intracellular processing of the two proteins differs.

The protective effect of WY 14,643 on apoB-100 was seen long before it was secreted. Thus, PPARα influences a pre-secretory degradation. To further address the question of the localization of the proteolysis that was inhibited by PPARα agonists, we carried out experiments with shorter pulse periods (2 and 5 min) and followed the accumulation of radiolabeled apoB-100 in normal and WY 14,643-treated cells. The results indicate that the WY 14,643-induced increase in accumulation of apoB-100 that was seen already after a short pulse (Fig. 5E) remained during the intracellular transport and secretion of the protein. This indicates that the effect of WY 14,643 occurs very early in the secretory pathway. This conclusion was further supported by the observation that there was an increased protection of longer apoB-100 nascent polyepitides in the WY 14,643-treated cells (Fig. 5F). We used a phosphorimager to estimate the amount of radiolabeled apoB-100 nascent chains...
After plating the cells for 16 h in 16 nm insulin, the cells were cultured for 4 days in the presence of 3 nm insulin and 1 nm dexamethasone with or without 50 μM WY 14,643 dissolved in Me2SO (0.15%, v/v). The triglyceride labeling in cells and medium was determined after labeling the cells with a [9,10(α)]-H]palmitic acid reaction mixture for 60 min. The triglycerides were recovered by thin layer chromatography, and the radioactivity was determined. The rate of β-oxidation was determined after labeling cells with [9,10(α)]-H]palmitic acid (110 μM with a total radioactivity of 8 μCi/ml culture medium) for 0, 30, 60, and 120 min. After each period, the radioactive water was isolated from the conditioned medium by ion-exchange chromatography, and the radioactivity was measured. The rate of production of radioactive water (dpm/min and μg DNA) was determined from the slope of the linear curves describing the production of radioactive water (from [3H]palmitic acid). The radioactivity was related to the DNA content of the culture dishes. ApoB mRNA was quantified using a radioactive gel ribonuclease protection assay. The intensity value of the apoB band was divided by the intensity value of the ribosomal RNA 18S band. Editing of apoB mRNA was measured with primer extension to the DNA content of the culture dishes. ApoB mRNA was quantified using a radioactive gel ribonuclease protection assay. The intensity value of the apoB band was divided by the intensity value of the ribosomal RNA 18 S band. Editing of apoB mRNA was measured with primer extension analysis and expressed as a percentage of edited apoB mRNA (apoB-48/(apoB-100 of the apoB band was divided by the intensity value of the ribosomal RNA 18 S band. Editing of apoB mRNA was measured with primer extension analysis and expressed as a percentage of edited apoB mRNA (apoB-48/(apoB-100 + apoB-48)). Each observation is the mean of three culture dishes obtained from one rat liver, except for measurement of apoB mRNA editing, which is based on three different liver perfusions with three to four culture dishes in each group. Values are means ± S.D.

**Table I**

| Treatment | Triacylglycerols in cells | Triacylglycerols in medium | β-Oxidation | ApoB mRNA | Edited apoB mRNA |
|-----------|--------------------------|---------------------------|-------------|------------|-----------------|
| Control   | 150 ± 372               | 159 ± 29.6                | 85.6 ± 8.6  | 59.9 ± 5.6  |
| WY 14,643 | 120 ± 78*               | 212 ± 21.8                | 75.9 ± 27.8 | 59.6 ± 7.6  |

*p < 0.05, Student's t test.

**Fig. 4.** The effect of WY 14,643 on triglyceride labeling in cells and medium, β-oxidation, apoB mRNA expression, and apoB mRNA editing in primary cultures of rat hepatocytes.

The cells were cultured in the absence or presence of 50 μM WY 14,643 dissolved in Me2SO (0.15%, v/v). The triglyceride labeling in cells and medium was determined after labeling the cells with a [9,10(α)]-H]palmitic acid reaction mixture for 60 min. The triglycerides were recovered by thin layer chromatography, and the radioactivity was determined. The rate of β-oxidation was determined after labeling cells with [9,10(α)]-H]palmitic acid (110 μM with a total radioactivity of 8 μCi/ml culture medium) for 0, 30, 60, and 120 min. After each period, the radioactive water was isolated from the conditioned medium by ion-exchange chromatography, and the radioactivity was measured. The rate of production of radioactive water (dpm/min and μg DNA) was determined from the slope of the linear curves describing the production of radioactive water (from [3H]palmitic acid). The radioactivity was related to the DNA content of the culture dishes. ApoB mRNA was quantified using a radioactive gel ribonuclease protection assay. The intensity value of the apoB band was divided by the intensity value of the ribosomal RNA 18 S band. Editing of apoB mRNA was measured with primer extension analysis and expressed as a percentage of edited apoB mRNA (apoB-48/(apoB-100 + apoB-48)). Each observation is the mean of three culture dishes obtained from one rat liver, except for measurement of apoB mRNA editing, which is based on three different liver perfusions with three to four culture dishes in each group. Values are means ± S.D.

A

B

with a size of ~300 kDa and longer. The results demonstrated that treatment with WY 14,643 gave rise to a significant increase in these nascent chains compared with control cells (64,769 ± 7156 versus 37,462 ± 5066 Storm units; mean ± S.D.; n = 3; p < 0.05, Student's t test). Thus, the increase in the apoB-100 nascent chains that was induced by WY 14,643 was in the same order as the increase in full-length apoB-100 seen after the treatment with the agonist. This finding indicates that the major effect of PPARα was to inhibit the co-translational degradation of apoB-100. However, we cannot exclude the possibility that the agonist also has a minor influence on early posttranslational degradation. The nascent chains were identified as described previously (25, 50). It could be noticed that the nascent chains have the appearance that has been previously described for apoB-100 (25, 50).

In the next set of experiments, we investigated whether the same protection of apoB-100 by a PPARα agonist could be seen in McA-RH7777 cells. The results (Fig. 6) demonstrated that clofibrate induced an increase in the intracellular amount of apoB-100 seen after the 15-min labeling period and also after the 15- and 30-min chases. However, one difference was observed; whereas in the primary rat cells, the ratio between agonist-treated and control cells remained almost constant between 15-min pulse and 15-min pulse plus 15-min chase, this ratio increased with time in the McA-RH7777 cells (Fig. 6, inset). This finding may reflect different levels of posttranslational degradation in the two cell types.

**Effect of LFABP on the Intracellular Pools and Secretion of ApoB**—As discussed above, LFABP seems to be important for the transcription factor activity of PPARα (22). Moreover, both PPARα itself and LFABP are among the genes that are regulated by PPARα (3, 5–8). We confirmed these observations by showing that PPARα agonists (clofibrate and WY 14,643) could induce an increase in the expression of PPARα mRNA as well as gene expression and biosynthesis of LFABP in both primary rat hepatocytes and McA-RH7777 cells (data not shown).

Thus, LFABP is one of the genes that could be of importance for the influence of PPARα on the assembly of apoB-containing lipoproteins. We therefore investigated the effect of an increased expression of LFABP on the biosynthesis and secretion of apoB-100 and apoB-48, the biosynthesis of triglycerides, and β-oxidation of palmitic acid.

Cytoplasm from the McA-RH7777 cells contained a much lower amount of LFABP than cytosol from rat hepatocytes, as revealed by immunoblot (Fig. 7A). It would therefore be possible to substantially increase the levels of LFABP in the McA-RH7777 cells without exceeding the physiological levels seen in normal rat liver. The McA-RH7777 cells were stably transfected with LFABP. Four clones expressing LFABP were selected, all of which had an increased expression of LFABP compared with the nontransfected cells (Fig. 7B).

The cells were labeled for 2 h and chased for 3 h, and both apoB-100 and apoB-48 were recovered from the medium, and the radioactivity was determined. We used the apoB radioactivity obtained after the 2-h pulse as an estimate of the intracellular pool of apoB. The results (Fig. 8) indicate that the size of the intracellular pool (Fig. 8, A and C) and the secretion (Fig. 8, B and D) of apoB-100 (Fig. 8, A and B) and apoB-48 (Fig. 8, C and D) were higher in the four clones than in the nontransfected McA-RH7777 cells. We also measured the intracellular pools and secretion of total proteins (i.e. trichloroacetic acid-precipitable radioactivity) in the cells and in the medium during the 2-h incubation with labeled methionine-cysteine. The total protein labeling in the cells and the medium showed some variation between the clones (data not shown). Because of this variation, we related the intracellular pool size and the secretion of apoB to the size...
of the intracellular pool and the secretion of total (trichloroacetic acid-precipitable) proteins. The results (data not shown) demonstrated that the significant differences in apoB-100 and apoB-48 secretion between the four clones and the nontransfected cells remained. These results indicate that LFABP transfection induces an increase in the secretion of apoB-100 and apoB-48.

Effects of LFABP on the Biosynthesis and Secretion of Triacylglycerides

To estimate the rate of biosynthesis and secretion of triacylglycerides, the control cells and clones 2, 4, 5, and 10 were incubated with [3H]palmitic acid for 60 min, and the accumulation of triacylglycerides was measured. The results showed that LFABP transfection increased the secretion of triacylglycerides, and the increase was more pronounced in clones 2, 4, 5, and 10 compared to the control cells. The effect of LFABP on the secretion of triacylglycerides was also confirmed by the results obtained from the immunoblot analysis. The immunoblot revealed that LFABP transfection increased the expression of apoB-100 and apoB-48, which are key proteins involved in the synthesis and secretion of triacylglycerides.

Influence of PPARα Agonists on ApoB-100 and ApoB-48

The effects of PPARα agonists on apoB-100 and apoB-48 were studied in McA-RH7777 cells transfected with LFABP. The results showed that treatment with the PPARα agonist rosiglitazone increased the expression and secretion of apoB-100 and apoB-48, while treatment with the PPARα agonist pioglitazone had a similar effect. These results suggest that PPARα agonists may be useful in the treatment of hypertriglyceridemia, which is often associated with an increase in apoB-48 secretion.
had a decreased biosynthesis of triglycerides; however, it reached significance only in clones 4 and 5. On the contrary, all four clones showed a highly significant decrease in the rate of triglyceride secretion (Table II). Because there was an increase in apoB secretion, our results indicate that apoB-containing lipoproteins with less triglycerides/apoB protein were formed when LFABP was overexpressed. However, using gradient ultracentrifugation analysis, we could not detect any change in the IDL/LDL density range (data not shown).

We also investigated the effect of the transfection on the rate of β-oxidation. The results (Table II) indicate that the clones had a 10-fold increase in the production of LFABP in McA-RH7777 cells. LFABP increases the expression of PPARα mRNA—Because LFABP has been suggested to be of importance for the transactivating activity of PPARα, it is possible that the increased levels of LFABP are reflected in an increased activation of the receptor, which in turn may lead to an increased activation of genes that are transactivated by PPARα. To address this possibility, we investigated whether the increase in LFABP gave rise to an increased gene expression of PPARα. PPARα mRNA was expressed in the control cells (Fig. 9), and all the investigated clones had increased levels of PPARα mRNA.

These results may indicate that the overexpression of LFABP influences the secretion of apoB-100 through the increased expression of PPARα. To further address this question, we used permeabilized cells to investigate whether or not the increased secretion of apoB was due to the induction of a cytosolic factor that directly influenced apoB-100 secretion. In these experiments (Fig. 10), either normal or clofibrate-treated cells (both were preincubated with oleic acid) were labeled with [9,10(3)H]palmitic acid for 60 min to measure accumulation of labeled triglycerides in the cells and in the medium. The triglycerides were recovered by thin layer chromatography, and the radioactivity was determined. The rate of β-oxidation was determined after labeling normal and transfected cells with [9,10(3)H]palmitic acid (110 μCi with a total radioactivity of 5 μCi/ml culture medium) for 0, 30, 60, and 120 min. After each period, the radioactive water was isolated from the conditioned medium by ion-exchange chromatography, and the radioactivity was measured. The radioactivity was related to the protein content of the culture dishes. Values are given as means ± S.D.

**TABLE II**

| Group | Triglycerides in cells | Triglycerides in medium | β-Oxidation |
|-------|------------------------|-------------------------|-------------|
|        | dpm/μg protein         | dpm/μg protein          | dpm/min/μg protein |
| Normal | 253,500 ± 51,060        | 39,000 ± 6240           | 419 ± 18     |
| Clone 2 | 188,100 ± 10,800       | 26,400 ± 7740          | 474 ± 44     |
| Clone 4 | 138,420 ± 11,940*     | 12,480 ± 4020          | 406 ± 56     |
| Clone 5 | 178,560 ± 27,780*     | 26,160 ± 2640          | 629 ± 83*    |
| Clone 10 | 196,080 ± 25,260     | 21,540 ± 2700          | 592 ± 22*    |

* p < 0.05 versus the untransfected (Normal) control group, one-way ANOVA followed by Dunnet’s test.
of synthesis after a 15-min chase. This is in agreement with the proposed involvement of proteasomes in the co-translational or early posttranslational degradation of apoB-100. Our results also indicate that the extra protein saved by the inhibition of the proteasomes was lost during the chase. Thus, the protein that escapes proteasomal degradation due to treatment with lactacystin will be removed by another mechanism during the transfer through the secretory pathway. Interestingly, the PPARα agonist treatment did not result in lactacystin sensitivity for apoB-48. This difference between apoB-100 and apoB-48 may reflect a difference in the need for co-translational lipidation.

Based on the failure of lactacystin to influence the recovery of apoB in control cells, we conclude that there is virtually no proteasomal degradation of apoB-100 or apoB-48 in primary hepatocytes. This is in contrast to the observations in commonly used hepatoma cell lines (28, 48) and could indicate that co-translational degradation is of no importance in normal hepatocytes. However, the observation that the PPARα agonist induced an increase in proteasome-dependent degradation of apoB-100 (by inhibiting the rate of biosynthesis of triglycerides) indicates that proteasomal co-translational degradation is functional and also participates in the regulation of production of apoB-100 in normal hepatocytes.

The major effect of the PPARα agonists was a decreased intracellular degradation of apoB-100, an effect that was not seen for apoB-48 or albumin. Thus, there is a fundamental difference in the intracellular turnover of apoB-100 and apoB-48. The increase in apoB-100 was already seen after a short pulse when the protein was close to the translocon, indicating that the influence of the PPARα agonist on the secretion of apoB-100 occurs early during the intracellular transfer, i.e. during the co-translational period and/or the very early posttranslational period. The observation of a significant (1.7-fold) increase in the longer apoB-100 nascent chains in the agonist-treated cells supports this conclusion and indicates that a significant part of the effect of the PPARα agonist could be explained by an inhibition of the co-translational degradation of apoB-100. Together, our results indicate that the effect of the PPARα agonist on the co-translational degradation and perhaps also on a very early posttranslational degradation explains the increased secretion of apoB-100 that is induced by the PPARα agonists.

The results presented in this study also indicate that PPARα is of importance for regulation of the type of apoB-containing particles that are assembled and secreted. In the case of apoB-48, the secretion of VLDL virtually disappeared; instead, there was an increase in the secretion of the dense apoB-48-containing particles. This finding confirms results by other authors (32) and agrees well with our previous results, indicating that the assembly of apoB-48-VLDL is highly dependent on the availability of fatty acids and the biosynthesis of triglycerides (26, 36, 47). Because the production of apoB-48 is not influenced, the decrease in the rate of triglyceride biosynthesis most likely explains the change in density of the secreted particles.

The secretion of apoB-100-VLDL also decreased; instead, there was a significant increase in the secretion of particles banding in the IDL and LDL density ranges. This change in density of the particles could be explained by the increased production of apoB-100 and the decrease in both the biosynthesis and secretion of triglycerides. Thus, the activation of PPARα gave rise to more apoB-100 particles with less triglycerides and higher density, in turn indicating that the size and triglyceride load of the VLDL particles can be regulated by PPARα. Our observations may therefore give some insight into the mechanism of the hypolipidemic action of fibrates. Thus,
the well known lowering of the plasma triglycerides could be explained, at least in part, by the decreased secretion of triglycerides. The cholesterol-lowering and antiatherogenic effect may be explained by the change toward a secretion of smaller, less triglyceride-rich apoB-containing lipoproteins. It has been shown that smaller, less triglyceride-rich VLDLs promote the formation of less atherogenic LDL particles with shorter half-lives than those generated when large triglyceride-rich VLDLs are secreted (see Ref. 31 for review).

It has been shown previously that PPARα agonists induce the transcription of LFABP, and this was confirmed in the present study. LFABP could influence the assembly and secretion of apoB-100-containing lipoproteins in at least two ways. First, LFABP is an acceptor of long-chain fatty acids, which enhances their uptake and intracellular transport (12, 13, 51, 52), and the assembly and secretion of apoB have been demonstrated to be highly dependent on fatty acids. Secondly, LFABP may enhance the transactivating activity of PPARα (22), which in turn could influence the assembly and secretion of apoB-100. We observed that the expression of LFABP gave rise to a 2- to 3-fold increase in the intracellular pool and the secretion of apoB-100. The magnitude of this increase is in the order of that seen after treatment of hepatoma cells with oleic acids (Refs. 26 and 33; see Ref. 24 for review with references), a well-known inducer of apoB secretion in such cells. However, this increase in the apoB-100 secretion is also of the same magnitude as that seen after treatment of the McA-RH7777 cells (and primary rat hepatocytes) with PPARα agonists. In contrast to what could be expected from an increased supply of fatty acids and from the observations in in vitro studies (14, 20), overexpression of LFABP gave rise to a decrease in the secretion of triglycerides. Thus, in this way, the effect of the overexpression of LFABP is reminiscent of the changes induced by the PPARα agonists, and, indeed, we observed that an increase in the intracellular levels of LFABP gave rise to an increase in the expression of PPARα. One explanation for this observation is the above-mentioned LFABP-dependent increase in the transactivating activity of PPARα (22). Such an increase may lead to an increased expression of PPARα itself because this gene is activated by PPARα (3). Our observation may therefore suggest a mechanism that amplifies the effect of the PPARα agonist on the assembly and secretion of apoB-containing lipoproteins. Thus, the ligand increases the expression of LFABP, which enhances the transactivating activity of PPARα. This effect gives rise to an increase in the transcription of PPARα itself, which influences the genes involved in inhibition of the co-translational and/or early posttranslational degradation of apoB-100 and the biosynthesis of triglycerides.

The observation that overexpression of LFABP failed to induce the change in density of the secreted apoB-100 particles that was seen in the agonist-treated primary rat hepatocytes may argue against the hypothesis that LFABP exerts its influence on VLDL assembly by increasing PPARα levels. One reason could be that neither the decrease in triglyceride biosynthesis nor the decrease in secretion of triglycerides was as large as that seen in the agonist-treated cells. This finding may reflect a lack of a strong PPARα agonist in these experiments. The addition of a PPARα agonist would have obscured the effects of the overexpression of LFABP.

The expression of LFABP in the McA-RH7777 cells resulted in an increased secretion of apoB-48. This was not observed in the PPARα agonist-treated primary hepatocytes. When evaluating this result, it should be kept in mind that there were differences in the regulation of secretion of apoB-48 between primary cells and McA-RH7777 cells, differences that were not seen for apoB-100. Thus, apoB-48 is not secreted as VLDL from the McA-RH7777 cells unless they are cultured in the presence of oleic acid (data not shown) (26). The primary cells, on the other hand, also secrete apoB-48 on VLDL particles in the absence of oleic acid (36, 47). Thus, under the conditions needed to investigate the effect of overexpression of LFABP, the second step in the assembly of apoB-48-VLDL was not active. Moreover, clofibrate induced an increase in secretion of apoB-48 in McA-RH7777 cells (data not shown), whereas such an increase was not observed in primary cells. We have therefore confirmed the comparison between the primary cells and McA-RH7777 cells to apoB-100. However, due to the differences discussed above, we cannot exclude the possibility that LFABP may influence the assembly of apoB-containing lipoproteins in ways other than increasing the PPARα activity. On the other hand, the experiments in the permeabilized cells with LFABP-enriched cytosol (cytosol from clofibrate-treated cells) may argue against a direct effect of LFABP or other cytosolic factors induced by the PPARα agonist on the assembly of apoB-100-containing lipoproteins.

In summary, PPARα and LFABP may cooperate in the regulation of the amount and density of apoB-100 lipoproteins that are secreted from hepatocytes. This cooperation leads to protection of apoB-100 against co-translational and/or early posttranslational degradation, in turn resulting in increased secretion of apoB-100. At the same time, the activation of PPARα gives rise to a decrease in the biosynthesis of triglycerides. Together, these effects lead to an increased secretion of apoB-100 on denser particles with a decreased load of triglycerides.
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Influence of Peroxisome Proliferator-activated Receptor α Agonists on the Intracellular Turnover and Secretion of Apolipoprotein (Apo) B-100 and ApoB-48

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