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PPARδ ligand L-165041 ameliorates Western diet-induced hepatic lipid accumulation and inflammation in LDLR$^{-/-}$ mice

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ABSTRACT

Although peroxisome proliferator-activated receptor delta (PPARδ) has been implicated in energy metabolism and the lipid oxidation process, detailed roles of PPARδ in lipid homeostasis under pathologic conditions still remain controversial. Thus, we investigated the effect of PPARδ ligand L-165041 on Western diet-induced fatty liver using low-density lipoprotein receptor-deficient ($LDLR^{-/-}$) mice. $LDLR^{-/-}$ mice received either L-165041 (5 mg/kg/day) or vehicle (0.1 N NaOH) with Western diet for 16 weeks. According to our data, L-165041 drastically reduced lipid accumulation in the liver, decreasing total hepatic cholesterol and triglyceride content compared to the vehicle group. Gene expression analysis demonstrated that L-165041 lowered hepatic expression of PPARγ, apolipoprotein B, interleukin 1 beta (IL-1β), and interleukin-6. In contrast, L-165041 increased hepatic expressions of PPARδ, lipoprotein lipase (LPL), and ATP-binding cassette transporter G1 (ABCG1). Our data suggest that L-165041 might be effective in preventing Western diet-induced hepatic steatosis by regulating genes involved in lipid metabolism and the inflammatory response.

Keywords:

Peroxisome proliferator-activated receptor delta

$LDLR^{-/-}$ mice

50 Steatosis

51 Inflammation

52

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of nuclear receptors that regulate various metabolic processes by acting as transcription factors. The peroxisome proliferator-activated receptors family consists of three structurally similar isoforms, α, δ, and γ (Takano et al., 2004). Among these different isoforms, the least studied PPARδ is known to be widely expressed in many different types of tissue (Ahmed et al., 2007). Although synthetic PPARδ ligands have not been fully approved for clinical applications, studies to elucidate their pharmacological effects have been ongoing since they were first synthesized and results are now accumulating.

PPARδ has been reported to be involved in developmental regulation (Braissant and Wahli, 1998), energy homeostasis (Berger et al., 2005), and lipid metabolism (Leibowitz et al., 2000). In the hepatic system, a synthetic PPARδ ligand has been reported to induce hepatic lineage cell proliferation (Hellemans et al., 2003; Glinghammar et al., 2003). In addition, some beneficial effects of PPARδ on hepatic lipid metabolism, including an attenuation of hepatic fat accumulation in leptin-deficient (ob/ob) mice (Wang et al., 2003) and an improvement in hepatic inflammation and subsequent steatohepatitis (Nagasawa et al., 2006), have been reported.

71 Nevertheless, a more defined role of PPARδ in hepatic lipid metabolism under
72 pathologic conditions such as hepatic steatosis remains to be determined.

73 A high-fat diet can lead to the hepatic accumulation of dietary fat and cause
74 inflammation (Lavoie and Gauthier, 2006). Moreover, a significant accumulation of fat
75 in the liver is considered to be one of the common features of metabolic syndromes such
76 as obesity, diabetes, and hyperlipidemia (Abdeen et al., 2006). In general, hepatic lipid
77 accumulation can compromise hepatic function, and further develop into cirrhosis or
78 hepatocellular carcinoma. Therefore, a need exists to clarify its regulatory mechanisms
79 and develop a pharmaceutical agent to control its development and subsequent hepatic
80 steatosis (Nagasawa et al., 2006).

81 In the present study, we evaluated the effect of a synthetic PPARδ ligand, L-165041
82 ([4-3-(4-Acetyl-3-dydroxyl-2-propylphenoxy propoxy) phenoxy] acetic acid) , on the
83 accumulation of hepatic lipids induced by a Western diet in low-density lipoprotein
84 receptor-deficient ($LDLR^{-/-}$) mice. According to our data, L-165041 treatment
85 significantly inhibited hepatic lipid accumulation, reducing the amount of total
86 cholesterol and hepatic triglycerides. In addition, L-165041 diminished the gene
87 expressions of PPARγ, apoB, and inflammatory cytokines induced by a Western diet.
88 Our data suggest that L-165041 might be an effective agent for preventing hepatic lipid

89 accumulation by modulating lipid metabolism and inflammation processes.

90

2. Materials and methods

2.1. Animals and diet.

The animals used for this study were 7–9-week-old female B6;129S-Ldlr^{tm1Her} mice (Orient Charles River Technology, Seoul, Korea) fed a Western diet (21% milk fat, 0.15% cholesterol, 19.5% casein, 0.3% DL-methionine, 15% cornstarch, 34.146% sucrose, 5% cellulose, 3.5% salt mix, 1% vitamin mix, 0.4% calcium carbonate, 0.0004 % ethoxyquin). A synthetic PPARδ ligand, L-165041, was purchased from Tocris (Ellisville, MO). LDLR$^{-/-}$ mice were divided into vehicle (0.1 N NaOH) and L-165041 (5 mg/kg/day) group (9 animals in each group). LDLR$^{-/-}$ mice received either NaOH or L-165041 via daily intraperitoneal injection (i.p) for 16 weeks with the Western diet. Body weight was measured once a week and the blood samples for a serum parameter analysis were collected using an eye-bleeding method every 4 weeks. At the end of the experiment, LDLR$^{-/-}$ mice were fasted for 24 hrs before sacrificed and the liver samples were either fixed in formalin or frozen at –70 °C for further analysis. All animal housed in polycarbonate cages in a room with a 12-h light/12-h dark cycle, and maintained at a constant temperature of 22˚C. All experiments were performed in

accordance with the Guidelines for the Care and Use of Laboratory Animals set forth by the Korea National Institute of Health (NIH-08-26).

2.2. Serum and liver lipid profile analysis.

The analysis of total serum cholesterol and triglycerides levels was performed by the Korean Research Institute of Biosciences and Biotechnology (KRIBB, Daejeon, Korea). Blood samples were collected and centrifuged at 3,000 rpm, room temperature, for 15 min to get serum samples. Unused serum samples were immediately frozen at −70 ˚C for later measurements. Serum samples were analyzed by a clinical chemistry analyzer (Hitachi 7020, Hitachi, Japan). Hepatic lipids were extracted using a previously described protocol (Folch et al., 1957). For the hepatic lipid extraction, the liver samples were homogenized with 1 ml distilled water for 5min. The homogenates were freshly added 2.5ml solvent mixture containing chloroform and methanol (2:1) and centrifuged at 3,000 rpm for 10min to separate phases. The lower phase was carefully removed and transferred to a new tube. Additional 1ml of solvent was added to the upper phase and the pellet, vortexed, then centrifuged at 3,000 rpm for 10min. The lower phase was repeatedly collected. Collected lower phase was combined with 1.5ml

127 of washing buffer containing chloroform, methanol, and 0.05 % $CaCl_2$ (3:48:47). After
128 vortexing and centrifugation, the lower phase containing lipids were evaporated under a
129 nitrogen stream. Dried pellet was resuspended in 0.5ml ethanol and analyzed using total
130 cholesterol and triglycerides kits (Bioclinical Systems Corp., Seoul, Korea).
131
132 *2.3. Histological examination*.
133
134 Liver samples fixed in 10% formalin were embedded in paraffin and sectioned at a
135 thickness of 7 μm before they were stained with hematoxylin and eosin (H&E). For the
136 Oil-Red O analysis, the liver samples frozen in embedding media were sectioned at a
137 thickness of 7 μm at –20 °C using a CM3050 cryostat (Leica, Wetzlar, Germany). The
138 slides were fixed and stained with Oil-Red O dye. After staining, slides were washed
139 three times with 85% 1,2-propranediol and then with deionized water. For the
140 macrophage staining, slides were incubated with blocking buffer containing 10 %
141 normal goat serum (NGS) in 0.3% triton X-100 for 60 min after permeabilized and
142 washed. Sections were then incubated with MOMA-2 primary antibody (Santa Cruz,
143 Santa Cruz, CA) diluted 1:200 in 5 % NGS for overnight at 4˚C. Sections were washed
144 three times in PBS for 5min each and incubated with the secondary antibody (HRP

conjugated) for 60min at room temperature. Sections were washed three times for 5min in PBS and then stained by 3-3' diaminobenzidine tetrahydrochloride (DAB) with metal enhancer (Sigma Chemical Co., St. Louis, MO) and mounted. For microscopic examinations, an upright light microscope (E600; Nikon, Melville, NY) was used, and the images were taken using a Nikon Coolpix 4300 digital camera.

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR).

Total RNAs from the liver tissue were isolated using an RNeasy lipid kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. For qRT-PCR, high-capacity cDNA reverse transcription kits and one-step SYBR PCR kits (Applied Biosystems, Foster city, CA) were used. qRT-PCR analysis was performed using an ABI prism 7900 (Applied Biosystems); the primers used are listed in Table 1. Individual gene expressions were normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression.

2.5. Statistical analysis.

The values are means ± S.E.M. The significance of differences was determined

using Student's *t*-test. A level of P value <0.05 was considered to be significant.

3. Results

3.1. Effects of L-165041 on the serum lipid profile of LDLR$^{-/-}$ mice fed a Western diet

After 16 weeks on the Western diet, the serum levels of total cholesterol, triglycerides, and low-density lipoproteins were significantly elevated in both the vehicle and L-165041 group compared to baseline. However, no significant difference was observed between the vehicle and L-165041 groups in terms of serum lipid parameters. In addition, no differences in the liver weight were detected (Table 2).

3.2. Effect of L-165014 on hepatic lipid accumulation

To examine the effect of general morphology, we performed histological analysis after hematoxylin and eosin staining. Animals developed a pronounced lipid accumulation after 16 weeks of Western diet and the lipid droplets were mainly distributed around the central vein of the liver (Fig. 1A). These lipid droplets were heterogeneous, consisting of both large and small droplets and showed a pattern typical of hepatic steatosis. In contrast, with L-165041 treatment, the formation of these lipid

droplets was significantly lower. This difference in hepatic lipid accumulation was more obvious with the Oil Red O staining (Fig. 1B).

3.3 Effects of L-165014 on the hepatic cholesterol and triglyceride levels

As shown in Fig. 2, L-165041 treatment significantly reduced the level of both the hepatic cholesterol and triglycerides. The results of the hepatic cholesterol and triglyceride levels correspond with the histological examination.

3.4. Expression of hepatic mRNA

Since L-165041 treatment inhibited hepatic lipid accumulation, we next examined the expression of the genes involved in hepatic lipid metabolism. mRNA expression of PPARα in L-165041-treated mice was not different from that of the vehicle group (Fig. 3A). Conversely, administration of L-165041 increased mRNA expression levels of PPARδ compared to the vehicle group (Fig. 3B), while administration of L-16541 significantly reduced mRNA expression levels of PPARγ (Fig. 3C). Lipoprotein lipase (LPL) expression in L-165041-treated mice was significantly higher than that in the

vehicle group (Fig. 4A). Apolipoprotein B (apoB) expression in L-165041-treated mice was significantly lower than that in vehicle mice (Fig. 4B). In addition, the expression of ABCG1, which is known to be involved in cholesterol efflux, increased dramatically with statistical significance (Fig. 4C). An inflammatory response in the liver due to a high-fat diet is one of the features of steatosis. Here, we investigated the expression of genes associated with inflammation in the liver. Treatment with L-165041 markedly reduced the expression of interleukin-1β (IL-1β) and IL-6 in comparison with the vehicle group (Fig. 5A and 5B). In contrast, the difference of tumor necrosis factor-alpha (TNF-α) expression in the both groups was not statistically significant, although a slight decreasing tendency was observed (Fig. 5C). To examine whether proinflammatory gene up regulation resulted in hepatic macrophage infiltration, we performed immunostaining using MOMA-2 antibody, a monocyte/macrophage marker. According to our data, macrophages were observed in the liver of vehicle group but this was reduced by treatment of L-165041 (Fig. 5D). These results suggest that L-165041 treatment may influence expression of inflammatory genes and subsequent macrophage recruitment in the liver.

4. **Discussion**

Ubiquitously expressed, PPARδ has been implicated in lipid metabolism and energy homeostasis of various tissue types including the liver. In lipid metabolism, PPARδ agonist has been reported to increase high-density lipoproteins in animal models (Graham et al., 2005) and regulate fatty acid oxidation in cardiomyocytes and skeletal muscles (Planavila et al., 2005). Although these previous studies demonstrated some beneficial effects of PPARδ on the lipid profile, much of the underlying mechanism of PPARδ in hepatic steatosis remains elusive. To address this issue, we examined the effect of a synthetic PPARδ ligand, L-165041, on Western diet-induced hepatic steatosis in $LDLR^{-/-}$ mice. According to our data, lipid accumulation was significantly reduced by treatment with L-165041 compared to the vehicle group. These data suggest the potential of PPARδ as a therapeutic target for high-fat-induced hepatic steatosis.

In the present study, both the vehicle and L-165041 treatment failed to inhibit the Western diet-induced increase in total plasma cholesterol, triglycerides, and low-density lipoproteins cholesterol. Although the synthetic PPARδ ligand GW501516 has been previously reported to improve the lipid profile and was particularly potent in raising high-density lipoproteins cholesterol in monkeys (Wallace et al., 2005), another

237 synthetic PPARδ ligand, GW0742X, had no significant effect on cholesterol, high-
238 density lipoproteins cholesterol, and low-density lipoproteins cholesterol (Graham et al.,
239 2005). Therefore, the debate on the lipid lowering effect of PPARδ is still open and
240 further studies are required to clarify this point.

241 Nevertheless, administration of L-165041 inhibited the hepatic lipid accumulation in
242 the Western-diet induced $LDLR^{-/-}$ mice and the hepatic cholesterol and triglyceride
243 levels were also significantly lowered with L-165041 treatment. Since recent studies
244 have suggested that peroxisome proliferator-activated receptors modulate the genes
245 involved in fatty acid oxidation in the liver (Furnsinn et al., 2007) and that PPARγ and
246 PPARα play important roles in lipogenesis and act as key modulators of high-fat diet-
247 induced liver steatosis (Inoue et al., 2005; Stienstra et al., 2007), we examined
248 peroxisome proliferator-activated receptors expression in the liver (Fig. 3). While
249 mRNA expression of PPARα in L-165041-treated mice was not different from that of
250 the vehicle group, administration of L-165041 increased mRNA expression levels of
251 PPARδ compared to the vehicle group. Expression of PPARγ was significantly reduced
252 by L-165041 treatment. These results indicate that downregulation of PPARγ
253 expression might be one of the mechanisms by which L-165041 attenuates lipid
254 accumulation in livers affected by Western diets.

In general, the mechanisms responsible for hepatic lipid accumulation could be an increase in lipid uptake and/or *de novo* synthesis of lipids or a reduction in fatty acid oxidation and lipoprotein secretion, or possibly both (Bradbury, 2006). Although hepatic low-density lipoproteins receptors are known to be responsible for the uptake of serum lipids into the liver, in our study, a high-fat diet induced significant hepatic lipid accumulation even without low-density lipoproteins receptors, suggesting that other lipid uptake systems might be activated. In fact, the deprivation of functional low-density lipoproteins receptors has been reported to induce other lipid uptake mediators such as SR-B1, VLDLR, FAT/CD36, and LPL to compensate for the impaired hepatic lipid uptake (Degrace et al., 2006). We examined the genes involved in the hepatic fatty acid uptake. According to our data, mRNA levels of VLDLR and CD36, which are known to be poorly expressed in a normal liver, were expressed in $LDLR^{-/-}$ mice regardless of L-165041 treatment. For VLDLR, no significant difference was observed between groups. In addition, L-165041 treatment had a tendency to further increase fatty acid translocase (CD36/FAT) expression but it was not statistically significant (data not shown). LPL mRNA expression was significantly increased and hepatic triglyceride levels were decreased by L-165041 treatment, suggesting that the increase in hepatic LPL expression by L-165041 might have contributed to the reduced lipid

accumulation in the liver.

LPL is known to catalyze the hydrolysis of triglyceride-rich lipoproteins and to modulate the binding between triglyceride -rich lipoproteins and very low-density lipoproteins receptors (Niemeier et al., 1996). Previous studies have demonstrated that a LPL activator, NO-1886, attenuated diet-induced steatohepatitis via anti-inflammation effects and induction of fatty acid oxidation (Yu et al., 2007) and suppressed hyperlipidemia by increasing LPL activity (Niho et al., 2005). However, we cannot definitively conclude whether the L-165041-induced increase in hepatic LPL mRNA is directly related to hepatic lipid metabolism because we have no relevant data pertaining to, for example, LPL activity, at this moment. This is one of the limitations of the present study and examining such a possibility would serve as an interesting subject for future studies.

Next, for the assembly of triglycerides into VLDL, apoB is required, which is an important regulatory protein in VLDL assembly and subsequent secretion (Lavoie and Gauthier, 2006). In the present study, L-165041 treatment decreased apoB expression and we speculate that this result is the outcome of decreased hepatic lipid accumulation or possibly coming from PPARδ-mediated non-genomic effect.

In addition, we observed that ABCG1 expression was significantly increased by L-

165041 treatment. Hepatic ABCA1 is involved in HDL production, whereas ABCG5/G8 and ABCG1 were proposed mediators of biliary efflux of cholesterol from the liver (Hoekstra et al., 2003). However, the role and mechanisms of ABCG1 and ABCG5/G8 by activation of PPARδ in the liver remain unknown. GW501516 has been shown to induce ABCA1 expression in HSKM cells (Sprecher, 2007). In contrast, another study reported that ABCA1 and ABCG1 expression levels in macrophages were unaffected by GW501516 and GW0742X treatment (Lee et al., 2003; Takata, Liu et al., 2008). Whether these contradicting results are due to species differences or other regulators is unclear. Our results suggest that inhibition of hepatic lipid accumulation by L-165041 might be affected by cholesterol efflux. This, however, is a topic for future study.

Another possible mechanism by which L-165041 may decrease hepatic lipid accumulation is through modulation of the inflammatory process. Excessive hepatic lipid accumulation has been known to trigger local inflammatory reactions (Otogawa et al., 2007) and the inflammatory processes mostly coincide with increased local fat accumulation as observed in nonalcoholic steatohepatitis (Stienstra et al., 2007). Another PPARδ agonist, GW501516, has been reported to improve hepatic steatosis via direct prevention of inflammation, and it also suppressed IL-6-mediated acute phase

309 reaction of hepatocytes (Kino et al., 2007). In our study, although L-165041 failed to
310 inhibit TNF-α and MCP-1 expression (data not shown), L-165041 treatment
311 significantly inhibited IL-1β and IL-6 expression compared to the vehicle group. These
312 observations indicate that activation of PPARδ might prevent hepatic lipid accumulation
313 by suppressing inflammation in the liver elicited by chronic consumption of western
314 style diets.

315 Although we are not able to provide direct evidence in the present study, we
316 believe both a direct effect of L-165041 and an indirect (secondary) effect work together
317 to attenuate lipid accumulation in the present study. Lipoprotein lipase (LPL) has been
318 known to have a PPRE in their promoter (Smith, 2002). Other recent study reported that
319 activated signaling of inflammation was shown to induce synthesis of hepatic apoB
320 which involved in lipid secretion from liver (Tsai et al., 2009). In addition, PPARδ
321 ligand suppressed transcriptional activity of STAT3 and NF-κB and subsequent
322 inhibition of the proinflammatory cytokines expression. Furthermore, IL-1β, IL-6, TNF-
323 α, and LPS inhibit lipid efflux by decreasing the expression of ABCA1 (Min et al.,
324 2007). All these studies suggest the existence of a highly complicated cross talk
325 between inflammatory signaling and lipid metabolism.

326 Due to the complexity of the mechanisms that cause hepatic lipid accumulation, we

were unable to identify a single major factor or mechanism responsible for the observed inhibitory effect of L-165041 on hepatic lipid accumulation in our experimental setting. In other words, there are many possible mechanisms that might explain the observe effect of L-165041 in the present study and such mechanisms include food intake, energy expenditure, or fat accumulation and expression of fatty acid oxidation enzymes in other tissues. Unfortunately we could not test all the hypothesis and that would be the major limitation of the present study. However, our findings suggest that the PPARδ ligand, L-165041, can be a potent agent in preventing the initiation and/or progression of pathologic hepatic conditions, including hepatic lipid accumulation. Further studies are thus warranted to clarify the role of PPARδ in hepatic lipid accumulation.

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447
448
449 **Figure Legends**

450

451 **Fig. 1.** Effect of L-165041 on the accumulation of lipid droplets in the liver.
452 Hematoxylin and eosin (H&E) staining of livers from control and L-165041- (5
453 mg/kg/day) treated mice. Numerous fatty droplets within hepatocytes were observed in
454 the livers of vehicle mice using H&E (A) and Oil-Red O stains (B).

455

456 **Fig. 2.** Effect of L-165041 on the hepatic lipid profile in mice fed a Western diet. (A)
457 Hepatic cholesterol content. (B) Hepatic triglyceride content. Data are presented as the
458 mean ± S.E.M. for nine mice in each group. * Statistically significant compared to
459 vehicle ($P < 0.05$).

460

461 **Fig. 3.** Effect of L-165041 on PPAR expression in the liver. (A) PPARα mRNA level
462 was analyzed by real-time PCR. (B) PPARδ mRNA level. (C) PPARγ mRNA level.
463 Results were quantified and normalized with respect to GAPDH. * Statistically
464 significant compared to vehicle ($P < 0.05$, $n = 5$ in each group).

465

466 **Fig. 4.** Hepatic gene expression in the mice administrated vehicle and L-165041.
467 Hepatocytes were lysed and lipid metabolism-related gene mRNA levels were analyzed
468 by real-time PCR analysis. (A) LPL, (B) apoB, (C) ABCG1. Results were quantified
469 and normalized with respect to GAPDH. * Statistically significant compared to vehicle
470 ($P < 0.05$, $n = 5$ in each group). LPL:lipoprotein lipase; apoB:apolipoprotein B;
471 ABCG1:ATP-binding cassette transporter G1.

472

473 **Fig. 5.** Effect of L-165041 on expression of inflammatory genes. mRNA expression
474 involved in liver inflammation using quantitative real time PCR analysis. (A)
475 Interleukin-1β; (B) Interleukine-6; (C) Tumor necrosis factor-alpha. Data are expressed
476 as relative to vehicle mice (n=5 mice per treated group). Results were quantified and
477 normalized with respect to GAPDH. *Statistically significant compared to vehicle (P <

0.05, n=5). (D), Photomicrographs of MOMA-2 immunohistochemical staining after 16 weeks in the liver of vehicle and L-165041 treated mice.

Table 1

Primers used for quantitative real-time PCR analysis of hepatic gene expression

Name		Sequence	Accession No.
PPARα	Sense	acgatgctgtcctccttgatg	NM013196
	Antisense	gtgtgataaagccattgccgt	
PPARδ	Sense	agatggtggcagagctatgacc	NM011145
	Antisense	tctcctcctgtggctgttcc	
PPARγ	Sense	ctgacccaatggttgctgattac	NM011146
	Antisense	tggagatgcaggttctactttga	
LPL	Sense	tggagaagccatccgtgtg	NM008509
	Antisense	tcatgcgagcacttcaccag	
apoB	Sense	tcaccatttgccctcaacctaa	NM009693
	Antisense	gaaggctctttggaagtgtaaac	
ABCG1	Sense	caagacccttttgaaagggatctc	NM009593
	Antisense	gccagaatattcatgagtgtggac	
IL-1β	Sense	caaccaacaagtgatattctccatg	NM008361
	Antisense	gatccacactctccagctgca	
IL-6	Sense	ctgcaagagacttccatccagtt	NM031168
	Antisense	gaagtagggaaggccgtgg	
TNF-α	Sense	ccagtgtgggaagctgtctt	NM013693
	Antisense	aagcaaaagaggaggcaaca	
GAPDH	Sense	tggcaaagtggagattgttgcc	NM008084
	Antisense	aagatggtgatgggcttcccg	

PPAR, peroxisome proliferator-activated receptor; LPL, lipoprotein lipase; apoB, apoprotein B; ABCG1, ATP-binding cassette transporters G1; IL-1β, interleukin-1 beta; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table 2

Effect of L-165041 on serum lipid profiles and tissue weight of $LDLR^{-/-}$ mice

Parameter	Vehicle (n = 9)		L-165041 (n = 9)	
	Baseline	16 weeks	Base line	16 weeks
Total cholesterol (mg/dl)	278.6 ± 46.8	845.2 ± 77.5	284.0 ± 30.5	750.0 ± 44
Triglyceride (mg/dl)	54.7 ± 21.6	261.0 ± 36.0	47.3 ± 14.6	231.0 ± 36.2
HDL-cholesterol (mg/dl)	62.7 ± 7.1	76.2 ± 3.3	59.4 ± 5.8	69.1 ± 3.7
LDL-cholesterol (mg/dl)	88.5 ± 16.0	328.6 ± 39.6	97.3 ± 15.1	267.5 ± 20.3
Body weight (g)	19.3 ± 0.25	21.31 ± 0.15	19.3 ± 0.23	21.29 ± 0.13
Liver/body weight (%)		3.60 ± 0.15		3.92 ± 0.13
Adipose/body weight (%)		7.14 ± 0.96		6.88 ± 0.99

Female $LDLR^{-/-}$ mice were treated with 5 mg/kg per day PPARδ ligand L-165041 or vehicle during 16 weeks. Results are expressed as means ± S.E.M. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Figure

Figure 1.

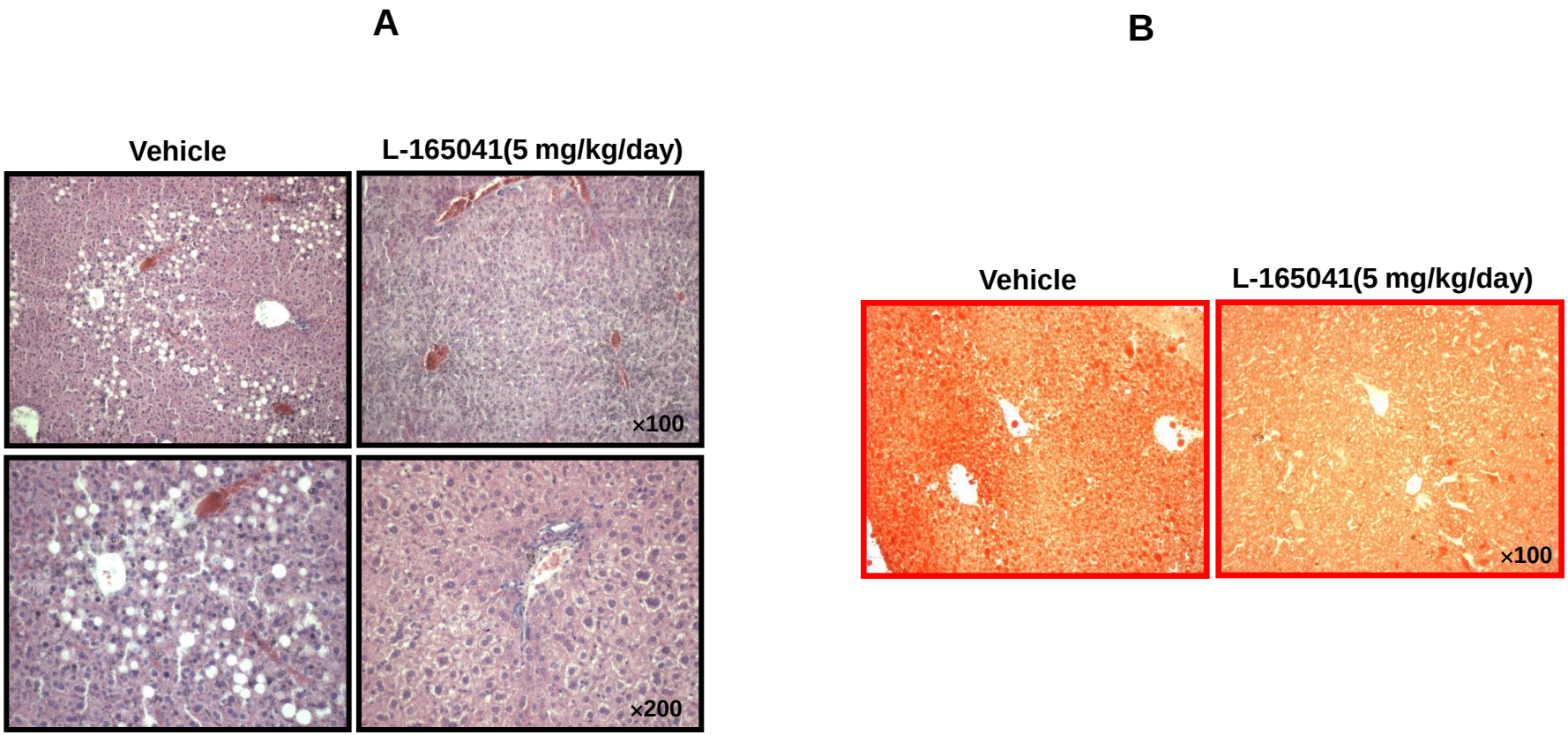


Figure 2.

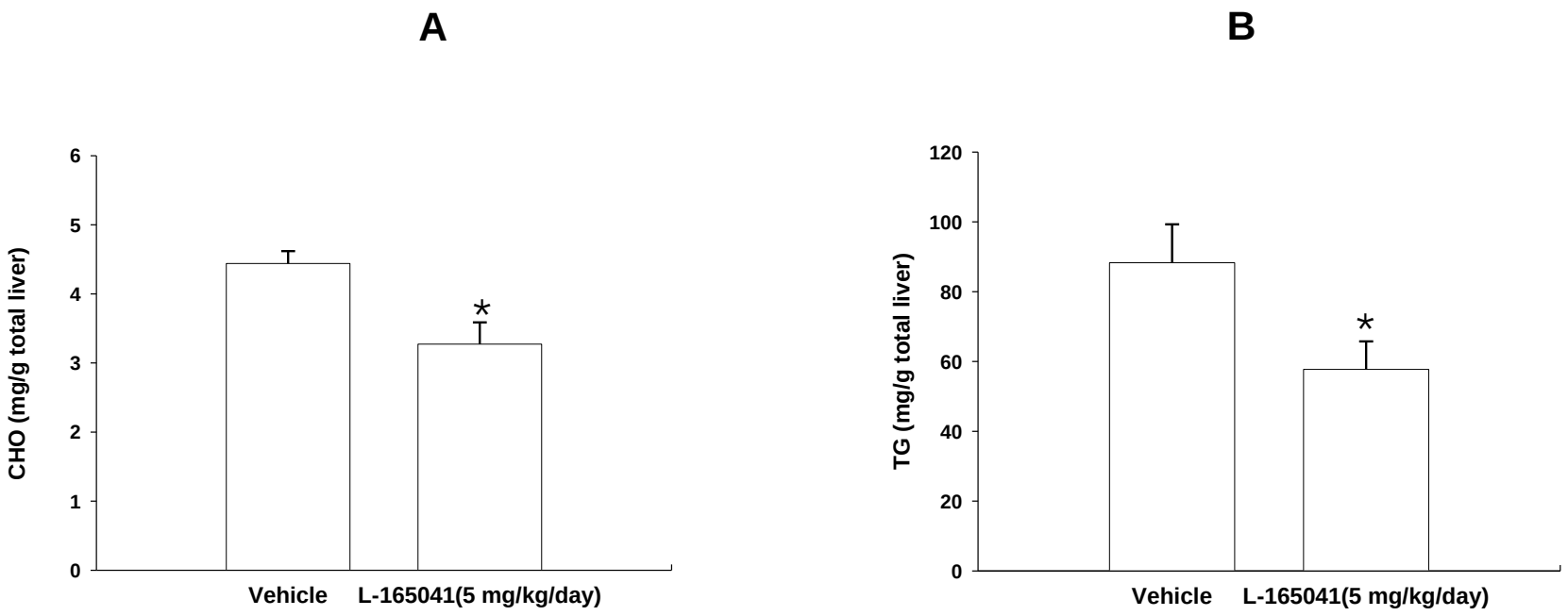

A
CHO (mg/g total liver)
6
5
4
3
2
1
0
*
Vehicle
L-165041(5 mg/kg/day)
B
TG (mg/g total liver)
120
100
80
60
40
20
0
*
Vehicle
L-165041(5 mg/kg/day)

Figure 3.

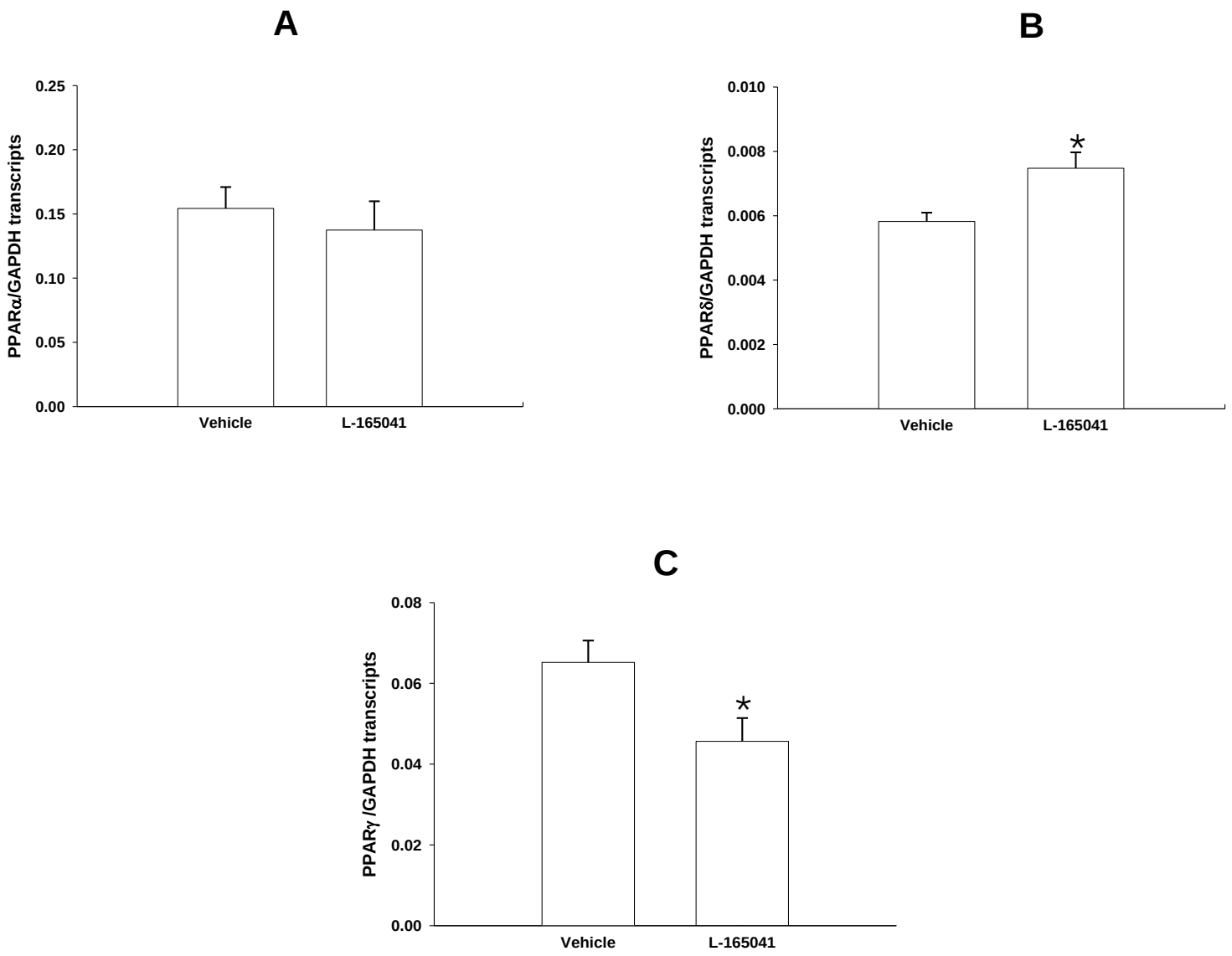
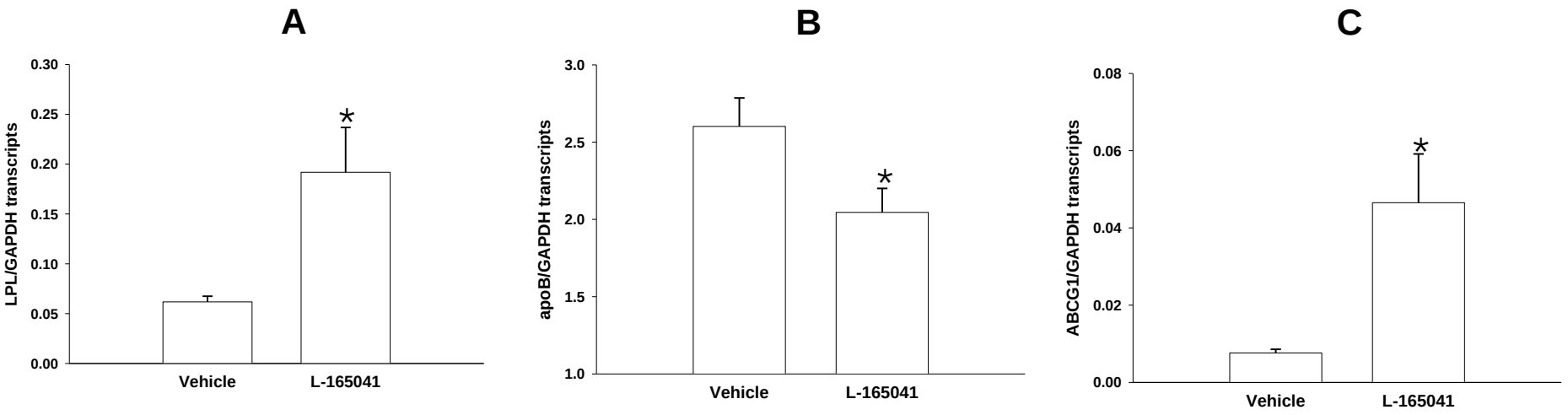

A
PPARα/GAPDH transcripts
0.25
0.20
0.15
0.10
0.05
0.00
Vehicle
L-165041
B
PPARδ/GAPDH transcripts
0.010
0.008
0.006
0.004
0.002
0.000
*
Vehicle
L-165041
C
PPARγ /GAPDH transcripts
0.08
0.06
0.04
0.02
0.00
*
Vehicle
L-165041

Figure 4.


A
LPL/GAPDH transcripts
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
Vehicle
L-165041
B
apoB/GAPDH transcripts
3.0
2.5
2.0
1.5
1.0
*
Vehicle
L-165041
C
ABCG1/GAPDH transcripts
0.08
0.06
0.04
0.02
0.00
*
Vehicle
L-165041

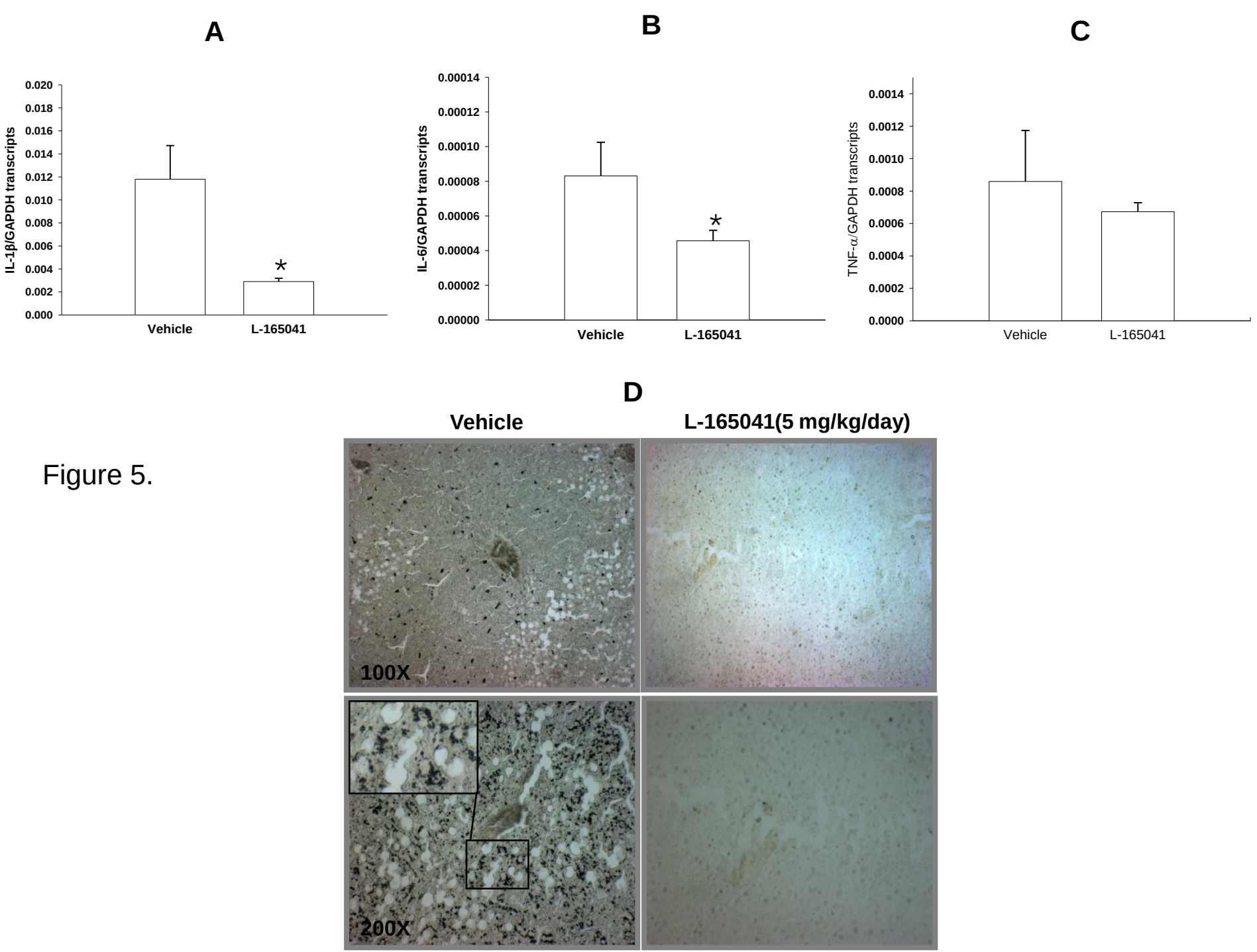

A
IL-1β/GAPDH transcripts
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
*
Vehicle
L-165041
B
IL-6/GAPDH transcripts
0.00014
0.00012
0.00010
0.00008
0.00006
0.00004
0.00002
0.00000
*
Vehicle
L-165041
C
TNF-α/GAPDH transcripts
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
Vehicle
L-165041
D
Vehicle
L-165041(5 mg/kg/day)
100X
200X

Figure 5.