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M. BARANOWSKI, A.U. BLACHNIO-ZABIELSKA, P. ZABIELSKI, E. HARASIM,
D. HARASIUK, A. CHABOWSKI, J. GORSKI

LIVER X RECEPTOR AGONIST T0901317 ENHANCED PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-DELTA EXPRESSION AND FATTY ACID OXIDATION IN RAT SKELETAL MUSCLE

Department of Physiology, Medical University of Bialystok, Bialystok, Poland

Liver X receptors (LXR) have been characterized as key transcriptional regulators of hepatic lipid and carbohydrate metabolism. LXR are expressed also in skeletal muscle, however, their role in this tissue is poorly investigated and the vast majority of available data comes from studies on cultured myotubes. Therefore, we aimed to examine effects of *in vivo* LXR activation on muscle lipid metabolism. The experiments were performed on male Wistar rats fed on a standard rodent chow. The animals were divided into two groups (n=10) receiving either LXR activator (T0901317, 10 mg/kg/day) or vehicle for one week. Samples of the soleus as well as red and white sections of the gastrocnemius muscle were excised. T0901317 increased muscle expression of peroxisome proliferator-activated receptor-δ and its target genes involved in fatty acid uptake and oxidation. In addition, LXR agonist enhanced palmitate oxidation (by 55%) in isolated soleus muscle. However, palmitate incorporation into triacylglycerol was decreased (by 38%), which was associated with reduced diacylglycerol acyltransferase expression (by 66%). Despite markedly increased plasma lipid concentration upon T0901317 treatment, muscle triacylglycerol level was elevated only in the red section of the gastrocnemius muscle. We conclude that T0901317 enhances muscle fatty acid oxidation, which prevents overt accumulation of intramuscular lipids that could be expected considering T0901317-induced hyperlipidemia.

Key words: liver X receptors, *diacylglycerol acyltransferase, fatty acid translocase/CD36, lipids, nuclear receptors, peroxisome proliferator-activated receptor, sterol regulatory element binding protein-1c, low density lipoprotein*

INTRODUCTION

Liver X receptors (LXR) are ligand-activated transcription factors belonging to the large superfamily of nuclear receptors. To date, two LXR subtypes termed α and β have been identified. High expression of LXR-α is restricted to liver, adipose tissue, intestine, kidney and lung, whereas LXR-β is ubiquitously expressed (1). Upon activation both isoforms form obligate heterodimers with the retinoid X receptor and regulate gene expression through binding to LXR response elements (LXRE) in the promoter regions of the target genes (2). Endogenous LXR agonists include a number of oxidized cholesterol derivatives, referred to as oxysterols (3). In addition to natural ligands, several potent synthetic LXR agonists have been developed. The two most commonly used in experimental studies are T0901317 and GW3965 which activate both LXR isoforms with similar potency (4).

LXR were shown to function as sterol sensors protecting the cells from cholesterol overload. When the amount of cholesterol increases there is a subsequent accumulation of cellular oxysterols which, in turn, activate LXR to induce transcription of a host of genes involved in reverse cholesterol transport and its conversion to bile acids in the liver (5, 6). This finding led to identification of synthetic LXR agonists as potent antiatherogenic agents in low density lipoprotein (LDL) receptor-deficient and apoE-deficient mice (7, 8). In addition, synthetic LXR agonists were found to stimulate hepatic lipogenesis *via* upregulation of sterol regulatory element binding protein (SREBP)-1c which results in liver steatosis and hypertriglyceridemia (4, 9). Administration of T0901317 and GW3965 was also reported to normalize plasma glucose level and improve whole-body insulin sensitivity in several rodent models of type 2 diabetes and insulin resistance (10, 11).

Both LXR isoforms were shown to be expressed in human and rodent skeletal muscle (12, 13). Similarly to other tissues, synthetic LXR activators upregulate expression of ATP-binding cassette transporter (ABC) A1 and enhance cholesterol efflux also in muscle cells (14) indicating normal response to LXR stimulation. To date only a few studies performed on cultured myotubes have dealt with the effect of T0901317 on muscle metabolism. However, considering the fact that *in vivo* LXR activation can dramatically alter plasma lipid profile, there is a need for re-evaluation of the action of LXR agonists on muscle tissue in whole-animal model. In the present study we characterize for the first time in details the effect of *in vivo* activation of LXR on lipid metabolism in skeletal muscle.

MATERIALS AND METHODS

Animals and study design

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the European Commission Directive 86/609/EEC and was approved by the Ethical Committee for Animal Experiments at the Medical University of Bialystok (permit no. 2006/05). Male Wistar rats (230–280 g) were housed under controlled conditions (21±1°C, 12 h light/12 h dark cycle, light on at 06:00 h) with unlimited access to water and standard laboratory rat chow (Agropol, Motycz, Poland). A total of 20 animals were randomly divided into two groups receiving either vehicle or a dual LXR-α/β agonist T0901317 (Cayman Chemicals, 10 mg/kg/d, suspended in 0.5% carboxymethylcellulose) for one week. The dosage and the length of treatment were chosen after Cao *et al.* (10). They reported that 10 mg/kg/day was the highest dose of T0901317 that could be administered to rats without inducing adverse effects on food consumption and weight gain. They also found that one week treatment with the drug was sufficiently long to induce strong effects on lipid metabolism in rats. The solutions were administrated once daily in the morning by an oral gavage. On the last day of the experiment, between 8 and 10 a.m., all rats were anaesthetized by intraperitoneal injection of pentobarbital in the dose of 80 mg/kg (in the fed state). Samples of the soleus as well as red and white sections of the gastrocnemius muscle were excised and immediately freeze-clamped with aluminum tongs precooled in liquid nitrogen. Blood taken from the abdominal aorta was collected in heparinized tubes, centrifuged, the plasma separated and flash-frozen in liquid nitrogen. All samples were stored at –80°C until analysis.

Muscle lipids

Samples were pulverized in an aluminum mortar precooled in liquid nitrogen. Lipids were extracted by the method of Folch. The fractions of total phospholipids, triacylglycerols, diacylglycerols, nonesterified fatty acids (NEFA), free cholesterol and cholesterol esters were separated by thin-layer chromatography (TLC) according to Roemen and van der Vusse (15). Lipid were then transmethylated in either 1 M methanolic sodium methoxide (Fluka) at room temperature for 10 minutes (triacylglycerols and phspholipids) or 14% methanolic boron trifluoride (Sigma) at 100°C for either 2 (NEFA) or 10 (diacylglycerols) minutes. The content of resulting fatty acid methyl esters was determined using gas-liquid chromatography as previously described in detail (16). Free cholesterol and cholesterol esters were eluted from the gel with chloroform, evaporated under nitrogen stream and redissolved in 2-propanol or diethyl ether, respectively. The content of free cholesterol and cholesterol esters was subsequently measured with commercially available cholesterol diagnostic kit (BioMaxima).

The content of ceramide was determined as described previously in detail (17). Briefly, tissue lipids were extracted into chloroform and the samples were then subjected to alkaline hydrolysis to deacylate ceramide. Free sphingosine liberated from ceramide was converted to o-phthalaldehyde derivative and analyzed using HPLC system. N-palmitoyl-D-erythro-sphingosine (C17 base) (a kind gift of Dr. Z. Szulc, Medical University of South Carolina) was used as an internal standard.

Soleus muscle incubations

Palmitate oxidation and esterification in incubated soleus muscle strips was determined using [9,10-^{3}H]-palmitate, as previously described in detail (18). Palmiate oxidation was estimated by measuring the release of $^{3}H_2O$ into the incubation buffer. To determine palmitate esterification, lipids were extracted from muscle strips and then separated by means of TLC. The lipid bands were scraped off the plates, and ^{3}H-palmitate incorporation into different lipid pools was measured by radioactivity counting.

Real-time PCR

Total RNA was isolated from the samples using TriReagent (Sigma) according to the manufacturer's instructions. Following RNA purification, DNase treatment (Ambion) was performed to ensure that there was no contaminating genomic DNA. Extracted RNA was solubilized in RNase-free water and stored at –80°C until use. The quality of each RNA sample was verified by running the agarose electrophoresis with ethidium bromide. The RNA was reverse transcribed into cDNA using First Strand cDNA Synthesis Kit (Fermentas) with oligo(dT)18. Oligonucleotide primers were designed using Beacon Designer Software 7.5 (Premier Biosoft). Real-time PCR was performed with SYBR Green JumpStart Taq ReadyMix (Sigma) using a Bio-Rad Chromo4 system. PCR efficiency was examined by serially diluting the template cDNA, and a melt curve was performed at the end of each reaction to verify PCR product specificity. A sample containing no cDNA was used as a negative control to verify the absence of primer dimers. The results were normalized to β-actin expression measured in each sample.

Western blotting

All primary antibodies were obtained from Abcam (LXR-α, ab28478; LXR-β, ab24361; SREBP-1, ab28481; peroxisome proliferator-activated receptor (PPAR)-α, ab2779; PPAR-δ, ab23673; fatty acid translocase/CD36 (FAT/CD36), ab36977; β-actin, ab129348). Briefly, protein extracts were fractionated on a 10% polyacrylamide gel, transferred to nitrocellulose membranes, and probed with antibodies specific to the proteins analyzed. After incubation with the secondary alkaline phosphatase-conjugated antibody (Sigma, A9019) protein bands were scanned and quantified using a Gel Doc EQ system (Bio-Rad). All samples were also probed with anti-β-actin antibody to verify equal loading.

Plasma measurements

Concentrations of plasma glucose, triacylglycerols, NEFA and total cholesterol were determined with the use of Glucose Oxidase Reagent Set (Pointe Scientific Inc.), Serum Triacylglycerol Determination Kit (Sigma), Wako NEFA C kit (Wako Chemicals) and Cholesterol kit (BioMaxima), respectively.

Statistical analysis

All data are presented as means ±S.D. Statistical comparisons between experimental groups were made by using Student's t-test. $P<0.05$ was considered statistically significant.

RESULTS

Administration of T0901317 affected neither body weight nor food consumption. Plasma NEFA and triacylglycerols concentration was markedly increased by T0901317, whereas total cholesterol level was reduced by the drug. Moreover, LXR activation resulted in a slight decrease in plasma glucose concentration (*Table 1*).

Animals treated with T0901317 were characterized by decreased muscle content of NEFA and free cholesterol. There

was also a marked reduction in the level of cholesterol esters, however, this effect was observed only in the soleus and red gastrocnemius (*Fig. 1*). On the other hand, the content of phospholipids was elevated upon LXR activation in all examined muscles. In addition, administration of T0901317 increased ceramide level in the soleus (*Fig. 1*). Animals treated with the LXR agonist were also characterized by accumulation of triacylglycerols in the red gastrocnemius but not in the soleus or white gastrocnemius, where no statistically significant changes were observed. The content of diacylglycerols in the soleus and red gastrocnemius was not affected by T0901317, whereas, in the white gastrocnemius it was reduced upon LXR activation (*Fig. 1*).

Data obtained from real-time PCR analysis indicate that mRNA level of LXR-β was higher than that of LXR-α in all examined muscles (estimated difference after correction for PCR reaction efficiency ranged from 3.5 to 7-fold depending on the muscle type, data not shown). Expression of LXR-α protein in the soleus and red gastrocnemius was similar, and approximately 30% higher, compared to the white gastrocnemius. Among the muscles examined, soleus was characterized by the highest level of LXR-β protein, followed by red and white gastrocnemius (*Fig. 2A*). T0901317 administration upregulated mRNA and protein expression of both LXR isoforms in all examined muscles, however, this effect was stronger for LXR-α compared to LXR-β (*Fig. 2A*, *Table 2*). As a result the LXR-α/LXR-β protein ratio increased in all examined muscles. It should be noted, however, that in the animals administered with T0901317 this ratio was markedly higher in the red gastrocnemius compared to the soleus and white gastrocnemius (*Fig. 2B*).

Table 1. General features and plasma measurements of the experimental animals.

	Vehicle	T0901317
Initial body weight (g)	256±13	271±11
Final body weight (g)	311±19	319±13
Food consumption (g/d/rat)	39.4±5	39±8.8
Epididymal fat pads weight (g)	3.58±0.69	4.23±0.96
Plasma measurements (fed state)		
Glucose (mg/dl)	156±17	133±11**
NEFA (nmol/ml)	183±32	246±44**
Triacylglycerols (nmol/ml)	1026±355	1874±782**
Total cholesterol (mg/dl)	63.4±14	48.8±12.9*

The results are means ±S.D. (n=10). One or two symbols indicate a significant difference versus the vehicle control at the $p<0.05$ or $p<0.01$ levels, respectively.
NEFA - nonesterified fatty acids.

Administration of T0901317 caused a marked increase in the level of stearoyl-CoA desaturase (SCD) 1 and SREBP-1c mRNA in all examined muscles. A similar, albeit much weaker, effect was observed for both precursor and mature SREBP-1 protein. Upregulation of SREBP-1 protein was more pronounced in the soleus as compared to the other two examined muscles. On the other hand, mRNA level of acetyl-CoA carboxylase (ACC) 1, another classical LXR target gene, was only modestly increased. Glycerol-3-phosphate acyltransferase (GPAT) mRNA level was slightly elevated upon LXR activation. Interestingly, expression of diacylglycerol acyltransferase (DGAT) 1 was strongly suppressed in all examined muscles by T0901317 (*Table 2*).

Skeletal muscles of animals treated with LXR agonist were characterized by elevated expression of PPAR-δ protein, whereas the level of PPAR-α remained stable. In addition, mRNA level of a number of PPAR-dependent genes including carnitine palmitoyltransferase (CPT) 1, malonyl-CoA decarboxylase (MCD), pyruvate dehydrogenase kinase (PDK) 4 and long-chain acyl-CoA synthetase (ACSL) 1 was significantly increased upon LXR activation. It should be noted, however, that expression of MCD was upregulated only in the soleus and red gastrocnemius (*Table 2*). T0903117 also increased muscle content of FAT/CD36 protein (*Fig. 3*).

Strips of the soleus muscle isolated from T0901317-treated animals were characterized by lower rate of ^{3}H-palmitate incorporation into triacylglycerols, whereas the rate of its incorporation into phospholipids was increased. There were no statistically significant changes in the amount of tracer incorporated into intramuscular NEFA or diacylglycerols (*Fig. 4A*). The rate of ^{3}H-palmitate oxidation in the soleus muscle

Table 2. Effect of T0901317 on mRNA level of selected genes in skeletal muscle.

	Soleus	Red gastrocnemius	White gastrocnemius
fold-change versus vehicle			
LXR-α	3.11±2.32*	11.3±1.94***	2.19±0.68*
LXR-β	2.96±2.33	5.82±1.49***	1.86±0.51*
SREBP-1c	5.91±0.49***	4.27±0.42***	5.34±0.38***
ACC1	1.55±0.07***	1.48±0.14***	1.28±0.15**
SCD1	12.8±0.14***	10.8±0.55***	7.18±0.35***
GPAT	1.07±0.02**	1.27±0.07***	1.3±0.16**
DGAT1	0.34±0.03***	0.3±0.03***	0.28±0.04***
CPT1	2.48±0.1***	3.09±0.34***	1.88±0.09***
MCD	2.28±0.09***	1.86±0.12***	0.92±0.05*
ACSL1	4.67±0.48***	3.32±0.45***	4.59±0.39***
PDK4	2.68±0.34***	3.01±0.24***	2.61±0.13***

The results are means ±S.D. (n=6). One, two or three symbols indicate a significant difference versus the vehicle control at the $p<0.05$, $p<0.01$ or $p<0.001$ levels, respectively.
ACC, acetyl-CoA carboxylase; ACSL, long-chain acyl-CoA synthetase; CPT, carnitine palmitoyltransferase; DGAT, diacylglycerol acyltransferase; GPAT, glycerol-3-phosphate acyltransferase; LXR, liver X receptor; MCD, malonyl-CoA decarboxylase; PDK, pyruvate dehydrogenase kinase; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element binding protein.
NEFA - nonesterified fatty acids.

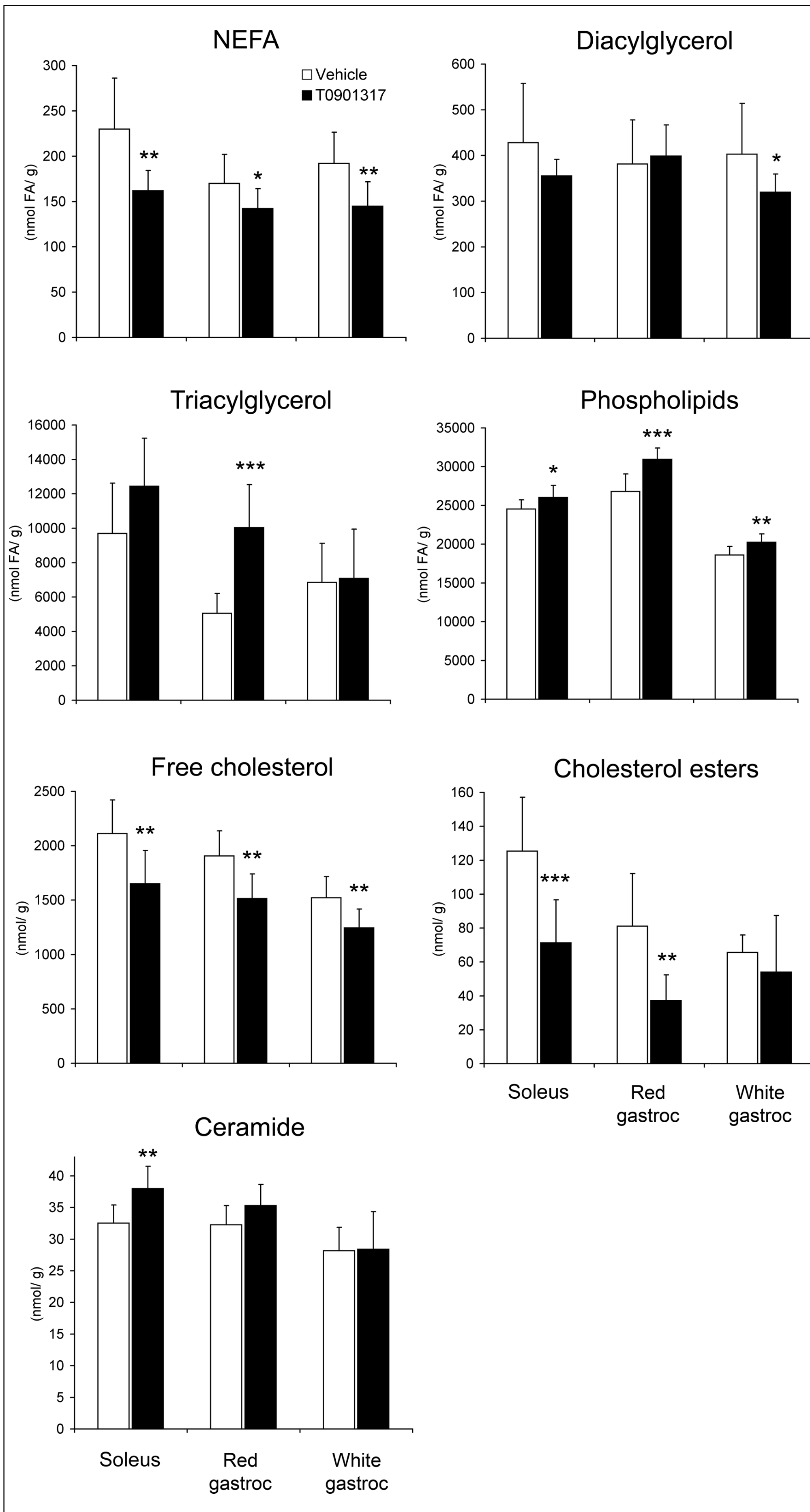

Fig. 1. Effect of LXR activation on muscle content of major lipid classes. Rats were treated with either T0901317 (10 mg/kg/d) or vehicle for one week (n=10).

One, two or three symbols indicate a significant difference versus the vehicle control at the $p<0.05$, $p<0.01$ or $p<0.001$ levels, respectively.

FA, fatty acids; NEFA, nonesterified fatty acids.

strips isolated from rats administered with LXR agonist was markedly higher as compared to the vehicle control (*Fig. 4B*).

DISCUSSION

In the present study we found that T0901317 increased muscle expression of PPAR-δ and its target genes, and stimulated palmitate oxidation in isolated soleus muscle. However, palmitate incorporation into triacylglycerol was decreased, which was associated with reduced DGAT expression. Despite markedly increased availability of plasma lipids upon T0901317 treatment, muscle triacylglycerol was elevated only in red gastrocnemius.

Our results indicate significant differences in LXR expression between muscles with different fiber type composition. Namely, protein level of both LXR isoforms was higher in muscles with high oxidative capacity (soleus and red gastrocnemius) compared to the glycolytic one (white gastrocnemius). A similar pattern was reported for PPARs expression in rat skeletal muscles (19).

In all available studies, including the present one, muscle SREBP-1c mRNA level was markedly increased upon administration of synthetic LXR agonist (14, 20-23). There is, however, conflicting evidence in the literature as to whether LXR activation enhances expression of other lipogenic genes in muscle cells. T0901317 was found to increase mRNA levels of ACC, SCD1 and fatty acid synthase in cultured human and murine myotubes (21-23), but not in murine skeletal muscle (14, 20). In our study, SCD1 expression was strongly induced in all muscles upon T0901317 administration. On the other hand, mRNA level of another lipogenic gene, ACC1 was barely

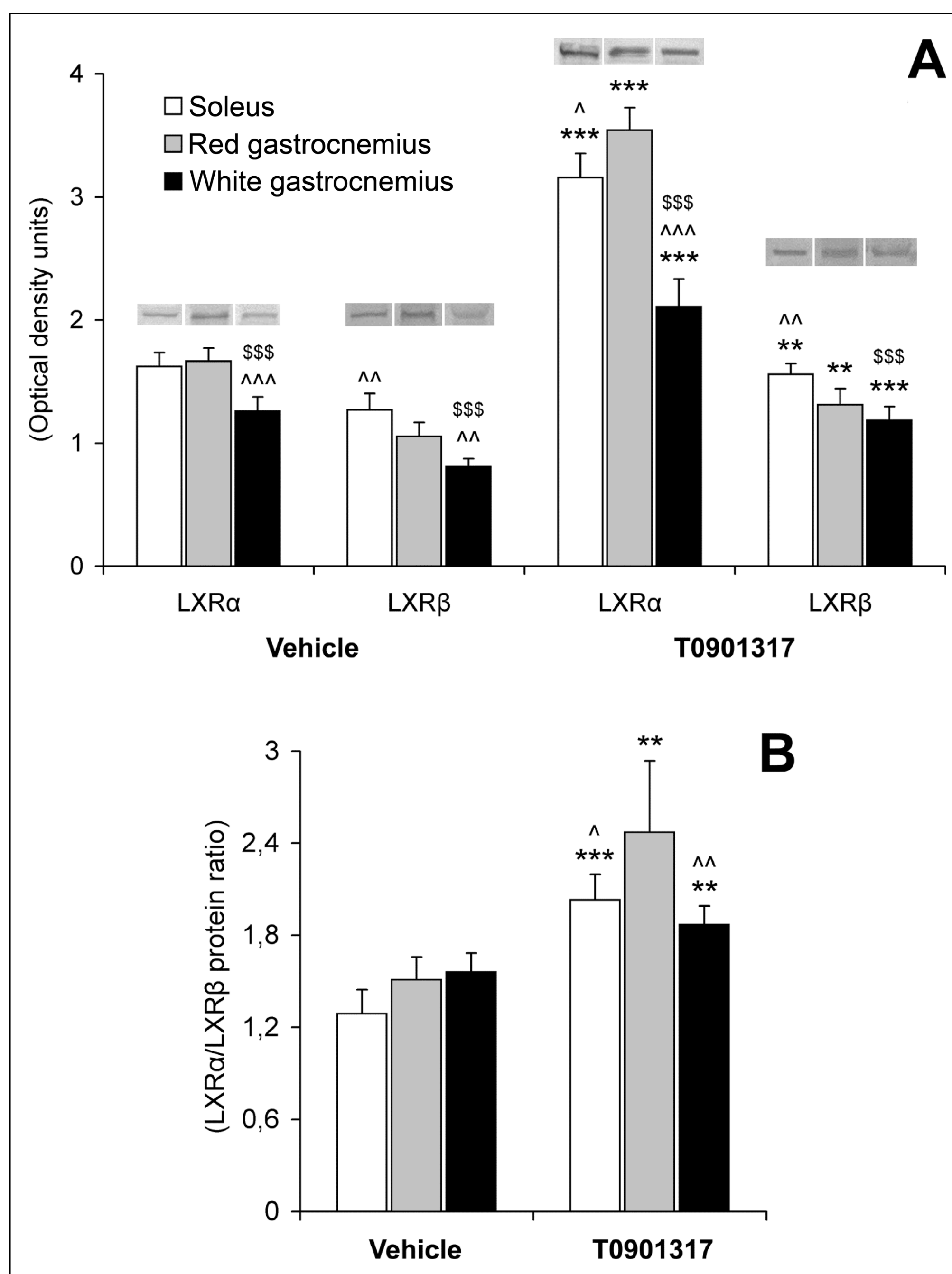

Fig. 2. Expression of LXR in rat skeletal muscles. Rats were treated with either T0901317 (10 mg/kg/d) or vehicle for one week (n=6). (*A*) content of LXR-α and LXR-β protein; (*B*) LXR-α/LXR-β protein ratio.
* statistically significant difference versus the respective muscle in vehicle control; ^ - statistically significant difference versus the red portion of the gastrocnemius muscle in the respective group; $ - statistically significant difference versus the soleus muscle in the respective group. One, two or three symbols indicate a significant difference at the $p<0.05$, $p<0.01$ or $p<0.001$ levels, respectively.

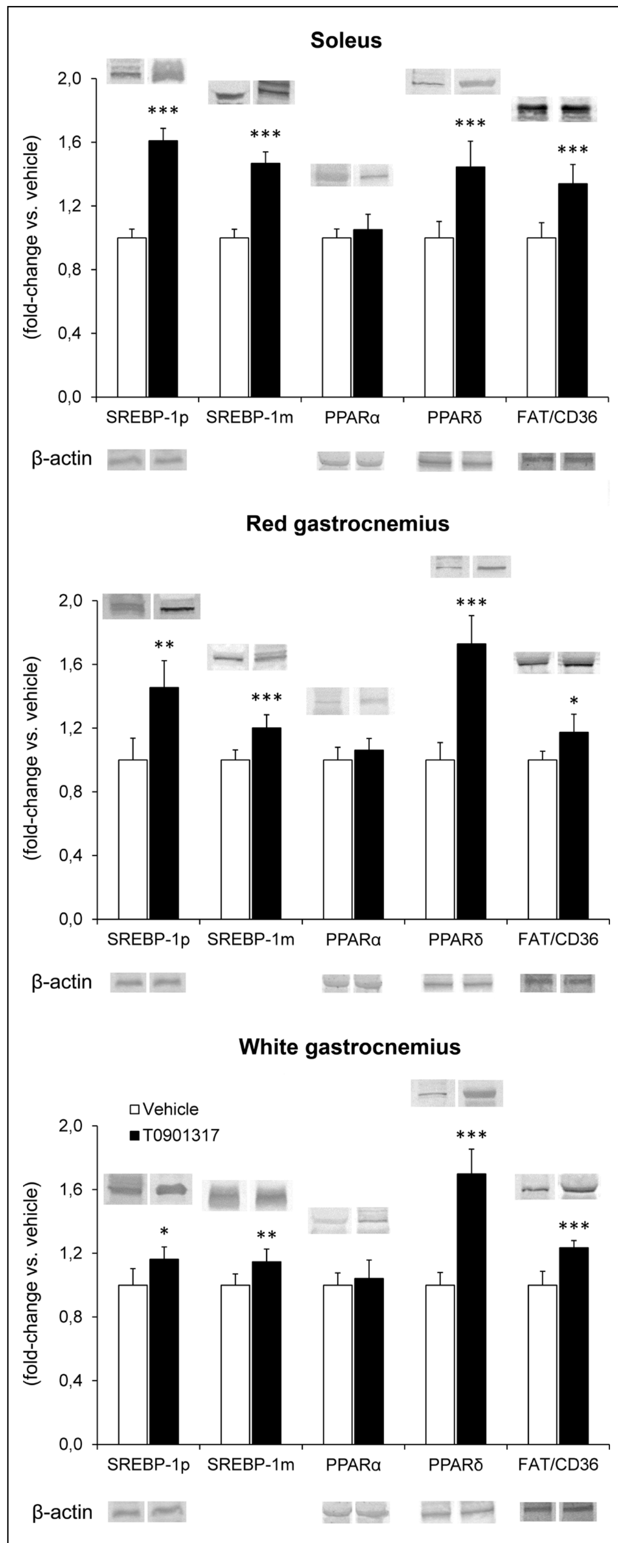

Fig. 3. Effect of T0901317 on protein expression of selected genes in skeletal muscle. Rats were treated with either T0901317 (10 mg/kg/d) or vehicle for one week (n=6).
* - statistically significant difference versus the vehicle control. One, two or three symbols indicate a significant difference at the p<0.05, p<0.01 or p<0.001 levels, respectively.
FAT/CD36, fatty acid translocase/CD36; PPAR, peroxisome proliferator-activated receptor;
SREBP-1p, precursor sterol regulatory element binding protein 1; SREBP-1m, mature sterol regulatory element binding protein 1.

increased. The same finding was reported for cultured human myotubes treated with T0901317 (21). This is in contrast to a response observed in the liver, where all lipogenic genes are coordinately and strongly upregulated upon LXR activation (9, 14). Liang *et al*. (9) found that SREBP-1c is crucial for the lipogenic action of LXR agonists in the liver. In their report transcriptional response of the lipogenic genes to LXR activation was either absent or severely blunted in SREBP-1c knockout mice. In our study muscle SREBP-1c mRNA increased as much as 5-fold after T0901317 administration, whereas the content of mature SREBP-1 protein was elevated by only 15 to 47%, depending on the muscle type. However, in the liver, LXR activation was repeatedly shown to markedly elevate both SREBP-1c mRNA and mature protein (9, 24, 25). Therefore, our results suggest that limited response of lipogenic pathway to synthetic LXR agonists, that is observed in skeletal muscle, is a consequence of their inability to effectively upregulate mature SREBP-1c protein therein.

We observed a significant increase in plasma triacylglycerols concentration after treatment with T0901317. Hypertriglyceridemia is a well-documented effect of T0901317 in rodents, and results from augmented hepatic very low density lipoprotein (VLDL)-triglyceride secretion (26). It should be noted that hypertriglyceridemic effect of T0901317 in mice is transient and limited to the first three days of the treatment (27, 28). In our study, it was still present after one week which provides additional evidence for the presence of differences between rats and mice in response to synthetic LXR agonists. Rats administered with T0901317 were also characterized by elevated plasma NEFA concentration. Similar finding was reported by other groups (28, 29). It was shown that LXR activation stimulates basal lipolysis in human adipocytes, and we have previously observed increased expression of hormone-sensitive lipase and adipocyte triglyceride lipase in adipose tissue of rats treated with T0901317 (30).

On the other hand, there was no clear tendency towards increased content of muscle lipids upon T0901317 administration. We observed a decrease in intramuscular NEFA level, and no change (soleus and red gastrocnemius) or a decrease (white gastrocnemius) in diacylglycerols concentration. Triacylglycerols were increased exclusively in red gastrocnemius, and only phospholipids content was moderately elevated in all examined muscles. There is very few data on the effect of LXR agonists on lipid content in muscle tissue. Hessvik *et al*. (31) found increased number of lipid droplets in differentiated human myotubes incubated with T0901317. However, Cozzone *et al*. (21), using the same experimental model, reported no such effect. Korach-Andre *et al*. (32) found that intramyocellular lipid content measured by magnetic resonance spectroscopy in tibialis anterior of wild-type mice was not affected by deficiency of either LXR-α, LXR-β or both LXR isoforms. This observation is in good agreement with our results, as we observed no effect of T0901317 on triacylglycerols level in white gastrocnemius, a muscle with fiber composition similar to murine tibialis anterior (33). Similarly, Commerford *et al*. (34) reported that triacylglycerols content in the gastrocnemius muscle was not affected by GW3965 in high-fat fed rats. Unfortunately, the authors did not indicate which portion of the gastrocnemius muscle they had used.

A surprising finding of our study is that T0901317 did not cause overt accumulation of intramuscular lipids despite significant increase in plasma NEFA and triacylglycerols concentration and enhanced muscle expression of genes involved in lipogenesis and fatty acid uptake. This observation suggests that, in contrast to liver which becomes fatty and enlarged, muscle lipid homeostasis is not perturbed by pharmacological LXR activation. Our results indicate that

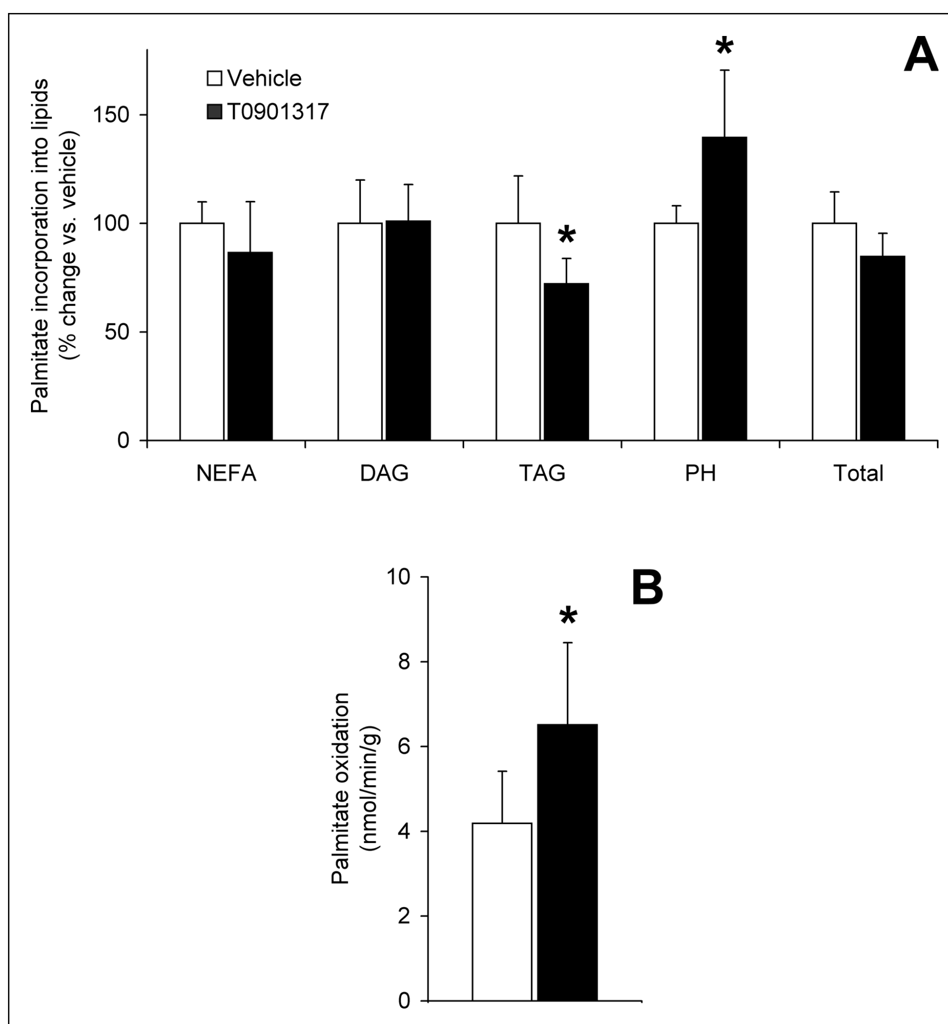

Fig. 4. LXR activation enhanced palmitate oxidation in isolated soleus muscle strips. Rats were treated with either T0901317 (10 mg/kg/day) or vehicle for one week (n=6). Strips of the soleus muscle were then isolated and incubated with [^{3}H]-palmitate as described in Materials and Methods. (*A*) incorporation of palmitate into intramuscular lipids, (*B*) palmitate oxidation to H_2O.
*- $p<0.05$ versus the vehicle control.
DAG, diacylglycerol; NEFA, nonesterified fatty acids; PH, phospholipids; TAG, triacylglycerol.

T0901317 not only increased fatty acid availability in skeletal muscle but also stimulated their oxidation therein, which prevented accumulation of lipids. This is supported by the fact that LXR agonist upregulated expression of genes favoring fatty acid β-oxidation in all three examined muscles. In addition, plamitate oxidation rate was markedly elevated in the soleus muscle strips isolated from rats administered with the LXR agonist. This finding is consistent with our previous report showing that T0901317 increased muscle fatty acid utilization during exercise (30). Similarly to the present work, studies on differentiated human myotubes showed that incubation with T0901317 enhanced fatty acid oxidation and increased expression of CPT1 and PDK4 (21, 22, 31, 35). However, one study found no effect of T0901317 on oleate oxidation rate in cultured myotubes derived from mice (23).

LXR agonist increased muscle expression of PPAR-δ protein and a number of PPAR-dependent genes regulating fatty acid uptake and oxidation. Upregulation of PPAR-dependent genes including FAT/CD36, CPT1, PDK4 and ACSL1 was observed also in differentiated human myotubes incubated with T0901317 (21, 22, 35, 36). PPAR-δ was found to be a key transcriptional regulator of fatty acid metabolism in skeletal muscle, and pharmacological activation or overexpression of this receptor increases muscle fatty acid oxidation rate in rodents (37). Therefore, it is very likely that LXR-induced stimulation of muscle palmitate oxidation observed in our study was a consequence of enhanced PPAR-δ expression. However, we can only speculate on the mechanism underlying PPAR-δ upregulation in response to LXR agonist. LXREs were identified in the promotor regions of PPAR-α and PPAR-γ genes (38, 39) but there is no information as to whether PPAR-δ is also directly regulated by LXR at the transcriptional level. In the study by Cozzone *et al*. (21). T0901317 did not affect mRNA expression of any PPAR subtype in cultured human myotubes. It should be noted, however, that gene set enrichment analysis of microarray data showed that PPAR signaling pathway was upregulated by T0901317 in cultured myotubes derived from mice (23). It is also possible that upregulation of muscle PPAR-δ and its target genes observed in our study was an indirect effect of LXR agonist. For instance, since long-chain fatty acids are endogenous agonists of PPAR-δ (37), T0901317-induced increase in plasma NEFA and triacylglycerol concentration could result in activation of this receptor.

An interesting finding of our study is that T0901317 markedly suppressed muscle DGAT1 expression which suggests reduced triacylglycerols synthesis rate. This is also supported by decreased incorporation of palmitate into triacylglycerols in the soleus muscle strips isolated from rats administered with the LXR agonist. Our observation is in contrast to reports showing that both palmitate incorporation into triacylglycerols and DGAT1 expression are increased in differentiated human myotubes incubated with T0901317 (21, 35, 36). In our study, decreased DGAT1 expression could be another factor besides enhanced fatty acid oxidation that limited

accumulation of intramuscular triacylglycerols in animals receiving LXR agonist.

Surprisingly, although the transcriptional response to LXR agonist was similar in all examined muscles, triacylglycerols level was increased only in the red gastrocnemius. Although we can only speculate as to the reason for this discrepancy, it is possible that it was a consequence of different proportions between LXR isoforms in examined muscles. T0901317 is a dual LXR-α/β agonist and there is some evidence indicating different regulatory functions of each isoform in skeletal muscle (23). Interestingly, in our study T0901317-induced triacylglycerols accumulation was observed in the muscle which was characterized by the highest post-treatment LXR-α/LXR-β protein ratio. On the contrary, in the white gastrocnemius, where this ratio was the lowest among examined muscles, there was, in general, a tendency for decreased intramuscular lipid content upon T0901317 administration. Differences in LXR signaling between various skeletal muscles were found also by Korach-Andre *et al*. (40).

We conclude that T0901317 stimulates muscle fatty acid oxidation (most likely *via* upregulation of PPAR-δ), which prevents overt accumulation of intramuscular lipids that could be expected considering T0901317-induced hyperlipidemia. In addition, our results indicate that effect of LXR activation on muscle lipid metabolism depends on the muscle type, which can be a consequence of differences in the pattern of LXR isoform expression.

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Author's address: Prof. Jan Gorski, Department of Physiology, Medical University of Bialystok, 2c Mickiewicza Street, 15-222 Bialystok, Poland.
E-mail: gorski@umb.edu.pl