Fenofibrate Ameliorates Insulin Resistance of Lipoprotein Lipase Knockout Heterozygous Mice

Yangxue Li
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Tingting Han
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Shuang Zheng
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Xingxing Ren
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Yaomin Hu (aminhu@yahoo.com)

Research article

Keywords: Fenofibrate, Lipoprotein lipase, Knockout Heterozygous, Hypertriglyceridemia, Insulin resistance

Posted Date: February 5th, 2020

DOI: https://doi.org/10.21203/rs.2.22680/v1

Abstract

Background

The benefits of fenofibrate (FB), a peroxisome proliferator-activated receptor-a agonist, against hyperlipidemia have been established. We investigated the effect of fenofibrate on insulin resistance of lipoprotein lipase knockout heterozygous (LPL+/-) mice, which represent inherited hypertriglyceridemia and impaired glucose tolerance.

Methods

Male LPL+/- mice were treated with FB (50 mg/kg, once daily) via gavage for 8 weeks. Plasma lipid, glucose tolerance test, systemic insulin sensitivity, insulin signaling of tissues, genes and proteins related to endoplasmic reticulum (ER) stress and oxidative stress were analyzed.

Results

Body weight of 40-week LPL+/- with FB were reduced by 30.3% ($P<0.05$), while the differences of 16- and 28-week LPL+/- with FB were not significant ($P>0.05$). FB improved the lipid profile of both 28 and 40-week LPL+/- ($P<0.001$ for both), while that of 16-week LPL+/- mice with FB was unaltered ($P>0.05$). Glucose tolerance of 40-week LPL+/- were improved by FB ($P<0.05$), while that of 16- and 28-week LPL+/- with FB kept unaltered ($P>0.05$). Fasting insulin of 40-week LPL +/- were improved by FB ($P<0.05$), thus HOMA-IR of 40-week LPL+/- was declined ($P<0.05$). HOMA-IR of 16- and 28-week LPL+/- with FB had no change. Insulin-stimulated phosphorylated Akt (Ser473) in liver and skeletal muscle of 28-week LPL+/- was enhanced by FB ($P < 0.001$ and $P<0.05$ respectively). ER stress biomarkers were detected decreased in liver of 16- to 40-week LPL+/- with FB whereas that in muscle of LPL+/- with FB unchanged. Reduced reactive oxygen species (ROS) levels and augmented mRNA expression of superoxide dismutase (SOD) and catalase (CAT) in skeletal muscle of 28- and 40-week LPL+/- mice with FB were observed. There was no significance on ROS levels and mRNA of SOD and CAT in liver between LPL+/- mice with and without FB.

Conclusions

Fenofibrate improved lipid profile, glucose tolerance, systemic and tissue-specific insulin resistance of LPL knockout heterozygous mice. This may be associated with alleviated endoplasmic reticulum stress in liver and reduced oxidative stress in muscle.

Background

The prevalence of type 2 diabetes (T2DM) is surging all across the world [1]. Combined with type 2 diabetes, obesity, hypertension, dyslipidemia and hyperuricemia are known as metabolic syndrome. The high prevalence and risk of metabolic syndrome have attracted the world's attention [2, 3]. We have known the common pathophysiological process of metabolic syndrome is insulin resistance [4], in which cells fail to respond to normal actions of insulin.

Lipoprotein lipase (LPL) is a key enzyme in lipid metabolism. LPL can hydrolyze triglyceride (TG) of TG-rich lipoproteins like chylomicrons and very low density lipoproteins into two free fatty acids and one monoacylglycerol molecule. There were over 400 lipoprotein lipase gene mutations reported [5]. Our previous meta-analysis showed that, Asn291Ser variant in the LPL gene which is a risk factor for dyslipidemia is associated with coronary heart disease and T2DM [6]. And through animal knockout approach, we found that lipoprotein lipase knockout heterozygous (LPL+/-) mice showed dyslipidemia, impaired glucose tolerance and insulin resistance [7].

Fenofibrate (FB), a hypolipidemic drug commonly used in the clinic, is a peroxisome proliferator-activated receptor- alpha (PPAR α) agonist. The famous FIELD study revealed that fenofibrate intervention did reduce total cardiovascular events of diabetic patients, mainly due to fewer non-fatal myocardial infarctions and revascularizations [8]. It's demonstrated that fenofibrate improved glucose tolerance and hepatic insulin resistance of mice when challenged with high-fat diet [9]. In high fructose-fed mice, researchers also found that treatment with FB ameliorated hepatic insulin resistance and steatosis [10].

High-fat and high-fructose are environmental factors which induces abnormal lipid profiles and insulin resistance. There are still many patients who still manifest dyslipidemia despite strict diets and exercises. Among those, LPL deficiency is an important genetic factor. And LPL+/- mice are representatives of these. So far, that how the hypolipidemic drug fenofibrate affects glucose metabolism and insulin sensitivity on condition of LPL deficiency is unclear. Thus, the present study elucidated that fenofibrate ameliorated lipid profile and

insulin resistance of LPL+/- mice. The underlying mechanism may be related with that fenofibrate can improve endoplasmic reticulum (ER) stress in liver and oxidative stress in muscle.

Methods

Animals - LPL-deficient mice of C57BL/6 background were rescued from neonatal death by intramuscular injection of an adenoviral vector coding a human LPL mutant, Ad-LPLS447X, as previously described [11]. LPL+/- mice were hybrids of LPL deficient mice and WT mice. Male LPL+/- mice and age-matched wild type littermates (WT) were provided by Animal Centre of Cardiovascular Sciences Institute of Peking University. They were divided into three groups by age: 16, 28 and 40 weeks. LPL+/- mice of the same age were divided into two subgroups. One was LPL+/- (W), the other was LPL+/- (FB). Mice in LPL+/- (FB) were gavage with fenofibrate (Laboratories FOURNIER SAS, France) 50mg/ kg/d for 8 weeks. Mice in LPL+/- (W) were orally fed with the same volume water as that in LPL+/- (FB) for 8 weeks. WT were fed with the same water with LPL+/- (W) for equal time. All mice were kept on a 12/12 h light/ dark cycle with free access to water and standard mouse chow. Ethical approval was granted by the animal ethics committee of Shanghai Jiaotong University affiliated Renji Hospital. All procedures implemented were according to Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. These were approved by the Animal Care and Use Committee of Shanghai Jiaotong University affiliated Renji Hospital.

Intraperitoneal glucose tolerance tests (IPGTT) - Mice were fasted overnight for 10–12 hours with free access to water, and then bolus of glucose injected intraperitoneally with 1 g/kg. Blood glucose of mice were measured from the tail using an automatic glucometer (ACCU-CHEK, Switzerland) at 0, 15, 30, 60 and 120 minutes after injection.

Fasting plasma glucose (FPG), fasting insulin (FINS) and homeostasis model assessment (HOMA) - Mice were fasted like above. Then blood was collected from angular veins. FPG was measured by biochemical method in clinical laboratory of Renji Hospital and FINS by ELISA (Shibayagi, Japan). HOMA is an approach of assessing insulin resistance and β-cell function from basal glucose and insulin [11]. HOMA-IR and HOMA-β were calculated via the formula of (FPG - FINS)/22.5 and 20*FINS/ (FPG–3.5) respectively.

Measurement of weight gains, plasma lipid profiles - Body weight were measured by electronic scale (Sartorius intec, Germany) before and after gavage. The difference between the two formulated weight gains during the gavage. Then plasma was collected from mice after overnight fasting. Plasma triglyceride and non-esterified fatty acid (NEFA) were measured using ELISA kits (Wako Chemicals, USA).

Insulin sensitivity in tissues - Basal liver and skeletal muscle were collected from 28-week mice after overnight fasting. After a 5 units/kg dose of insulin (Humulin R, USA), administered via the intraperitoneal injection, mice were anesthetized by intraperitoneally injected with 1.5g/kg dose of carbamate and euthanized by cervical dislocation based on Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. These were approved by the Animal Care and Use Committee of Shanghai Jiaotong University affiliated Renji Hospital. Mice were unconscious and dead in no time. Then liver and skeletal muscle were collected, flash-frozen in liquid nitrogen and stored at 80°C until further analysis. For western blot analysis, tissues were homogenized in cell lysis buffer. A total of 60 ug protein was electrophoresed on 8% precast SDS-PAGE gels and transferred onto a PVDF membrane. Blots were probed with antibody for phospho-Akt (Ser 473) (Cell Signaling Technology) and then with fluorescently-labeled secondary antibody (LI-COR, USA). Results were detected by odyssey infrared imaging system. And β-actin (anti-β-actin, Cell Signaling Technology) was for standardization of protein expression levels.

Reactive oxygen species (ROS) level in liver and muscle—ROS level was measured in situ by dihydroethidium (DHE) fluorescent probe, which reacts with ROS and forms ethidium bromide binding to DNA. Flash-frozen livers and muscles were cut into 10 μm thick and incubated with 10 μM DHE for 30 minutes in a humidified chamber of 37°C. Images were taken by a laser scanning confocal microscope (Leica Microsystems, Germany). The fluorescence intensities were measured with Image J software in randomly selected areas of images captured and presented as integrated optical density per unit area.

Lipid peroxidation and anti-oxidant capacity level in plasma - Lipid peroxidation was determined by the reaction of malondialdehyde (MDA) with thiobarbituric acid to form a colorimetric product, proportional to the MDA present. MDA levels in the plasma were examined by calorimetric kit (Sigma, USA). Absorbance at 532nm was detected via Multiscan Spectrum (Biotek, USA). Total antioxidant capacity (TAOC) in the plasma represents the capacity to deal with oxidative stress. Cu^{2+} ion is converted to Cu^{+} by both small molecule and protein. The reduced Cu^{+} ion is chelated with a colorimetric probe giving a broad absorbance peak around 570 nm, proportional to the total antioxidant capacity. TAOC was detected through calorimetric kit (Biovision, USA). Absorbance at 570nm was examined via Multiscan Spectrum (Biotek, USA).

Quantitative real-time PCR - Liver and skeletal muscle were collected, flash frozen, and stored at –80°C until processing. Total RNA was extracted from homogenized tissue using TRIZOL reagent (Invitrogen). The process of reverse transcription was performed using Prime Script RT reagent Kit (TaKaRa, Japan). Then real-time PCR was performed using SYBR Premix Ex Taq Kit (TaKaRa, Japan), and reactions were run in duplicate on a Light Cycler Real Time PCR Detection System (Roche Diagnostics, Basel, Switzerland). The cycling conditions comprised 15minutes' cDNA synthesis at 37°C, 5 seconds' reverse transcriptase inactivation at 85°C, and 40 cycles at 95°C for 5 seconds and 60°C for 30 seconds. Results of mRNA were normalized to levels of glyceraldehyde–3-phosphate dehydrogenase (GAPDH) using the comparative threshold cycle method. Primers of genes were represented in Table S1.

Statistical analysis - All results except that in Fig. 3 are expressed as mean ± SEM. Statistical differences of results were analyzed via ANOVA using SPSS 22.0. $P < 0.05$ was considered significant.

Results

Weight gains during the gavage.

As we found in previous research, LPL+/- mice put on weight as they grew old [7]. Thus in the present research we observed weight gains during 8-week gavage. We found that 28- and 40-week LPL+/- mice had an exquisite weight gains versus their WT controls in 8-week time (Fig 1, $P<0.05$). Gavage of fenofibrate to 40-week LPL+/- cause weight loss by 30.3% compared with their LPL+/- controls ($P<0.05$). However, weight loss in 28-week LPL+/- did not achieve statistical difference ($P>0.05$). There were no significant difference among three groups at 16w in weight gains ($P>0.05$).

Lipid profile.

[Insert Figure 1 here.]

[Insert Figure 2 here.]

LPL+/- mice manifest obvious hypertriglyceridemia [7], which is a risk factor of insulin resistance [12]. In the present study, we evaluated the effect of FB on lipid profile. As expected, plasma triglyceride of both 28- and 40-week old LPL+/- (W) are higher than their WT littermates (Fig 2, $P<0.001$), while that of 16-week has no difference with the control ($P>0.05$). The effects of FB on 28- and 40-week old mice are obvious. As we can see, plasma TG of 28- and 40-week LPL+/- (FB) is lower than that without FB by 34.4% and 46% apiece ($P<0.001$ for both). Consistently, plasma non-esterified fatty acid (NEFA) of 28- and 40-week LPL+/- decreased to 40.1% and 23.5% of that in LPL+/- without FB respectively ($P<0.001$ for both). Interestingly, plasma TG and NEFA of 16-week mice with FB have no comparable changes ($P>0.05$ for both).

Intraperitoneal glucose tolerance.

[Insert Figure 3 here.]

Glucose tolerance of LPL+/- (W) mice at 16w and 28w showed no difference compared with WT and LPL+/- (W) mice at 40w displayed impaired glucose tolerance with WT. These were consistent with our previous research [7]. The glucose of 16-week LPL+/- (FB) at 15min and 30min were higher while LPL+/- (FB) of 40w had an improvement in glucose tolerance than that without FB (Fig 3, $P<0.05$). Mice at 28w of three groups had no obvious differences with each other ($P>0.05$).

FPG, FINS, HOMA-IR, HOMA-β and AUCG.

Table 1 summarized details related to glucose metabolism. LPL+/- at 16w with FB emerged higher fasting plasma glucose compared with that without FB (7.69±0.78 vs. 5.47±0.54, $P<0.05$). However, mice at 28w and 40w did not show the same trend. Fasting insulin of LPL+/- (W) at 28w and 40w both were higher than their WT (7.11±0.08 vs. 6.34±0.14, 9.21±1.21 vs. 4.15±0.24, $P<0.05$ for both). LPL+/- (FB) at 40w showed lower fasting insulin level than that without FB (3.59±0.34 vs. 9.21±1.21, $P<0.05$), whereas LPL+/- (FB) at 28w did not show any difference with their LPL+/- controls (6.22±0.04 vs. 7.11±0.08, $P>0.05$). HOMA-IR of LPL+/- mice in 28 and 40-w showed the same trend, which meant 28- and 40-week old LPL+/- developed systematic insulin resistance (2.12±0.23 vs. 1.62±0.13, 2.34±0.32 vs. 0.97±0.07, $P<0.05$ for both), while only FB interference in 40-week old LPL+/- improved systemic insulin resistance (1.04±0.07 vs. 2.34±0.32, $P<0.05$). HOMA-β of three ages with FB did not show any significance ($P>0.05$ for all). Neither did LPL+/- (FB) do much better, nor did they do worse. AUCG of glucose tolerance quantized the results of IPGTT. AUCG of LPL+/- (W) in 40-w was increased compared with WT (23.76±3.23 vs. 18.95±1.78, $P<0.05$), though 28- and 40-week LPL+/- mice gavage with FB did not show any difference with

LPL+/- in water. Interestingly, higher area did not appear in LPL+/- (W) at 16w compared with WT whereas AUCG of 16-w mice with FB was much larger than that of LPL+/- in water (37.98+4.29 vs. 25.81+4.39, P<0.05).

	16w			28w			40w		
	LPL+/-(W)	LPL+/-(FB)	WT	LPL+/-(W)	LPL+/-(FB)	WT	LPL+/-(W)	LPL+/-(FB)	WT
FPG（mmol/L）	5.34±0.62	7.69±0.78[b]	5.47±0.54	6.72±0.30	7.08±0.92	5.78±0.75	5.62±0.43	6.67±0.5	5.73±0.64
FINS(mU/L)	6.47±0.38	6.30±0.38	5.80±0.34	7.11±0.08[a]	6.22±0.04	6.34±0.14	9.21±1.21[a]	3.59±0.34[b]	4.15±0.24
HOMA-IR	1.64±0.33	1.85±0.53	1.57±0.09	2.12±0.23[a]	1.95±[i]0.51	1.62±0.13	2.34±0.32[a]	1.04±0.07[b]	0.97±0.07
HOMA-β	81.8±19.35	28.50±2.81	54.5±18.95	44.16±1.99	34.75±0.54	55.61±3.55	92.25±18.74	26.51±6.15	28.49±4.39
AUCG	25.81±4.39	37.98±4.29[b]	24.44±0.96	21.19±1.93	25.61±1.53	20.19±2.99	23.76±3.23[a]	21.44±0.77	18.95±1.78

Tab 1. a, P<0.05, vs. WT of the same age; b, P < 0.05 vs. LPL+/- (W) of the same age, n = 6 for LPL+/- (W), LPL+/- (FB) and WT mice at 16, 28 and 40 weeks. Data are represented as means ± STE

Insulin sensitivity in liver and muscle.

Our previous results showed that insulin signaling in 28-week-old LPL+/- mice is impaired [7]. Our present study evaluated the insulin signaling of 28-week LPL+/- with or without FB. The picture told us that insulin sensitivity of LPL+/- mice was impaired in liver and muscle (Fig 4, P<0.05 vs. WT), which was consistent with our previous demonstration [7]. The current results also displayed that liver of mice with FB improved in insulin sensitivity versus LPL+/- (W) (P<0.001). Insulin-stimulated Ser473 Akt was also augmented in muscle of LPL+/- (FB) versus LPL+/- (W) (P<0.05). Thus we demonstrated that fenofibrate interference in 28-w LPL+/- mice can enhance insulin sensitivity of liver and muscle.

[Insert Figure 4 here.]

ER stress in liver and muscle.

We can see that ER stress biomarkers like Bip, ATF−6 and CHOP in liver of LPL+/- (W) all increased than WT (P<0.05). This is consistent with our previous research [7]. Our present data elucidated that mRNA of Bip and ATF−6 in liver of LPL+/- (FB) at 16w decreased by 24% and 25% than LPL+/- (W) respectively (P<0.05 for both). Expression of Bip and ATF−6 in liver of LPL+/- (FB) at 28w dropped to 23%, 9% of that in LPL+/- (W) (P<0.001 for both) and CHOP to 38% versus LPL+/- (W) (P<0.05). Bip and ATF−6 in liver of LPL+/- (FB) declined to 40% and 47% of that in LPL+/- (W) at 40w (P<0.05 for both). On the contrary, Bip, ATF−4, ATF−6 and CHOP in muscle of LPL+/- (W) has nothing significant with their WT controls at three ages (P>0.05). Furthermore, fenofibrate interference did not cause any statistical change in ER stress biomarkers of muscle (P>0.05). Therefore we concluded that FB interference improve ER stress in liver, which anticipated the improvement in insulin resistance.

[Insert Figure 5here.]

Oxidative stress in circulation and in tissues.

Lipid peroxidation and anti-oxidant capacity can represent oxidative stress in vivo [13]. Our research showed that MDA and TAOC levels of all the mice changed as they grew older. MDA of LPL+/- (W) at 40w were significantly higher than that of WT mice whereas TAOC decreased by 34% than WT mice of the same age (Fig 3, P<0.05). MDA of LPL+/- (W) at 28w doubled with WT of the same age (P<0.001). But significance in TAOC of LPL+/- (W) at 28w did not make sense compared with WT. This consisted with our former investigation [7]. Interestingly, both MDA and TAOC of 16-week LPL+/- (W) showed nothing significant compared with WT. What surprised us was that LPL+/- (FB) interference of all ages revealed no significant changes in MDA and TAOC compared with LPL+/- (W) of the same age.

On the other hand, ROS levels in liver and muscle of 16- to 40-week mice showed differences with results above. ROS levels in liver and muscle of LPL+/- (W) from 16w to 40w obviously higher than WT of the same age (P<0.001 for all) (Pic 2 to 4), which were identical with

our previous results [7]. 28- and 40-week LPL+/- (FB) expressed lower ROS in muscle compared with LPL+/- mice (W) (Pic 2 and Pic 3, $P<0.05$ for both), while there was no significance between LPL+/- mice (FB) and LPL+/- (W) in liver at 28w and 40w ($P>0.05$ for both). ROS in liver and muscle of 16-week mice showed no difference between LPL+/- with FB and without it (Pic 1, $P>0.05$ for both).

To further explore the association between FB interference and oxidative stress, we examined mRNA expression of superoxide dismutase (SOD) and catalase (CAT) in liver and muscle of 28- and 40-week mice. The results showed that SOD and CAT in muscle of LPL+/- (W) at 28w decreased compared with their WT controls ($P<0.05$ for both). That in 40-week LPL+/- (W) showed the same trend ($P<0.05$ for both). And levels of SOD and CAT in liver of LPL+/- (W) were elevated significantly compared with their WT controls at 28w and 40w ($P<0.05$ for both). Those results were consistent with our research before [7]. The current revealed that expression of SOD and CAT in muscle was increased by about twofold than their LPL+/- controls at 40w via FB interference ($P<0.001$ for both). Levels of anti-oxidant mRNA in muscle of LPL+/- (FB) double that in muscle of LPL+/- (W) at 28w ($P<0.001$ for both). What astonished us were that gavage of FB did not affect the expression of SOD and CAT in liver when compared with their LPL+/- controls of the same age ($P>0.05$ for all). Then we drew the conclusion that oxidative stress in muscle of LPL+/- mice could be alleviated by fenofibrate.

[Insert Figure 6 here.]

[Insert Figure 7 here.]

Discussion

Dyslipidemia is often seen in diabetic patients and even their offspring who do not have T2DM [14]. We observed that mutations of Lys312insC, Thr361insA and Lys312insC+Asn291Ser in LPL contribute to hypertriglyceridemia observed in offspring of type 2 diabetic patients [15]. LPL is a key enzyme in lipid metabolism. It can hydrolyze TG into NEFA. Our previous meta-analysis proved that LPL Asn291Ser variant, which resulted in dyslipidemia was related with T2DM, and coronary heart diseases [6]. Through animal knockout approach, we found that LPL knockout heterozygous mice which showed reduced LPL expression in different tissues and decreased activity presented impaired glucose tolerance and insulin resistance [7].

Fenofibrate was commonly used in clinic to lower triglyceride and total cholesterol [16]. Our results verified the hypolipidemic effect of FB. Of interest, FB interference do not affect lipid profile of 16-week LPL+/- mice, which did not even develop hyperlipidemia. Furthermore, LPL+/- with FB were lost weight during the period of gavage. This effect was verified in high-fat fed mice. Sunhyo Jeong and his colleagues found that obese mice fed with high-fat and FB had lower weight compared with mice fed with high-fat only[17]. Thus our current indicated that FB can effectively improve body weight and lipid profile of LPL+/- mice, but it did not lower normal lipid profile.

However, the effects of FB did not limit to this one. Fenofibrate was reported to ameliorate insulin resistance, hypertension and novel oxidative stress markers in patients with metabolic syndrome [18]. Sunhyo Jeong et al demonstrated that FB improved insulin sensitivity of high-fat induced obese mice via enhancing β oxidation in visceral adipose tissue [17]. Stanley M. H. Chan and his colleagues found that FB improve hepatic insulin resistance in high-fructose fed mice by reducing deleterious lipids accumulated in liver [10]. In our research, 40-week LPL+/- developed hyperinsulinemia and impaired glucose tolerance, thus increasing HOMA-IR. FB interference on 40-week LPL+/- decreased their fasting insulin level, resulting in decreased HOMA-IR. As for 28-week mice, we can see insulin signaling of LPL+/- controls were obviously blocked in liver and muscle compared with WT. FB interference improved blocked insulin signaling of liver and muscle in LPL+/- mice. Therefore, our results indicated that FB can enhance insulin sensitivity of LPL+/-, which represented patients with inherited hypertriglyceridemia.

Our explorations before elucidated that endoplasmic reticulum stress and oxidative stress may be linked to insulin resistance of LPL+/- mice. Since we have known that FB can enhance insulin resistance of LPL+/-, how did that work? Did FB work on oxidative stress or endoplasmic reticulum stress? Then we start our further explorations.

The ER plays a key role in metabolism like protein folding, lipid synthesis and so on. Furthermore, it is a sensor of both intra- and extracellular stress. When unfolded proteins and excessive energy accumulates in the lumen of the ER, unfolded protein response namely ER stress occurs [10, 19]. In our current research, we observed ER stress emerged in livers of young, senior and old LPL+/- mice. These were consistent with our previous study [7]. And FB interference improve ER stress in liver of 16 to 40-week LPL+/- mice. Although 16-week LPL+/- with FB did not show improved glucose tolerance and insulin resistance, ER stress in livers were alleviated. Nan Zhang also reported that FB improved insulin sensitivity in liver of C57 mice fed with high-calorie and high-cholesterol diet by alleviating ER stress [20]. Chan SM et al. found that FB increased rather than decreased ER stress though insulin resistance was improved. This inconformity with our research may be related to feeding with high-fructose diet and fenofibrate administration for a shorter time [10]. Moreover, Yan F

and her colleagues reported that hepatic steatosis occurred after C57 mice were directly administered orally with fenofibrate for 10 days [21]. This could attribute to the direct toxicity of fenofibrate in the normal liver and not to protective effects on mice with hyperlipidemia. Therefore, our reports elucidated FB improved ER stress in liver of LPL+/- prior to improvement in glucose tolerance and insulin resistance.

The insulin sensitivity of muscle was also observed improved, though ER stress biomarkers of muscle were not affected. Then we guessed that there were other mechanisms underlying the improvement.

Oxidative stress may be the answer. Reactive oxygen species have a causal role in insulin resistance. Reviewing the documents, Dorit Samocha-Bonet et al. proposed that metabolic impairments of people contribute to insulin resistance via increased oxidative stress [22]. ROS were reported increased in both two kinds of insulin resistance, and using six treatments designed to alter ROS levels all ameliorated insulin resistance to varying degrees [23]. Thus anti-oxidative became another therapy to treat diabetes and its complications [24]. By reducing systemic and hepatic oxidative stress, apocynin were found to ameliorate insulin resistance in high-fat fed mice [25]. The results of FIELD suggested that FB in type 2 diabetic individuals reduces the need of laser treatment for diabetic retinopathy [26]. Its effect of PPAR-a activated antioxidant enzymes, such as SOD and CAT [27], thus ameliorating oxidative stress, a well-established pathogenic factor in developing diabetic retinopathy [8]. Our results elucidated that SOD and CAT were activated and expressed more in muscle of LPL+/-, then ROS in muscle lessened. Although plasma MDA and TAOC were unaltered, oxidative stress was alleviated in muscle tissues, which resulted in improvement in insulin signaling of muscle.

Previously, we revealed that LPL+/- showed insulin resistance and impaired glucose tolerance. At present, we explored the effect of FB, a hypolipidemic drug, on glucose metabolism and insulin sensitivity. Through activation of PPAR-a, it obviously lessen TG and NEFA of LPL+/- mice [28]. Although FB did not significantly decrease fasting glucose, it did improve glucose tolerance, systemic and tissue-specific insulin sensitivity. And the mechanisms underlying were different between livers and muscles. FB alleviate insulin resistance in liver of LPL+/- via reliving the ER stress of liver while oxidative stress in muscle of LPL+/- with FB was improved which resulted in the improvement in insulin sensitivity of muscle. However, there were limitations in our study. FB was known to have the effect of anti-inflammation. On the other hand, inflammation was another canonical pathway causing insulin resistance. And inflammation overlapped with ER stress and oxidative stress. Inflammation in LPL+/- and anti-inflammation effect of FB on LPL+/- mice may be our explorations in the future. In summary, our findings suggested that patients with LPL deficiency and severe hypertriglyceridemia could benefit from FB, which improved insulin resistance and blocked oxidative stress and ER stress, thereby reducing the risk of T2DM.

List Of Abbreviations

FB fenofibrate

LPL+/- mice lipoprotein lipase knockout heterozygous mice

ROS reactive oxygen species

SOD superoxide dismutase

CAT catalase

T2DM type 2 diabetes

LPL lipoprotein lipase

TG triglyceride

PPAR α peroxisome proliferator-activated receptor- alpha

ER endoplasmic reticulum

IPGTT intraperitoneal glucose tolerance tests

FPG fasting plasma glucose

FINS fasting insulin

HOMA homeostasis model assessment

MDA malondialdehyde

TAOC total antioxidant capacity

GAPDH glyceraldehyde–3-phosphate dehydrogenase

NEFA non-esterified fatty acid

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the animal ethics committee of Shanghai Jiaotong University affiliated Renji Hospital.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

No competing interest.

Funding

This study was supported by the National Natural Science Foundation of China (No. 81270946, 81170758, 30670988) and the Foundation from Renji Hospital, School of Medicine, Shanghai Jiaotong University (RJZZ14–003). They provide funds for our experiments.

Authors' contributions

LYX feed the mice, collect samples, analyze data and write the manuscript. HTT designed the experiment and write the manuscript. ZS and RXX both feed the mice and collect samples. HYM design the experiment and revised the manuscript. All authors have read and approved the manuscript to be published.

Acknowledgements

Not Applicable.

Reference

1.Cefalu WT, Buse JB, Tuomilehto J, Fleming GA, Ferrannini E, Gerstein HC, Bennett PH, Ramachandran A, Raz I, Rosenstock J et al: Update and Next Steps for Real-World Translation of Interventions for Type 2 Diabetes Prevention: Reflections From a Diabetes Care Editors' Expert Forum. Diabetes care 2016, 39(7):1186–1201.

2.Vinicor F, Bowman B: The metabolic syndrome: the emperor needs some consistent clothes. Diabetes care 2004, 27(5):1243; author reply 1244.

3.Marott SC, Nordestgaard BG, Tybjaerg-Hansen A, Benn M: Components of the Metabolic Syndrome and Risk of Type 2 Diabetes. The Journal of clinical endocrinology and metabolism 2016, 101(8):3212–3221.

4.Nolan CJ, Ruderman NB, Kahn SE, Pedersen O, Prentki M: Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. Diabetes 2015, 64(3):673–686.

5.Goldberg IJ, Merkel M: Lipoprotein lipase: physiology, biochemistry, and molecular biology. Frontiers in bioscience: a journal and virtual library 2001, 6:D388–405.

6.Hu Y, Liu W, Huang R, Zhang X: A systematic review and meta-analysis of the relationship between lipoprotein lipase Asn291Ser variant and diseases. Journal of lipid research 2006, 47(9):1908–1914.

7.Li YX, Han TT, Liu Y, Zheng S, Zhang Y, Liu W, Hu YM: Insulin resistance caused by lipotoxicity is related to oxidative stress and endoplasmic reticulum stress in LPL gene knockout heterozygous mice. Atherosclerosis 2015, 239(1):276–282.

8.Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P et al: Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet (London, England) 2005, 366(9500):1849–1861.

9.Chan SM, Zeng XY, Sun RQ, Jo E, Zhou X, Wang H, Li S, Xu A, Watt MJ, Ye JM: Fenofibrate insulates diacylglycerol in lipid droplet/ER and preserves insulin signaling transduction in the liver of high fat fed mice. Biochimica et biophysica acta 2015, 1852(7):1511–1519.

10.Chan SM, Sun RQ, Zeng XY, Choong ZH, Wang H, Watt MJ, Ye JM: Activation of PPARalpha ameliorates hepatic insulin resistance and steatosis in high fructose-fed mice despite increased endoplasmic reticulum stress. Diabetes 2013, 62(6):2095–2105.

11.Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985, 28(7):412–419.

12.Holzl B, Paulweber B, Sandhofer F, Patsch JR: Hypertriglyceridemia and insulin resistance. Journal of internal medicine 1998, 243(1):79–82.

13.Mateos R, Lecumberri E, Ramos S, Goya L, Bravo L: Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress. Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. Journal of chromatography B, Analytical technologies in the biomedical and life sciences 2005, 827(1):76–82.

14.Szalat A, Durst R, Leitersdorf E: Managing dyslipidaemia in type 2 diabetes mellitus. Best practice & research Clinical endocrinology & metabolism 2016, 30(3):431–444.

15.Hu Y, Ren Y, Luo RZ, Mao X, Li X, Cao X, Guan L, Chen X, Li J, Long Y et al: Novel mutations of the lipoprotein lipase gene associated with hypertriglyceridemia in members of type 2 diabetic pedigrees. Journal of lipid research 2007, 48(8):1681–1688.

16.McKeage K, Keating GM: Fenofibrate: a review of its use in dyslipidaemia. Drugs 2011, 71(14):1917–1946.

17.Jeong S, Yoon M: Fenofibrate inhibits adipocyte hypertrophy and insulin resistance by activating adipose PPARalpha in high fat diet-induced obese mice. Experimental & molecular medicine 2009, 41(6):397–405.

18.Ueno H, Saitoh Y, Mizuta M, Shiiya T, Noma K, Mashiba S, Kojima S, Nakazato M: Fenofibrate ameliorates insulin resistance, hypertension and novel oxidative stress markers in patients with metabolic syndrome. Obesity research & clinical practice 2011, 5(4):e267–360.

19.Su J, Zhou L, Kong X, Yang X, Xiang X, Zhang Y, Li X, Sun L: Endoplasmic reticulum is at the crossroads of autophagy, inflammation, and apoptosis signaling pathways and participates in the pathogenesis of diabetes mellitus. Journal of diabetes research 2013, 2013:193461.

20.Zhang N, Lu Y, Shen X, Bao Y, Cheng J, Chen L, Li B, Zhang Q: Fenofibrate treatment attenuated chronic endoplasmic reticulum stress in the liver of nonalcoholic fatty liver disease mice. Pharmacology 2015, 95(3–4):173–180.

21.Yan F, Wang Q, Xu C, Cao M, Zhou X, Wang T, Yu C, Jing F, Chen W, Gao L et al: Peroxisome proliferator-activated receptor alpha activation induces hepatic steatosis, suggesting an adverse effect. PloS one 2014, 9(6):e99245.

22.Samocha-Bonet D, Heilbronn LK, Lichtenberg D, Campbell LV: Does skeletal muscle oxidative stress initiate insulin resistance in genetically predisposed individuals? Trends in endocrinology and metabolism 2010, 21(2):83–88.

23.Houstis N, Rosen ED, Lander ES: Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006, 440(7086):944–948.

24.Rani V, Deep G, Singh RK, Palle K, Yadav UC: Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sci 2016, 148:183–193.

25.Meng R, Zhu DL, Bi Y, Yang DH, Wang YP: Anti-oxidative effect of apocynin on insulin resistance in high-fat diet mice. Annals of clinical and laboratory science 2011, 41(3):236–243.

26.Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E et al: Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. Lancet (London, England) 2007, 370(9600):1687–1697.

27.Bordet R, Ouk T, Petrault O, Gele P, Gautier S, Laprais M, Deplanque D, Duriez P, Staels B, Fruchart JC et al: PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases. Biochemical Society transactions 2006, 34(Pt 6):1341–1346.

28.Shipman KE, Strange RC, Ramachandran S: Use of fibrates in the metabolic syndrome: A review. World journal of diabetes 2016, 7(5):74–88.

Figures

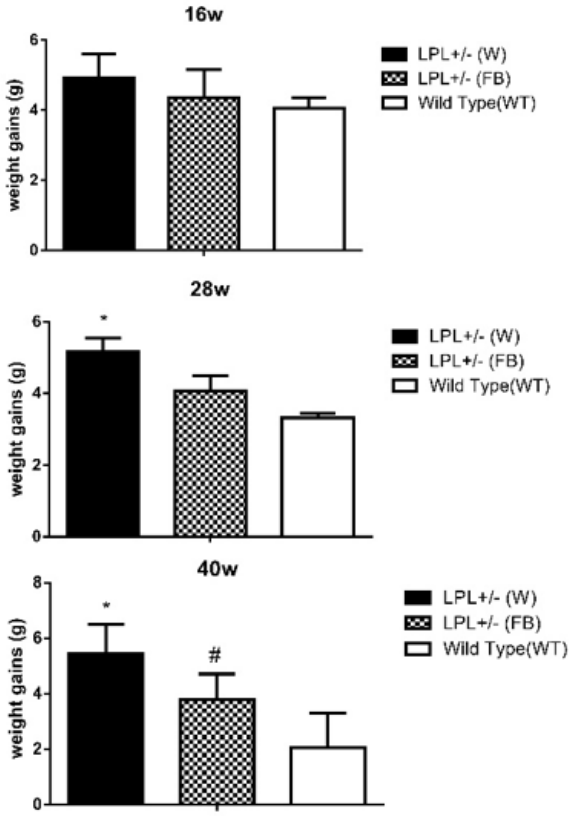

Fig 1. Weight gains from gavage start to the end of LPL+/- (W), LPL+/- (FB) and Wild Type (WT). Weight gains equal to final body mass minus initial body mass. *, P<0.05 vs. WT of the same age; #,P<0.05 vs. LPL+/- (W); n=6 for each group at each age. Data were represented as mean ± SEM.

Figure 1

Weight gains from gavage start to the end of LPL+/- (W), LPL+/- (FB) and Wild Type (WT). Weight gains equal to final body mass minus initial body mass. *, P<0.05 vs. WT of the same age; #, P<0.05 vs. LPL+/- (W); n=6 for each group at each age. Data were represented as mean ± SEM.

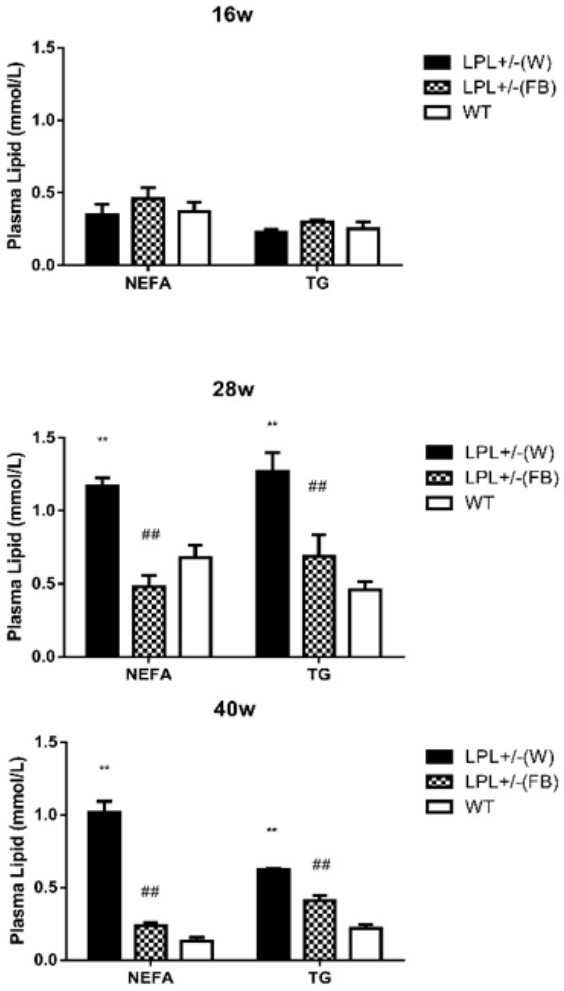

Fig 2. TG and NEFA levels in plasma of 16-, 28- and 40-week LPL+/- (W), LPL+/- (FB) and WT mice. *, P<0.05, **, P<0.001 vs. WT of the same age; #, P<0.05, ##, P<0.05 vs. LPL+/- (W) in the same age. n=6 for each group at 16, 28 and 40 weeks. Results are mean ± SEM.

Figure 2

TG and NEFA levels in plasma of 16-, 28- and 40-week LPL+/- (W), LPL+/- (FB) and WT mice. *, P<0.05, **, P<0.001 vs. WT of the same age; #, P<0.05, ##, P<0.05 vs. LPL+/- (W) in the same age. n=6 for each group at 16, 28 and 40 weeks. Results are mean ± SEM.

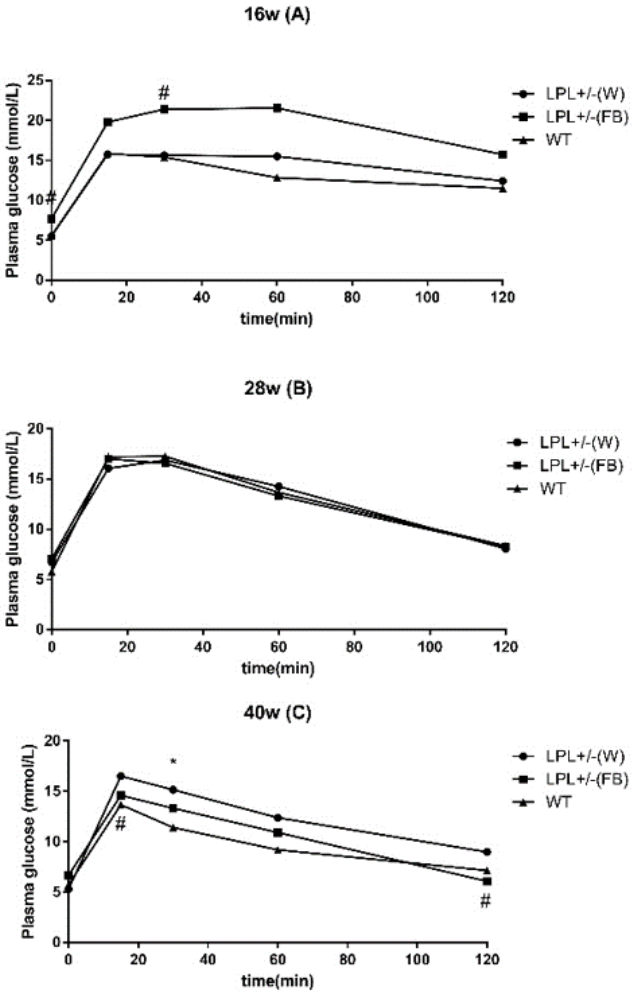

Fig 3. Intraperitoneal glucose tolerance tests. Time course of blood glucose levels in 16 (A), 28 (B) and 40-week (C) LPL+/- (water), LPL+/- (FB) and WT mice after intraperitoneal injection of 1 g/kg glucose are shown. *, P < 0.05, vs. WT mice of the same age at the same time point; #, P < 0.05, vs. LPL+/- (water) of the same age at the same time point. n = 6 for LPL+/- (water), LPL+/- (FB) and WT at 16, 28 and 40 weeks.

Figure 3

Intraperitoneal glucose tolerance tests. Time course of blood glucose levels in 16 (A), 28 (B) and 40-week (C) LPL+/- (W), LPL+/- (FB) and WT mice after intraperitoneal injection of 1 g/kg glucose are shown. *, P < 0.05, vs. WT mice of the same age at the same time point; #, P < 0.05, vs. LPL+/- (W) of the same age at the same time point. n = 6 for LPL+/- (W), LPL+/- (FB) and WT at 16, 28 and 40 weeks. Data are represented as mean.

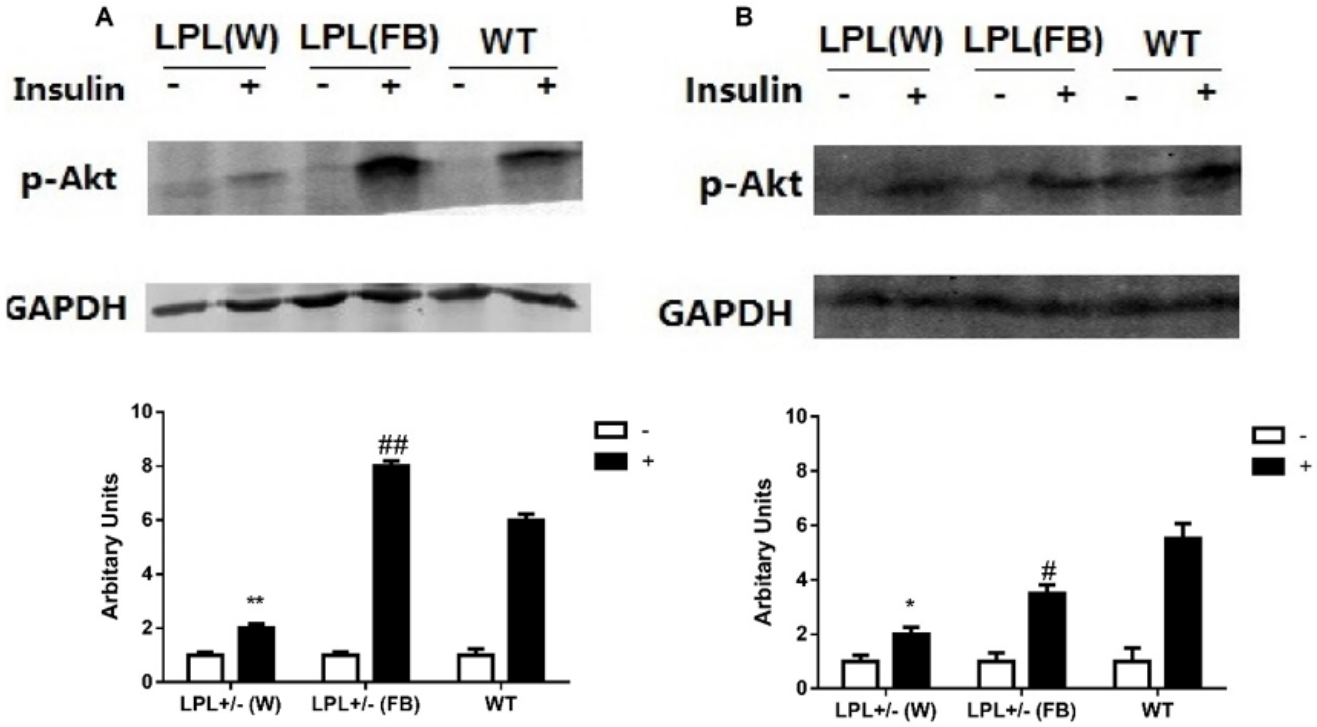

Fig. 4. Ser473 phosphorylation level of Akt was compared for the liver (A) and skeletal muscle (B) of 28-week LPL+/- (W) (LPL(W)) , LPL+/- (FB) (LPL(FB)) and WT mice before and after insulin stimulation. *, P < 0.05; **, P < 0.001 vs. WT; #, P<0.05; ##, P<0.001, vs. LPL+/- (W); n =6 per group. Results are mean ± SEM.

Figure 4

Ser473 phosphorylation level of Akt was compared for the liver (A) and skeletal muscle (B) of 28-week LPL+/- (W) (LPL (W)), LPL+/- (FB) (LPL (FB)) and WT mice before and after insulin stimulation. *, P < 0.05; **, P < 0.001 vs. WT; #, P<0.05; ##, P<0.001, vs. LPL+/- (W); n =6 per group. Results are mean + SEM.

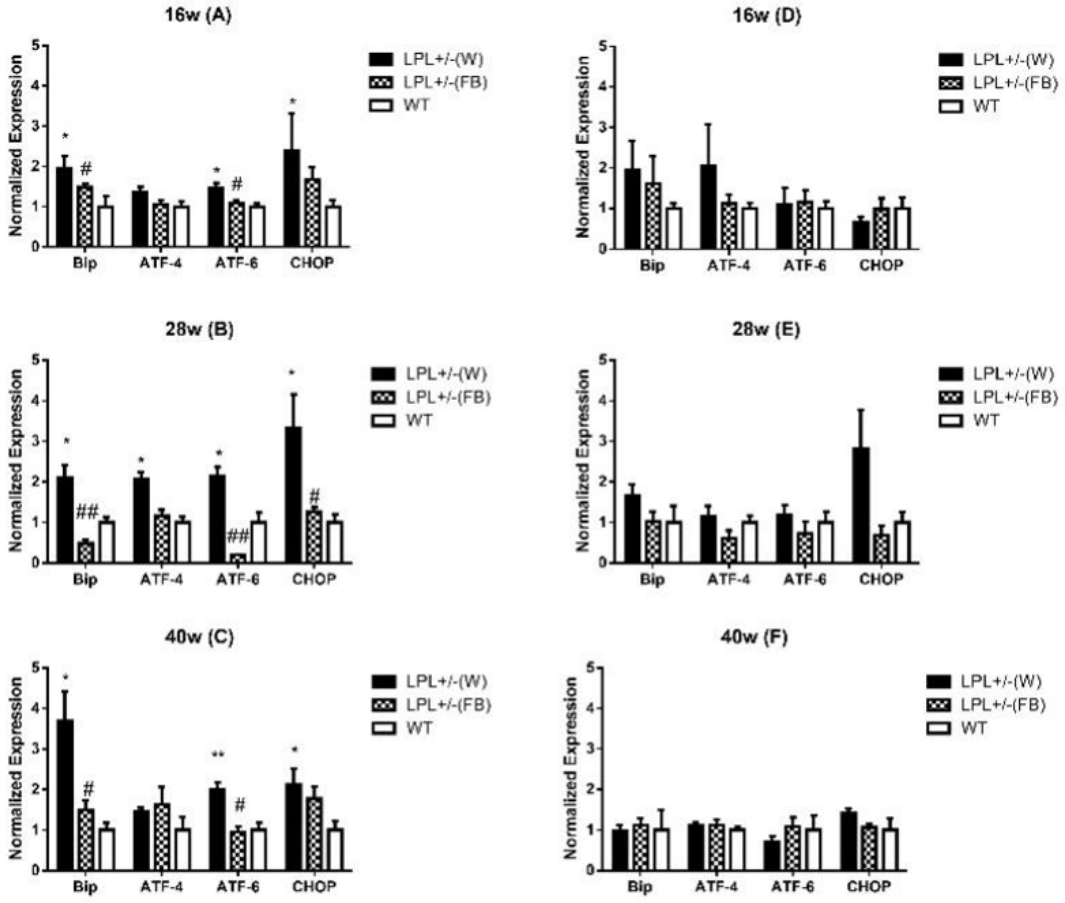

Fig 4. Liver (A-C) and muscle (D-F) mRNA expression of ER stress biomarkers in LPL+/- (W), LPL+/- (FB) and WT at 16, 28 and 40w; n=6 per group; *, P<0.05, **, p<0.001 vs. WT of the same age; #, P<0.05, ##, p<0.001 vs. LPL+/- (W) of the same age. Results are represented as mean ± SEM.

Figure 5

Liver (A-C) and muscle (D-F) mRNA expression of ER stress biomarkers in LPL+/- (W), LPL+/- (FB) and WT at 16, 28 and 40w; n=6 per group; *, P<0.05, **, p<0.001 vs. WT of the same age; #, P<0.05, ##, p<0.001 vs. LPL+/- (W) of the same age. Results are represented as mean ± SEM.

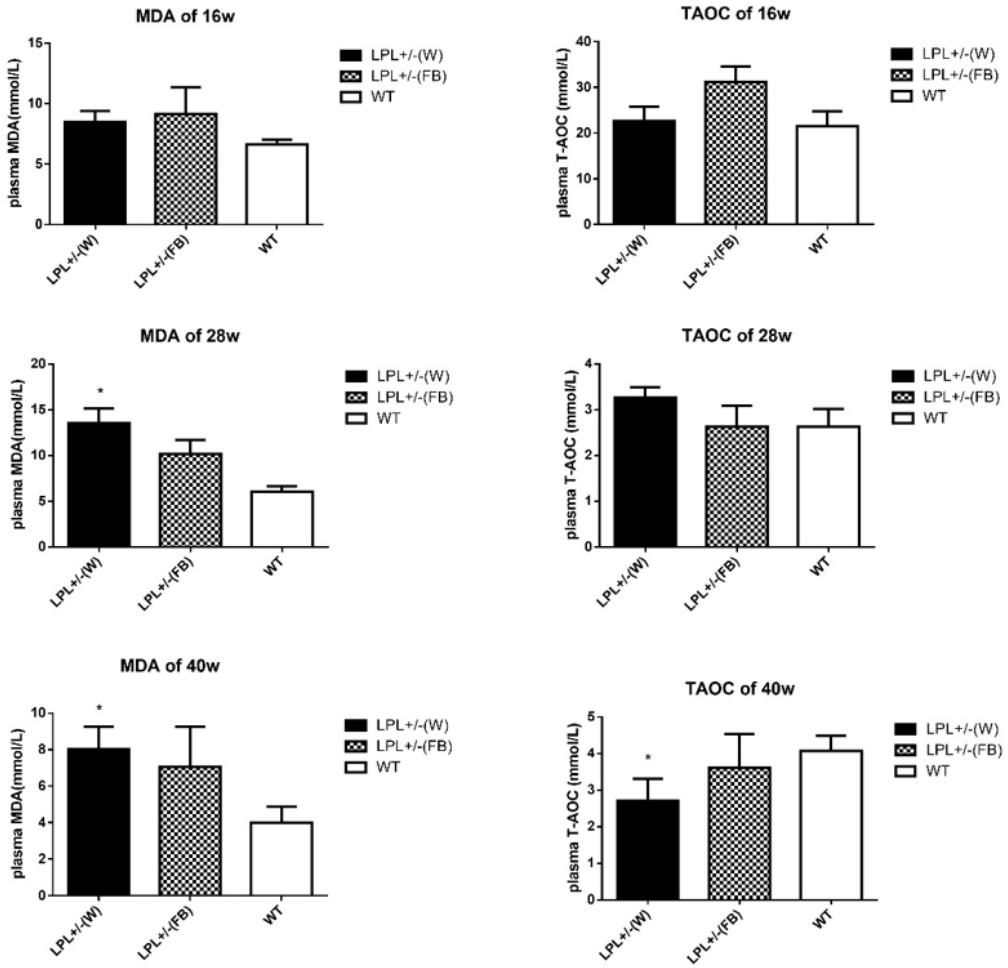

Fig 6. Plasma MDA and TAOC in 16- ,28- and 40-week LPL+/- (W), LPL+/-(FB) and WT. *, P < 0.05 vs.WT mice at the same age. n=6 for every group at 16,28, and 40 weeks.Results are mean ±SEM.

Figure 6

Plasma MDA and TAOC in 16-, 28- and 40-week LPL+/- (W), LPL+/- (FB) and WT. *, P < 0.05 vs.WT mice at the same age. n=6 for every group at 16, 28, and 40 weeks. Results are mean ± SEM. Pic 1. ROS level in the liver (A-C) and skeletal muscle (D-F) of 16-week LPL+/- (W), LPL+/- (FB) and WT mice; **, P<0.001 vs. WT; n = 6 per group. Data were mean ± SEM. Pic 2. ROS level in the liver (A-C) and skeletal muscle (D-F) of 28-week LPL+/- (W), LPL+/- (FB) and WT mice; **, P<0.001 vs. WT; #, P<0.05 vs. LPL+/- (W); n = 6 per group. Results were mean ± SEM. Pic 3. ROS level in the liver (A-C) and skeletal muscle (D-F) of 40-week LPL+/- (W), LPL+/- (FB) and WT mice; **, P<0.001 vs. WT; #, P<0.05 vs. LPL+/- (W); n = 6 per group. Results were represented as mean ± SEM.

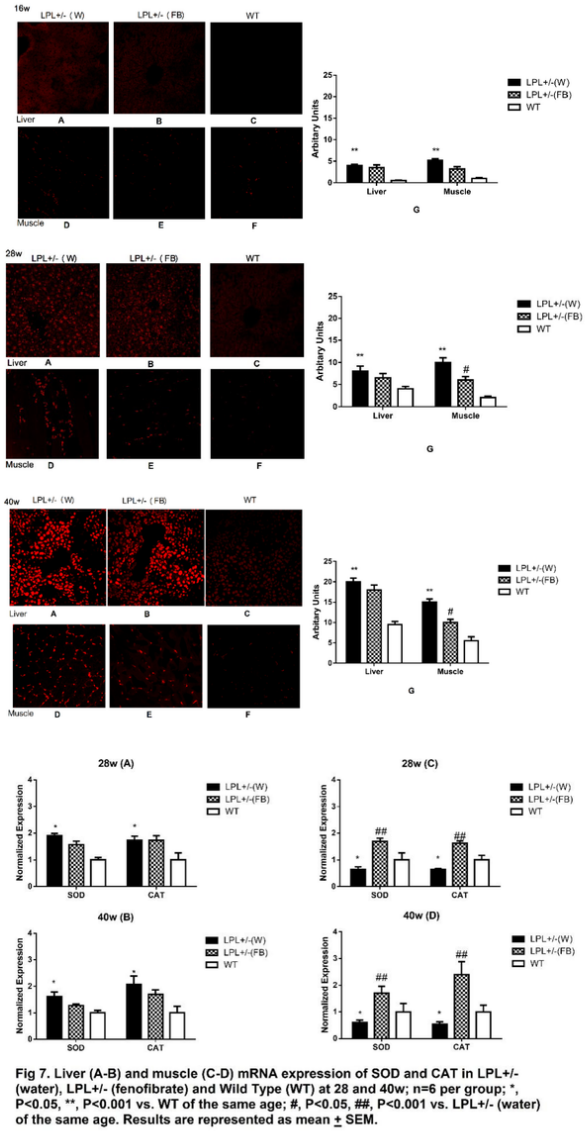

Fig 7. Liver (A-B) and muscle (C-D) mRNA expression of SOD and CAT in LPL+/- (water), LPL+/- (fenofibrate) and Wild Type (WT) at 28 and 40w; n=6 per group; *, P<0.05, **, P<0.001 vs. WT of the same age; #, P<0.05, ##, P<0.001 vs. LPL+/- (water) of the same age. Results are represented as mean ± SEM.

Figure 7

Liver (A-B) and muscle (C-D) mRNA expression of SOD and CAT in LPL+/- (W), LPL+/- (FB) and Wild Type (WT) at 28 and 40w; n=6 per group; *, P<0.05, **, P<0.001 vs. WT of the same age; #, P<0.05, ##, P<0.001 vs. LPL+/- (W) of the same age. Results are represented as mean ± SEM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklistfillable.pdf
- TableS1.docx