Hepatic AQP9 expression in male rats is reduced in response to PPARα agonist treatment

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Lebeck J, Cheema MU, Skowronski MT, Nielsen S, Praetorius J. Hepatic AQP9 expression in male rats is reduced in response to PPARα agonist treatment. Am J Physiol Gastrointest Liver Physiol 308: G198–G205, 2015. First published December 4, 2014; doi:10.1152/ajpgi.00407.2013.—The peroxisome proliferator receptor α (PPARα) is a key regulator of the hepatic response to fasting with effects on both lipid and carbohydrate metabolism. A role in hepatic glycerol metabolism has also been found; however, the results are somewhat contradictory. Aquaporin 9 (AQP9) is a pore-forming transmembrane protein that facilitates hepatic uptake of glycerol. Its expression is inversely regulated by insulin in male rodents, with increased expression during fasting. Previous results indicate that PPARα plays a crucial role in the induction of AQP9 mRNA during fasting. In the present study, we use PPARα agonists to explore the effect of PPARα activation on hepatic AQP9 expression and on the abundance of enzymes involved in glycerol metabolism using both in vivo and in vitro systems. In male rats with free access to food, treatment with the PPARα agonist WY 14643 (3 mg·kg⁻¹·day⁻¹) caused a 50% reduction in hepatic AQP9 abundance with the effect being restricted to AQP9 expressed in perportal hepatocytes. The pharmacological activation of PPARα had no effect on the abundance of GlyK, whereas it caused an increased expression of hepatic GPD1, GPAT1, and L-FABP protein. In WIF-B9 and HepG2 hepatocytes, both WY 14643 and another PPARα agonist GW 7647 reduced the abundance of AQP9 protein. In conclusion, pharmacological PPARα activation results in a marked reduction in the abundance of AQP9 in perportal hepatocytes. Together with the effect on the enzymatic apparatus for glycerol metabolism, our results suggest that PPARα activation in the fed state directs glycerol into glycerolipid synthesis rather than into de novo synthesis of glucose.

aquaporin-9; immunohistochemistry; WY 14643; hepatocytes; glycerol metabolism

Peroxisome proliferator receptor α (PPARα) is a ligand-activated transcription factor that belongs to the superfamily of nuclear hormone receptors (39). After binding of ligands such as free fatty acids and fibrates, PPARα and its coactivators influence the transcription of target genes either by binding to response elements in the corresponding promoter region (PPREs) or by interfering with other transcription factors (39). Fibrates are used in the treatment of hypertriglyceridemia and PPARα regulates the expression of several proteins involved in lipid metabolism that promotes the removal of triglycerides (TG) from the circulation and enhances β-oxidation and ketogenesis in the liver (11). The induction of these pathways is particularly vital during starvation and PPARα knockout (KO) mice respond to fasting with hypoketonemia, hypothermia, and hypoglycemia (12). Several mechanisms have been proposed to explain the fasting-induced hypoglycemia, including decreased hepatic glucose production (12, 21) as well as increased extrahepatic glucose utilization (13, 38). Along these lines, PPARα has been shown to affect hepatic glycerol gluconeogenesis; however, the results obtained are contradictory. Patsouris and coworkers (21) found a decreased hepatic glucose production when comparing fasted wild-type (WT) and PPARα KO mice and showed that this was paralleled by a lack of increase in the expression of several genes involved in hepatic glycerol gluconeogenesis including aquaporin 9 (AQP9), glycerol kinase (GlyK), and cytosolic glycerol phosphate dehydrogenase (GPD1). In contrast, tracer studies performed in fasted PPARα KO mice showed that the KO mice had increased hepatic glucose production, which was at least partly due to a marked increase in hepatic gluconeogenesis from glycerol (38). Thus further studies are needed to clarify the role of PPARα in regulation of hepatic glycerol metabolism.

During states of low energy supply, glycerol released from adipose tissue is transported to the liver, where it serves as a precursor for de novo synthesis of glucose and recycling of fatty acids into glycerolipids (26). Aquaporin 9 (AQP9) is a broad-selectivity neutral solute channel that facilitates the hepatic uptake of glycerol (3, 29, 32, 33). The importance of AQP9 as a facilitator of hepatic glycerol uptake is illustrated by the increased plasma glycerol levels found in AQP9 KO mice (29). AQP9 is expressed in the basolateral plasma membrane domain of hepatocytes with the highest expression level in the perivenous region (5, 29). In male rodents, the hepatic expression of AQP9 is inversely regulated by insulin, with an increased abundance of AQP9 in response to fasting and diabetes (4, 15). These findings have led to the assumption that AQP9 plays an important role in supplying glycerol for the increased gluconeogenic rate in these states. In support, AQP9-deficient Leprdb/Lepdb mice, which become obese and develop Type 2 diabetes, have lower blood glucose levels compared with their AQP9 WT littermates (19). As outlined in Fig. 1, once glycerol is inside the hepatocyte, GlyK phosphorylates it into glycerol-3-phosphate (G3P). GPD catalyzes the reversible oxidation of G3P into dihydroxyacetone phosphate (DHAP) that can be channeled into either the gluconeogenic or glycolytic pathway. Alternatively, G3P is used for the synthesis of glycerolipids, with the initial step being catalyzed by glycerol-3-phosphate acyltransferase (GPAT).

To clarify whether PPARα activation suppresses or stimulates the initial hepatic handling of glycerol, we here investigate the direct effect of PPARα activation on the hepatic...
expression of AQP9 using in vivo and in vitro models and evaluate the impact on the enzymes involved in the initial hepatic handling of glycerol.

**METHODS**

**Animal experiment.** Male Wistar rats (Taconic) with a body weight of 318 ± 3 g (n = 10) were kept in individual cages at 21°C with a 12:12-h artificial light-dark cycle and 55% humidity. The experimental group (n = 5) received 3 mg·kg\(^{-1}\)·day\(^{-1}\) of the PPAR\(\alpha\) agonist WY 14643 administered in their chow for 10 days before the termination of the experiment. WY 14643 was dissolved in DMSO and WY 14643 administered in their chow for 10 days before the termination of the experiment. Gel samples were prepared after 24-h exposure to the experimental medium indicated in Fig. 5. The presented data were obtained from 3 experimental days, and n indicates the total number of wells.

**Immunohistochemistry.** The perfusion-fixed tissues were postfixed for 1 h at 4°C. After washing and dehydration, the tissues were embedded in paraffin wax and 2-μm sections were cut with a rotary microtome (Leica). Immunoperoxidase and immunofluorescence labeling for AQP9 were performed as previously described (16, 17). Microscopy of immunoperoxidase labeling was performed by using a bright-field microscope (Leica, DMIRE2) equipped with a Leica DM300 digital camera, whereas a Leica TCS SL (SP2) laser scanning confocal microscope was used for immunofluorescence detection. Quantitative analysis was performed with Image-Pro Analyzer 6.2 software. For each image (control: n = 5, WY 14643 treated: n = 3), immunofluorescence labeling was detected in a squared area of same size in the perivenous and perportal area, and from this the ratio of AQP9 expression was calculated.

**Immunocytochemistry.** Cells grown on coverslips were fixed for 10 min in 4% paraformaldehyde in PBS. After washing and permeabilization with 0.2% saponin, unspecific binding was blocked with 10% doner bovine serum and 0.1% BSA for 30 min and 1% BSA for another 30 min. Cells were then incubated with AQP9 antibody diluted in 0.5% BSA and 0.05% saponin in PBS overnight at 4°C. After washing, the primary antibody was visualized by use of a fluorescent secondary antibody and TO-PRO3 (Invitrogen) was used for nuclear counterstaining. After washing, the coverslips were mounted with a coverslip in Glycergel Antifade Medium (Dako). Microscopy was performed with a Leica TCS SL (SP2) laser scanning confocal microscope.

**Semiquantitative immunoblotting.** Liver and cultured hepatocyte samples were immediately homogenized and prepared for storage as previously described (16, 17). Proteins were separated by SDS-PAGE, and for the samples obtained in the animal experiment the initial gels were stained (Gelcode Coomassie Blue Stain Reagent, Thermo Scientific) to adjust protein loading. Separated proteins were electrotransferred onto either nitrocellulose (Amersham Pharmacia Biotech) or Immunobilon FL (Merck Millipore) membranes. Antibody binding was visualized by use of either ECL (Amersham Biosciences) or Odyssey Infrared Imaging System (LI-COR Biosciences). ECL detection was performed as previously described (17). For Odyssey detection the membranes were blocked with Odyssey blocking buffer (LI-COR Biosciences) in 0.1 M PBS (50% vol/vol) and incubated overnight at 4°C with primary antibody. After washing in PBS-T, the blots were incubated for 1 h with fluorescent-labeled secondary antibody. For a final wash in PBS the bound antibody was visualized with Odyssey Infrared Imaging System (LI-COR Biosciences). For both detection systems semiquantification was performed after background subtraction and each lane was adjusted to the band intensity of actin from the same membrane. The band intensities were measured within the linear range by using ImageJ software as outlined in the ImageJ documentation.

**Antibodies.** The primary antibodies used were anti-AQP9 (RA2674-685) (29), anti-AQP9 (Alpha Diagnostics, AQP91-A), anti-GlyK (Abcam, ab70029), anti-GPD1 (Pierce, PA5-31051), anti-GPAT1 (Abcam ab68925), anti-liver fatty acid binding protein (L-FABP; Abcam, ab7874), anti-20S proteasome a2, and anti-actin (Sigma A2066), all raised in rabbits. The secondary antibodies used were horseradish peroxidase-conjugated goat anti-rabbit IgG (Dako) for immunoperoxidase labeling and ECL detection. For immunofluorescence labeling Alexa Fluor-488 goat anti-rabbit (Invitrogen) was used. Fluorescence
detection was performed with a donkey anti-rabbit IRDye 800CW antibody (Odyssey).

RT-PCR. Total RNA was extracted from liver homogenate or WIF-B9 hepatocytes by using TRizol Reagent Solution (Ambion) according to the manufacturer’s instructions. To generate cDNA, RT-PCR was performed after DNase treatment with 10 U/μl SuperScript II Reverse Transcriptase (Invitrogen). PCR for the PPARα transcript was performed on the obtained cDNA by using HotStarTaq Master Mix (Qiagen), 10% cDNA, and 0.5 pM of each primer 5’-CCACGAAGCTCTAGAAGAC-3’ and 3’-CAATCTCTCTCTGAAACCTTCT-5’ with an expected product size of 584 bp. After heat activation at 95°C, 30 PCR cycles were performed with denaturation for 20 s at 95°C, annealing for 30 s at 60°C, and elongation at 72°C for 60 s. PCR controls included omission of RT as negative controls and PCR for GAPDH to validate each template (not shown). PCR products were separated by 1% agarose gel electrophoresis with controls and PCR for GAPDH to validate each template (not shown).

RESULTS

Wy 14643 has no effect on body weight or plasma levels of glucose, glycerol, and insulin. Male rats were treated for 10 days with 3 mg·kg⁻¹·day⁻¹ of the PPARα agonist Wy 14643 to assess the role of PPARα in regulation of hepatic glycerol handling. No effect of PPARα agonist treatment was observed on body weight throughout the experiment (Fig. 2A), and the plasma levels of glucose and glycerol remained unaffected (Fig. 2, B and C). The plasma level of insulin was similar in the two groups measured at the termination of the experiment (Fig. 2D), indicating that any effect on hepatic AQP9 expression would be independent of insulin signaling.

Wy 14643 treatment changes the hepatic expression pattern of hepatic AQP9. The expression of AQP9 was examined by immunohistochemistry. Representative images of immunoperoxidase labeling are shown in Fig. 3 and, as previously described, labeling for AQP9 in the liver is primarily localized to the basolateral plasma membrane domain of hepatocytes surrounding the central veins (Fig. 3, A and B), with decreased labeling toward the portal triad. No apparent effect on the expression of AQP9 in the perivenous area was observed in response to PPARα agonist treatment (Fig. 3, A and B). However, a marked change in the distribution of AQP9 was observed when the overall expression pattern was evaluated (Fig. 3, C and D). As illustrated in Fig. 3, C and D, the periportal area with no labeling for AQP9 was markedly extended in the treated animals. This shows that the PPARα agonist treatment reduces the expression of AQP9 in periporal but not perivenous hepatocytes. Furthermore, the Wy 14643-treated animals did not display any apparent histological changes in the liver parenchyma. To make a semiquantitative evaluation of the changed expression pattern of AQP9, we used immunofluorescence labeling. The ratio of AQP9 immunofluorescence signal in pericentral vs. periportal hepatocytes was 1.9-fold higher in the PPARα agonist-treated rats (controls: n = 4, treated: n = 3, P < 0.05; Fig. 3G) as a result of the low AQP9 expression level in the periporal area of the treated rats.

Wy 14643 changes the hepatic expression of proteins involved in glycerol metabolism. Immunoblotting for AQP9 in liver homogenates revealed that the overall abundance of AQP9 was reduced by more than 50% in response to PPARα agonist treatment (controls: n = 5, treated: n = 5, P < 0.01; Fig. 4, A and E). We found no differences between the two groups in the hepatic protein abundance of GlyK, the first enzyme to metabolize glycerol into G3P (not significant; Fig. 4, B and F). By contrast, we found a twofold increase in the expression level of GPD1 (P < 0.001; Fig. 4, C and G), the enzyme that catalyzes the reversible conversion of DHAP and reduced nicotinic adenine dinucleotide (NADH) to G3P and NAD⁺ (Fig. 1). In addition, a threefold increase in hepatic GPAT1 protein expression was found (P < 0.0001; Fig. 4, D and H). GPAT1 is localized to the outer mitochondria membrane where it catalyzes the initial step of triacylglycerol synthesis. The GPAT1 antibody was tested on liver samples from GPAT1 WT and KO mice to evaluate the specificity of the detected band. In the GPAT1 KO mice the band observed in the WT animals was almost abolished (Fig. 4I). Immunoblotting for AQP9 was used to confirm that a comparable amount of protein was loaded in the two lanes. As a positive control for the effect of the Wy 14643 treatment, we evaluated the effect on L-FABP and found a marked increase in the protein abundance (controls: n = 5, treated: n = 4; P < 0.05; Fig. 4J), as previously reported (reviewed in Ref. 1).

Wy 14643 and GW 7647 both cause a decreased expression of AQP9 in WIF-B9 hepatocytes. To establish whether the reduced expression of AQP9 in Wy 14643-treated male rats was due to a direct effect on the hepatocytes, we analyzed the effect of PPARα activation in cultured hepatocytes. WIF-B9 hepatocytes are rat hepatoma and human fibroblast hybrid cells that natively expresses rat and human AQP9 mRNA (9). As
illustrated in Fig. 5A, the AQP9 immunoreactivity in WIF-B9 hepatocytes is predominantly found in the plasma membrane domain. The presence of PPARα mRNA in WIF-B9 hepatocytes was confirmed by RT-PCR using rat liver cDNA as positive control (Fig. 5B). The WIF-B9 hepatocytes responded to WY 14643 exposure by decreasing the abundance of AQP9 protein in a concentration-dependent manner ($n = 11–17$, $P < 0.01$ and $P < 0.05$, respectively; Fig. 5C), thus confirming that PPARα activation in hepatocytes reduces the expression of AQP9. As illustrated in Fig. 5D, a marked increase in L-FABP was observed in the same settings, thus confirming the effect of the agonist ($n = 11–18$; $P < 0.001$ and $P < 0.001$, respectively). The most pronounced effect of WY 14643 on AQP9 and L-FABP protein abundance was seen at 1 μM where the abundance of AQP9 was reduced by 34%. However, the selectivity profile of WY 14643 with $EC_{50}$ of 0.63 μM for PPARα and $EC_{50}$ of 32 and 100 for PPARγ and PPARδ, respectively, does not rule out effects on other PPAR molecules in the liver such as PPARδ, which is expressed to a similar extend as PPARα (6). Therefore, we wanted to evaluate the effect of another PPARγ agonist (GW 7647) with a higher specificity for PPARα (2). As illustrated in Fig. 5E we found that 100 nM of GW 7647, similarly to WY 14643, reduced the AQP9 protein abundance by 43% ($n = 9$, $P < 0.05$). No significant effect was observed in response to 10 and 1,000 nM of GW 7647. Again we used the effect on L-FABP expression as a positive control and found a marked increase in the protein abundance at 10 and 100 nM, whereas the response to 1,000 nM GW 7647 was greatly diminished ($P < 0.05$ and $P < 0.01$, respectively; Fig. 5F).

GW 7647 causes a decreased expression of AQP9 in HepG2 hepatocytes. To examine whether the observed effect of PPARα activation on hepatic AQP9 protein abundance would also apply to human hepatocytes, we analyzed the effect of GW 7647 in the human hepatocyte cell line HepG2. The HepG2 hepatocytes responded to GW 7647 exposure by decreasing the abundance of AQP9 protein ($n = 6$, $P < 0.01$; Fig. 6A), thus suggesting that the effect of PPARα activation on
hepatic AQP9 expression is comparable between species. As illustrated in Fig. 6A we found that 100 nM of GW 7647 caused a 24% reduction in AQP9 protein abundance. However, in contrast to what was observed in WIF-B9 hepatocytes, GW 7647 did not significantly increase the protein abundance of L-FABP in HepG2 hepatocytes (n = 4; P = 0.10; Fig. 6B).

DISCUSSION

We here demonstrate that the expression of hepatic AQP9 protein is markedly reduced in response to pharmacological activation of PPARα in male rats with free access to food. A reduced abundance of AQP9 protein was also observed in WIF-B9 and HepG2 hepatocytes when exposed to PPARα agonists. These findings are in line with the increased hepatic glycerol gluconeogenesis demonstrated in fasted PPARα KO mice (38) but seem to contradict the paradigm of PPARα being essential for the increased hepatic AQP9 abundance during fasting in male rodents (21, 22, 25).

The marked reduction in hepatic AQP9 expression induced by PPARα activation was confined to periportal hepatocytes, whereas the abundance in perivenous hepatocytes remained unaffected. According to the hepatic zonation model, hepatic metabolism is divided into two different zones, with the periportal region being dedicated to gluconeogenesis and the perivenous region being the preferential site for glycolysis and lipogenesis (10, 23). With the changes in hepatic AQP9 expression demonstrated here, it seems plausible that PPARα activation in fed male rats lowers the hepatic availability of glycerol for gluconeogenesis, whereas the supply of glycerol for either glycolysis or lipogenesis remains unaffected. Again, this is in line with the inhibitory effect of PPARα activation on hepatic glycerol gluconeogenesis suggested by the results obtained in fasted PPARα-KO animals (38). However, in another tracer study a marked increase in hepatic glycerol gluconeogenesis was found in fasted male rats treated with WY 14643 (30). Multiple factors could explain the differences in these results, such as differences in species, genetic background, and dosage of the PPARα agonist applied. In addition, it is a well-known phenomenon that there are discrepancies in the conclusions made from studies in PPARα-KO mice and experiments using PPARα-ligands (22). Furthermore, as previously noted, the abundance of PPARα is increased by fasting (12) and the relative PPARα expression level, together with changes other signaling pathways, will likely influence the transcriptional response to PPARα activation (24). Thus the reduction in perportal AQP9 abundance in response to PPARα activation found here in ad libitum fed rats will not necessarily apply to fasted rats.

Among the enzymes involved in the initial hepatic metabolism of glycerol, we found no effect of WY 14643 treatment on hepatic GlyK protein abundance, which opposes the previous observed increased GlyK mRNA levels in response to pharmacological PPARα activation (20, 21, 24). The increased expression of GPD1 protein is in line with the observations made by Patsouris and coworkers (21). GPD1 catalyzes the reversible oxidation of G3P into DHAP, and its increased expression in this study could both indicate channeling of G3P toward gluconeogenesis/glycolysis as well as support an increased synthesis of G3P for glycerolipid synthesis as GDP1 catalyzes the final step of glyceroneogenesis (31). To investigate this matter further, we analyzed the effect of WY 14643 treatment on hepatic GPAT1 protein expression. GPAT1 is one of four known GPAT isoforms that catalyzes the initial step in glycerolipid synthesis. In the liver, GPAT1 is responsible for 30–50% of total GPAT activity, and this isoform is known to be regulated by nutritional status, with an increased abundance

Fig. 4. Semiquantitative immunoblots with antibody against AQP9, glycerol kinase (GlyK), glycerol phosphate dehydrogenase 1 (GPD1), glycerol-3-phosphate acyltransferase 1 (GPAT1), and fatty acid binding protein (FABP) in liver homogenates from control and WY 14643 (WY)-treated male rats. A: AQP9 expression in control and WY 14643-treated male rats. B: GlyK expression in control and WY 14643-treated male rats. C: GPD1 expression in control and WY 14643-treated male rats. D: GPAT1 expression in control and WY 14643-treated male rats. E–H: densitometric analysis of the immunoblots presented in A–D. Expression of all proteins was normalized to actin or 20S proteasome α2. I: Immunoblot with antibody against GPAT1 in liver homogenates from wild-type and GPAT1 knockout mice (top); immunoblot for AQP9 was used as a control for similar loading of protein in the 2 lanes (bottom). J: densitometric analysis of the immunoblot for FABP. The protein expression was normalized to actin. Mean band densities ± SE were corrected to mean control level. *Statistical significant differences by Student’s t-test.
Thereon needs to be transported to this cellular compartment. Lysophosphatidic acid synthesized by GPAT1 glycerophospholipids are located in the endoplasmic reticulum hepatic GPAT1 mRNA expression (20, 24). This agree with the emerging evidence that PPAR activation increases hepatic generation of glycerolipids in ad libitum fed rats.

Previous studies in HepG2 have indicated that the inverse regulation of AQP9 by insulin described in rodents does not apply to humans (27). On the other hand, recent results obtained in humans (28) and mice (7) both show a reduced AQP9 abundance in relation to nonalcoholic fatty liver disease, suggesting comparable regulation of AQP9 expression between species. Here we find that incubation with PPAR agonists results in a reduced AQP9 abundance in both rat WIF-B9 and human HepG2 hepatocytes. The effect of PPAR activation on AQP9 protein abundance was less pronounced in the human cell line, which is in line with a previous notion that PPAR activation has a more limited effect in HepG2 hepatocytes compared with a rat hepatoma cell line (36). Our results are in contrast to a previous study using HepG2 hepatocytes in which WY 14463 caused a marked increase in AQP9 abundance (18). This discrepancy could at least partially be due to differences in the cell medium composition. Here we use 0.5% FBS in the experimental medium, which we previously have noted as a vital parameter for transcriptional regulation of AQP9 expression by estradiol in WIF-B9 hepatocytes (16). In all, our findings in HepG2 hepatocytes suggest that the results obtained in rats by pharmacological PPAR activation do have human relevance.

In conclusion, we here show that pharmacological PPAR activation in male rats with free access to food markedly reduces the abundance of the glycerol channel AQP9 in perivenous hepatocytes, whereas the expression in perivenous hepatocytes remains unaffected. This is paralleled by an increased abundance of both GPD1 and GPAT1. These findings suggest that WY 14463 treatment in the fed state increases the capacity for hepatic glycerolipid synthesis and that the supply of G3P for this is supported by an increased rate of glyceroneogenesis via GPD1 and an unchanged AQP9 expression in perivenous hepatocytes.

and activity in the fed state (reviewed in Refs. 8, 37). Here we find that pharmacological PPAR activation results in a threefold increase in hepatic GPAT1 protein expression, suggesting an increased hepatic glycerolipid synthesis in these animals. This agrees with the emerging evidence that PPAR activation, in addition to its promotion of fatty acid catabolism, stimulates fatty acid elongation and TG synthesis (14, 20, 25), and it extends the recent studies showing that PPAR activation increases hepatic GPAT1 mRNA expression (20, 24).

The enzymes that catalyze the final steps to form TG or glycerophospholipids are located in the endoplasmic reticulum membrane. Lysophosphatidic acid synthesized by GPAT1 therefore needs to be transported to this cellular compartment. This transport has been suggested to be mediated by L-FABP (34, 35) and therefore the increased abundance of L-FABP found in this study also supports that PPAR activation increases hepatic generation of glycerolipids in ad libitum fed rats.
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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.L., S.N., and J.P. conception and design of research; J.L., M.U.C., and M.T.S. performed experiments; J.L., M.U.C., and M.T.S. analyzed data; J.L. and J.P. interpreted results of experiments; J.L. prepared figures; J.L. drafted manuscript; J.L. and J.P. edited and revised manuscript; J.L., M.U.C., M.T.S., S.N., and J.P. approved final version of manuscript.

REFERENCES


