Effects of the beta3-adrenergic receptor agonist KTO-7924 on improvement of diabetic pathology in Zucker diabetic fatty rats

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Abstract

Background and Aims

Beta3 adrenergic receptor agonists have been shown to improve lipid metabolism and diabetes in a rodent diabetes model presumably by augmenting lipolysis and inducing uncoupling protein 1 in adipose tissue that led hypertrophic adipose cells to convert to small adipose cells. The present study investigated the effects of KTO-7924, a selective beta3 adrenergic receptor agonist, on a Zucker diabetic fatty rat model.

Methods

Zucker diabetic fatty rats were assigned into a control group or a KTO-7924 treatment group (30 mg/kg twice a day for 28 days). Plasma glucose, insulin, triglycerides (TGs), free fatty acids (FFAs), and adiponectin were measured and oral glucose tolerance testing was performed. We also determined organ weight and the mRNA expression of adiponectin receptors 1 and 2.

Results

There was a clear tendency for lower glucose levels in the KTO-7924 group versus controls. Insulin levels tended to remain constant until the study end point in treated animals. KTO-7924 administration had significantly decreased plasma TG and FFA levels as compared with the control group on day 7, both of which persisted until day 28. Plasma adiponectin level was significantly higher in KTO-7924-treated animals from 7 days onwards. Insulin sensitivity in the KTO-7924 group was significantly improved according to the oral glucose tolerance test. Retroperitoneal white adipose tissue weight was significantly lower, and soleus muscle weight was higher, in the treated mice. The mRNA expression of adiponectin receptor 2 in the soleus muscle of the KTO-7924-treated group was significantly lower than in controls.

Conclusion

KTO-7924 improved the pathology of diabetes in Zucker diabetic fatty rats by enhancing lipid metabolism, adipose tissue weight loss, and muscle weight gain while decreasing adiponectin receptor 2 expression in muscle.

1. Introduction

Many beta3-adrenergic receptor agonists have been developed to date (1). One report described that administration of AJ-9677 to KK-Ay/Ta mice significantly decreased adipose tissue weight along with plasma glucose, insulin, free fatty acid (FFA), and triglyceride (TG) levels while upregulating the mRNA expression of uncoupling protein-1 (UCP1) in both brown adipose tissue (BAT) and white adipose tissue (WAT) (2). In another study, the administration of CL-316,243 to db/db mice decreased plasma glucose and insulin levels and improved type 2 diabetes (3).

Belonging to the G protein-coupled receptor family, beta adrenergic receptors mediate physiological actions through the activation of adenylyl cyclase (4). While beta1 adrenergic receptor stimulation evokes an increase in heart rate, that of beta2 adrenergic receptors causes relaxation of bronchial smooth muscle. Adrenergic receptors are present in WAT that stores energy as TGs and in BAT containing abundant mitochondria. The findings of impaired beta3 adrenergic receptors in genetic and dietary obese mice and increased total body fat in beta3 adrenergic receptor-deficient mice strongly indicate a correlation between the receptor and obesity (5, 6).

In humans, obesity and fat accumulation are accompanied by elevated plasma plasminogen activator inhibitor-1 tumor necrosis factorα, and leptin and a decreased plasma level of adiponectin (7-17). We earlier showed that the newly-developed beta3 adrenergic receptor agonist KTO-7924 ameliorated insulin resistance in obese (fa/fa) Zucker rats with hyperinsulinemia (18, 19, 20). However, the pharmacological effect of this drug on diabetic rats with regard to hyperglycemia is unknown. In the present study, we

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analyzed the pathological changes in Zucker diabetic fatty rats, with a particular focus on hyperglycemia, with KTO-7924.

2. Materials and methods

Preparation of KTO-7924

KTO-7924, the pharmacological properties of which have been described elsewhere (18, 19, 20), was synthesized at Kissei Pharmaceutical Co., Ltd. (Nagano, Japan).

Animal studies

Male 5-week-old ZDF/Gmi-fa/fa and ZDF/Gmi-lean rats were purchased from Charles River Genetic Models, Inc. (USA). The animals were given standard chow (Purina 5008, Japan SLC, Inc., Japan) and tap water ad libitum and assigned to control or treatment groups approximately matched for mean body weight and plasma glucose level. The treatment group was given 30 mg/kg of KTO-7924 twice daily by gavage in the morning and evening for 28 days while the control group received a 0.5% methyl cellulose solution from 9-week old. Blood samples were drawn from the tail vein and plasma was prepared by centrifugation at 1880×g for 10 min. All rats were sacrificed at the end of the experimental period and the interscapular BAT, retroperitoneal WAT, soleus muscle, pancreas, and liver were harvested. All procedures involving laboratory animals in this study were conducted according to the guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co., Ltd.

Determination of plasma glucose, insulin, TGs, FFAs, and adiponectin

Plasma glucose levels were determined with a Glucose C-IITest (Wako Pure Chemical Industries, Osaka, Japan). Insulin levels were measured using a Morinaga insulin assay kit (Morinaga Bioscience Laboratory, Yokohama, Japan). TG levels were assessed by means of a

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Triglyceride E Test (Wako Pure Chemical Industries). FFA levels were determined with a nonesterified fatty acids (NEFA) C-Test (Wako Pure Chemical Industries). Adiponectin levels were quantified using a Mouse/Rat adiponectin ELISA Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The manufacturer's protocol was followed for all tests.

Oral glucose tolerance test (OGTT) trials

OGTT trials were performed after fasting the rats for 16 hours. Briefly, animals were administered a 2 g/kg bolus orally, and blood samples were obtained from the tail vein at -3, 15, 30, 60, 120, 240 minutes post-glucose dosage. Plasma glucose and insulin levels were determined as described above. Composite insulin sensitivity index (CISI) was calculated using the formula: (10,000/$\sqrt{}$ [(fasting glucose×fasting insulin)×(mean glucose ×mean insulin)]) during OGTT.

Tissue RNA isolation

To quantify the mRNA expression of adiponectin receptors 1 and 2, frozen tissues were homogenized for 1 min in TRIZOL® Reagent (Invitrogen, California, USA) using an Ultra-turrax T8 homogenizer (IKAWerke GmbH and CO.KG, Staufen, Germany). Subsequent procedures were carried out according to the manufacturer's instructions. Isolated total RNA samples were purified using an RNeasy Mini Kit (Qiagen, Japan).

Reverse transcription (RT) and real-time quantitative polymerase chain reaction (PCR)

RT of total RNA to cDNA was performed using a Super Script First-Strand Synthesis System for RT-PCR (Invitrogen, California, USA) according to the manufacturer's directions. All forward primers, reverse primers, and Taqman probes were designed with Primer Express software version 1.0 (Applied Biosystems,

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Foster City, USA). Real-time PCR-based 5' nuclease assays (Taqman assays) were performed using a DNA Engine Opticon System (MJ Research, Inc.). The following primers were used: adiponectin receptor 1, forward 5'-ACGTTGGAGAGTCATCC-CGTAT-3', reverse 5'-TCTTGAAG-CAAGCCCGAAAG-3', and fluorogenic probe FAM-5'-AAAGACAACGACTAC-CTGCTACATGGCCAC-3'-TAMRA; adiponectin receptor 2, forward 5'-AGCCTCTATATCACCGGAGCTG-3', reverse 5'-GCTGATGAGAGTGAAA-CCAGATGT–3', and fluorogenic probe FAM-5'-CCTGAGCGCTTCTTTCCTGG CAAA-3'. Rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control reagents (Applied Biosystems) were used as primers for GAPDH. For each reaction, cDNA from individual tissues was mixed with Platinum Quantitative PCR SuperMix-UDG (Invitrogen), forward and reverse primers, Taqman probe, and diethyl pyrocarbonate (DEPC)-treated water (Nacalai Tesque, Kyoto, Japan). Standards consisted of 10-fold serial dilutions of pCR-Blunt II Topo® Vector (Invitrogen, USA) that contained the PCR product amplified by forward and reverse primers for the analyzed gene. At the end of reaction, the cycle threshold (Ct) values (i.e., the cycle numbers at which fluorescent signals were obtained) were determined for standard and unknown samples. A standard curve was constructed by plotting the Ct values as a function of the log concentration of standard cDNA. On the basis of the Ct values of the unknown samples, relative mRNA concentration was determined and corrected using GAPDH mRNA as an internal standard.

Statistical analysis

Values are presented as mean ± standard error (SE). Statistical analysis of data was performed using one-way

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analysis of variance followed by unpaired t-testing. A probability level of less than 0.05 was accepted as statistically significant. Excel Tokei (version 5.0; Esumi Co., Ltd.) and Prism (version 3.0; GraphPad Software, Inc.) software packages were employed for statistical testing.

3. Results

Body weight and food intake

There were no significant differences between the KTO-7924 treatment group and control group for body weight or food intake during the study period (Fig. 1A, 1B).

Organ weight

The weight of retroperitoneal WAT was significantly decreased in the KTO-7924 group as compared with the control group (Table 1). Soleus weight was significantly increased in the KTO-7924 group over controls.

Plasma parameters

Plasma glucose level in the control group increased time-dependently throughout the experimental period (Fig. 2A). In the KTO-7924 group, glucose level tended to be lower than controls from day 7 onwards.

Plasma insulin level in the control group decreased time-dependently over the study period (Fig. 2B). In the KTO-7924 group, insulin level tended to remain stable.

Plasma TG level in the control group increased markedly during the first week and remained high until day 28 (Fig. 2C). In the KTO-7924 group, TG level became significantly decreased on day 7, which persisted until the 28^{th} day.

Plasma FFA level in the control group remained high during the study period (Fig. 2D). In the KTO-7924 group, FFA level was decreased significantly at 7 days of administration and tended to

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remain low until the end point.

Plasma adiponectin level in the control group decreased time-dependently during the study period (Fig. 2E). In the KTO-7924 group, adiponectin level was significantly higher than in controls from day 7 onwards.

OGTT

At the end of the administration period, OGTT revealed significantly lower plasma glucose levels for the KTO-7924 group versus controls (Fig. 3A). Regarding plasma insulin, there were no significant differences after glucose loading between the control and KTO-7924 groups (Fig. 3B). CISI analysis showed that insulin sensitivity in KTO-7924 rats had become significantly ameliorated over controls (Fig. 4).

Adiponectin receptors 1 and 2 expression

The mRNA expression of adiponectin receptor 2 in the soleus muscle of the KTO-7924 treatment group was significantly lower than that in the vehicle control group at the end of observation (Fig. 5).

4. Discussion

We earlier reported that KTO-7924 ameliorated lipid metabolism in terms of significant reductions in TG and FFA levels in a db/db diabetic mouse model that was accompanied by a significant increase in plasma adiponectin (21). These findings established a possible effect of KTO-7924 on hyperglycemia and insulin resistance.

We have also described the impact of KTO-7924 on obese (fa/fa) Zucker rats (18). It is generally accepted that obese (fa/fa) Zucker and Zucker diabetic fatty rats differ with regard to the pathological phenomenon of blood glucose. In obese (fa/fa) Zucker rats, hyperlipidemia and hyperinsulinemia increase with age (data from Charles River Laboratories

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International, Inc.). Moreover, we found that obese (fa/fa) Zucker rats developed severe insulin resistance but did not exhibit hyperglycemia (18). Thus, obese (fa/fa) Zucker rats are particularly recognized as a model of hyperinsulinemia. On the other hand, Zucker diabetic fatty rats appear to more closely resemble human type II diabetes in that they develop hyperglycemia.

This study aimed to determine the effect of KTO-7924 on Zucker diabetic fatty rats from the viewpoint of lipid metabolism and especially glucose tolerance. We witnessed that KTO-7924 evoked significant decreases in lipid parameters (TGs and FFAs), which resembled the earlier results of db/db mice administered KTO-7924. These findings confirmed that beta3 adrenergic receptor agonist administration improved lipid metabolism, in agreement with previous studies (2,3). Plasma glucose levels increased time-dependently in the control group. KTO-7924 treatment resulted in markedly lower plasma glucose levels compared with controls, presumably in a manner resembling that in db/db mice (21). Regarding plasma adiponectin level, that in control rats decreased time-dependently during the 28-day study period, which was comparable to the db/db report (21). Similar parallels could be drawn for the observed abrogation of this decrease by KTO-7924 in this study. Taken together, it is likely that KTO-7924 induces the same improvements across multiple rodent diabetes models, including Zucker diabetic fatty rats and db/db mice with accompanying hyperglycemia. Concerning OGTT findings, KTO-7924 treatment has been shown to ameliorate insulin resistance in db/db mice and obese (fa/fa) Zucker rats (18,21). Similar results were observed here in Zucker diabetic fatty rats.

This study evaluated the expression

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of adiponectin receptors 1 and 2 in the KTO-7924 group. These receptors have already been cloned (22). Adiponectin receptor 1 is highly expressed in human muscle (23,24), while adiponectin receptor 2 is predominantly expressed in the liver. We earlier reported that CL-316,243, a beta3 adrenergic receptor agonist, decreased hepatic adiponectin receptor 2 and improved diabetes and lipid metabolism in db/db mice (3). In this series, a significant decrease in adiponectin receptor 2 expression was seen in the soleus muscle of the KTO-7924 group, possibly due to a reduced need for signaling from adiponectin receptor 2 in the test group or by a direct effect of plasma adiponectin levels.

A recent report described that beta3 adrenergic receptor agonists could stimulate human BAT thermogenesis and represented a promising treatment for metabolic disease (25). In general, beta3 adrenergic receptor agonists induce the up-regulation of UCP-1 in adipose tissue and convert hypertrophic adipose cells into small adipose cells. We surmise that KTO-7924 may also lead to an improvement in thermogenesis in humans.

In conclusion, this study examined the pharmacological effects of KTO-7924, a newly–developed beta3-adrenergic receptor agonist, on Zucker diabetic fatty rats. Plasma levels of TGs and FFAs were both significantly reduced in the KTO-7924 group as compared with controls. Plasma adiponectin level decreased with time in the control group but was significantly higher in the KTO-7924 group throughout the study period. Furthermore, KTO-7924 led to an improvement in glucose tolerance in OGTT trials after 28 days of drug administration. Retroperitoneal WAT weight was significantly reduced, while that of the soleus muscle was significantly

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increased in the KTO-7924 treatment group. The mRNA expression of adiponectin receptor 2 in the soleus muscle of KTO-7924-treated rats was significantly lower than that in the control group. To our knowledge, this is the first study demonstrating that KTO-7924 improves glucose tolerance and normalizes lipid metabolism in Zucker diabetic fatty rats.

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Legends

Figure 1. Body weight (A) and food intake (B) in control, KTO-7924, and lean groups. Mean ± SE of n=5-6 rats in each group.

Figure 2. Effects of KTO-7924 on plasma glucose (A), insulin (B), TGs (C), FFAs (D), and adiponectin (E) in each group. Mean ± SE of n=5-6 rats in each group. *$P < 0.05$ and **$P < 0.01$ vs. control group.

Figure 3. Effects of KTO-7924 on OGTT trials in Zucker diabetic fatty rats (A, plasma glucose; B, plasma insulin). Mean ± SE of n=5-6 rats in each group. *$P < 0.05$ and **$P < 0.01$ vs. control group.

Figure 4. CISI was calculated using plasma glucose and insulin levels from OGTT. *$P < 0.05$ and **$P < 0.01$ vs. control group.

Figure 5. Effects of KTO-7924 on adiponectin receptor 1 and adiponectin receptor 2 mRNA levels in the soleus muscle and liver of Zucker diabetic fatty rats. Mean ± SE of n=5-6 rats in each group. *$P < 0.05$ vs. control group.

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Table 1

Table 1. Effect of KTO-7924 on organ weight in Zucker Diabetic Fatty rats			
	fa/fa		Lean
		KTO-7924	
	Vehicle	30 mg/kg	
Liver (g)	19.0 ± 0.6	18.4 ± 1.0	10.9 ± 0.2 **
Retroperitoneal WAT (g)	13.6 ± 0.7	10.3 ± 0.9 *	2.3 ± 0.2 **
Intrascapular BAT (g)	1.03 ± 0.08	1.56 ± 0.24	0.38 ± 0.02 **
Soleus (g)	0.28 ± 0.01	0.34 ± 0.01 **	0.32 ± 0.00 **
Pancreas (g)	1.00 ± 0.01	1.04 ± 0.08	1.19 ± 0.03 **
Data are expressed as the mean ± S.E. of 5-6 animals. Tissues were harvested under feeding conditions on day 30. *P<0.05 and **P<0.01 vs control group.			
WAT, white adipose tissue; BAT, brown adipose tissue.			

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Fig. 1

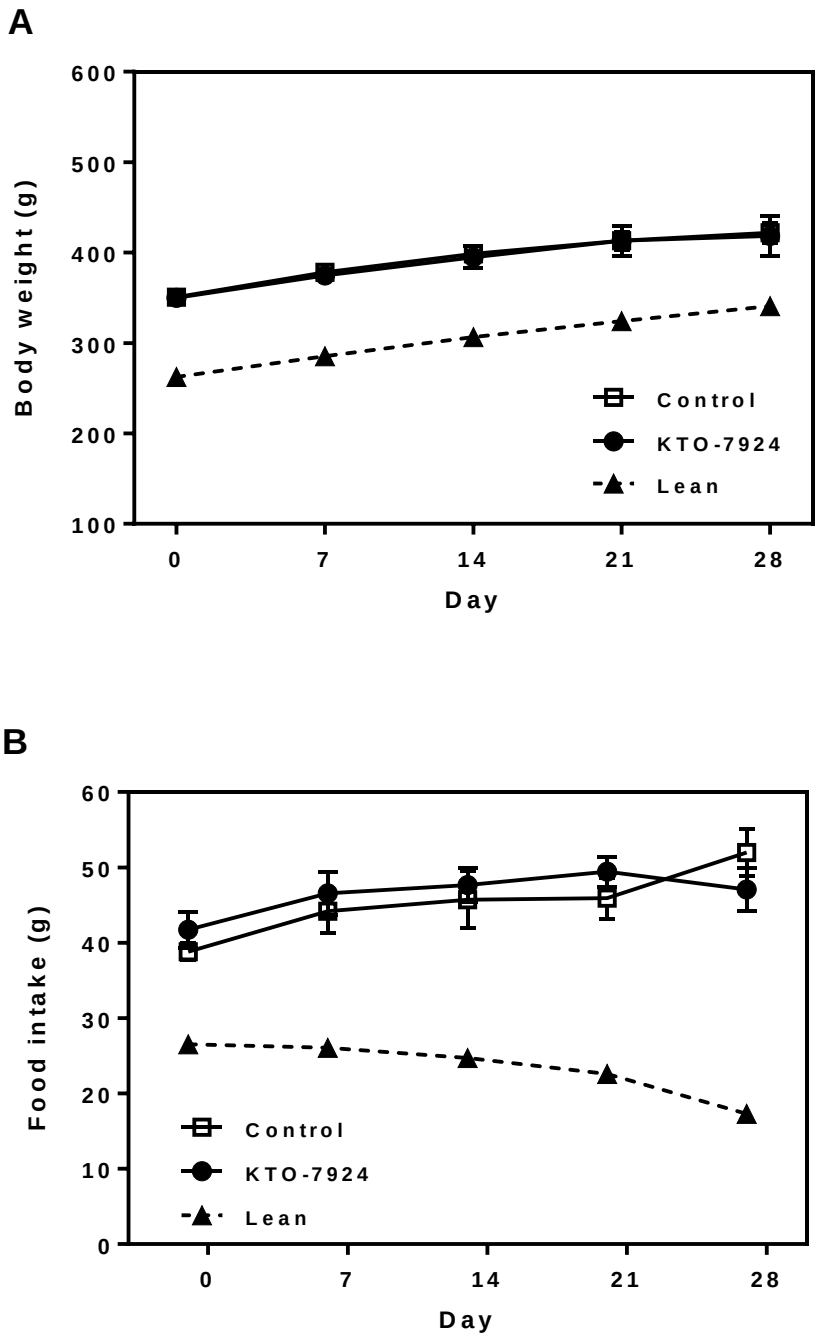

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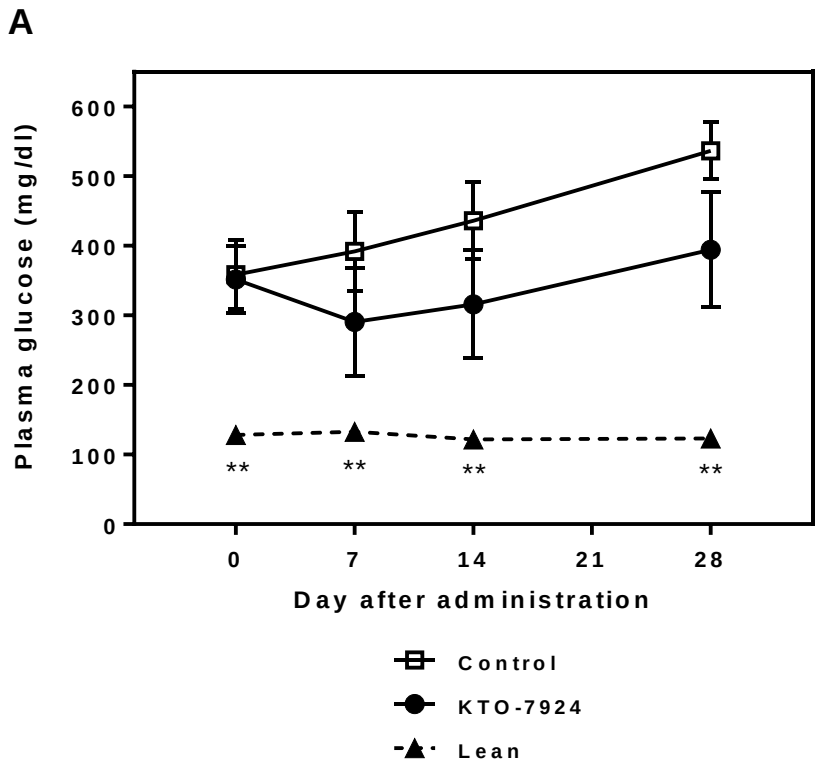
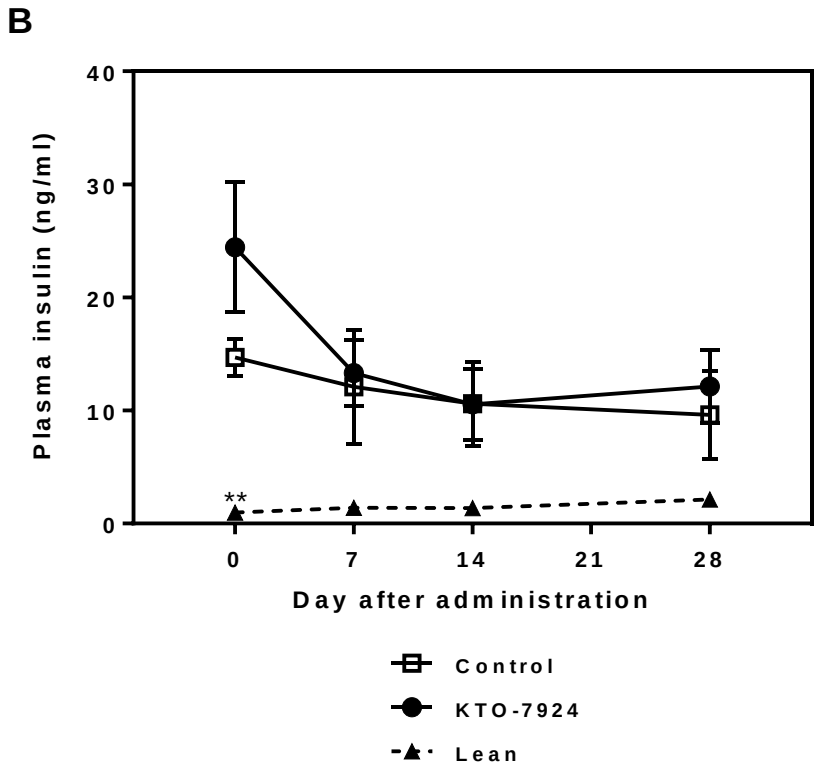
Fig. 2





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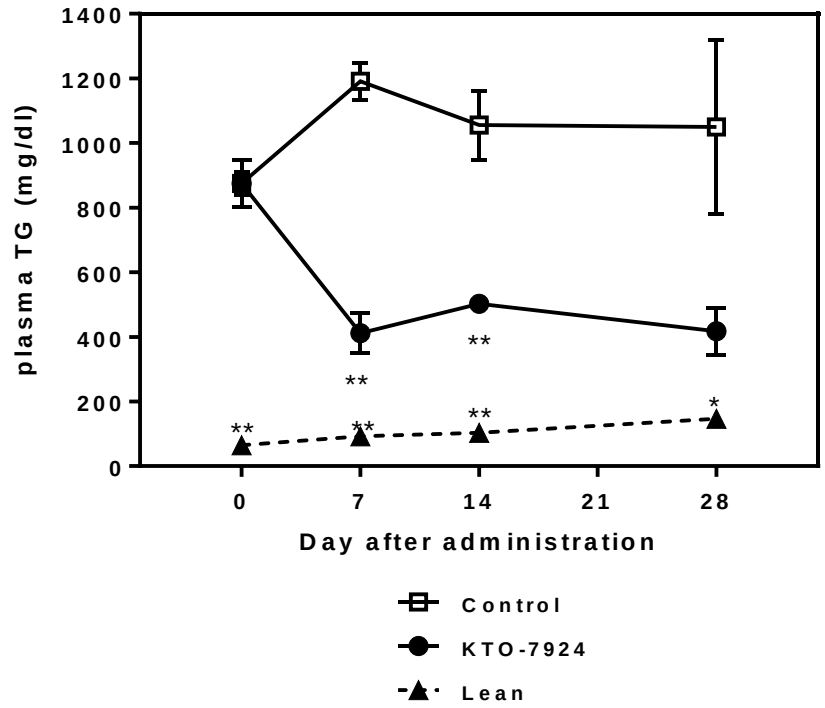

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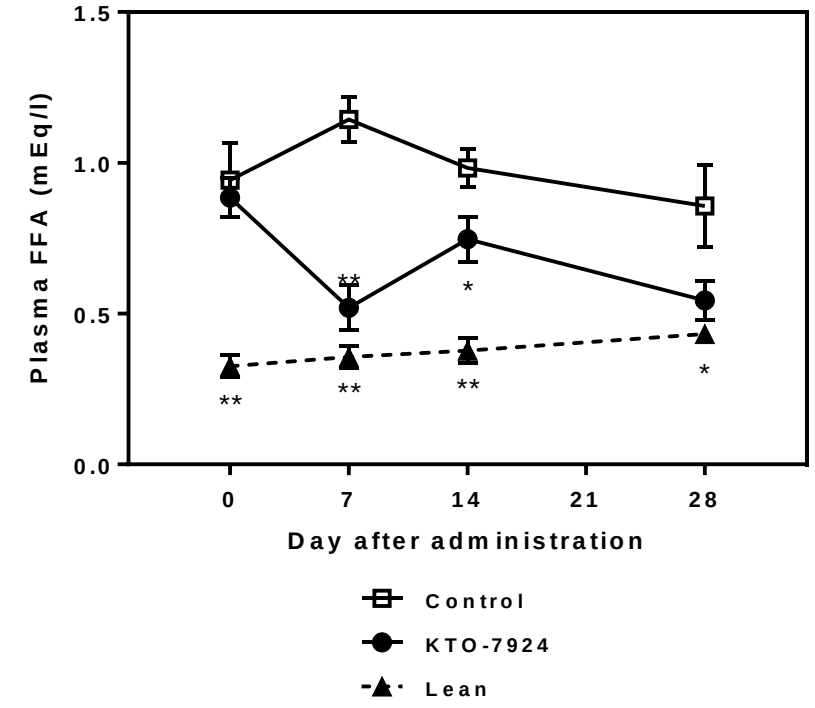

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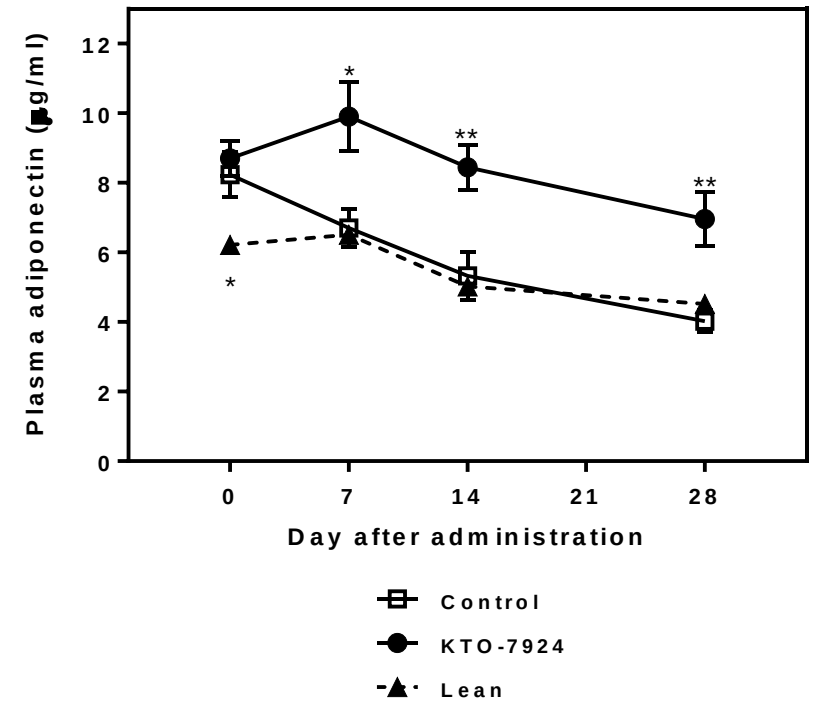

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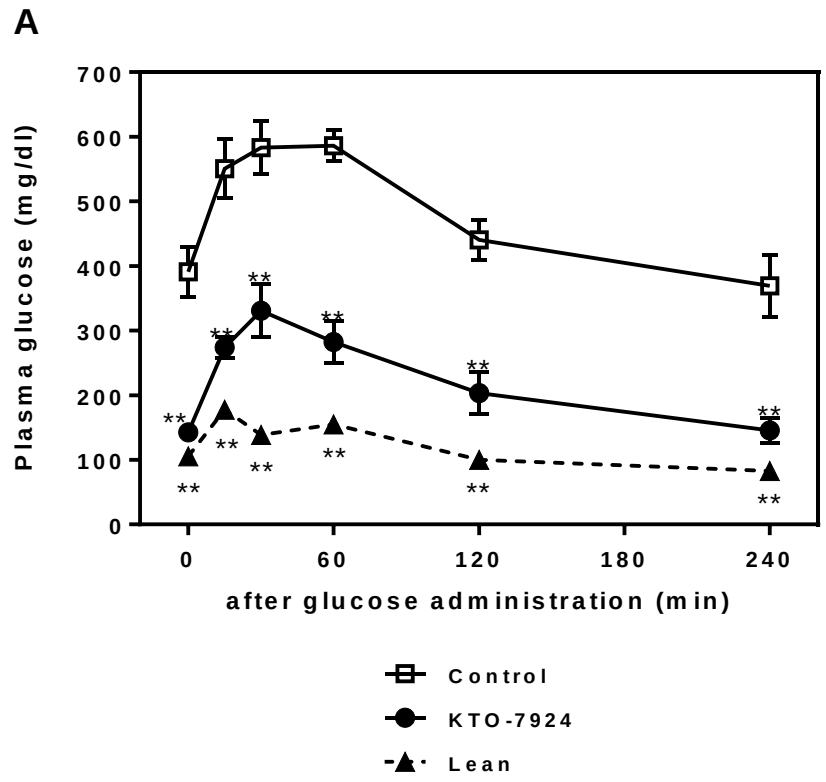
Fig. 3



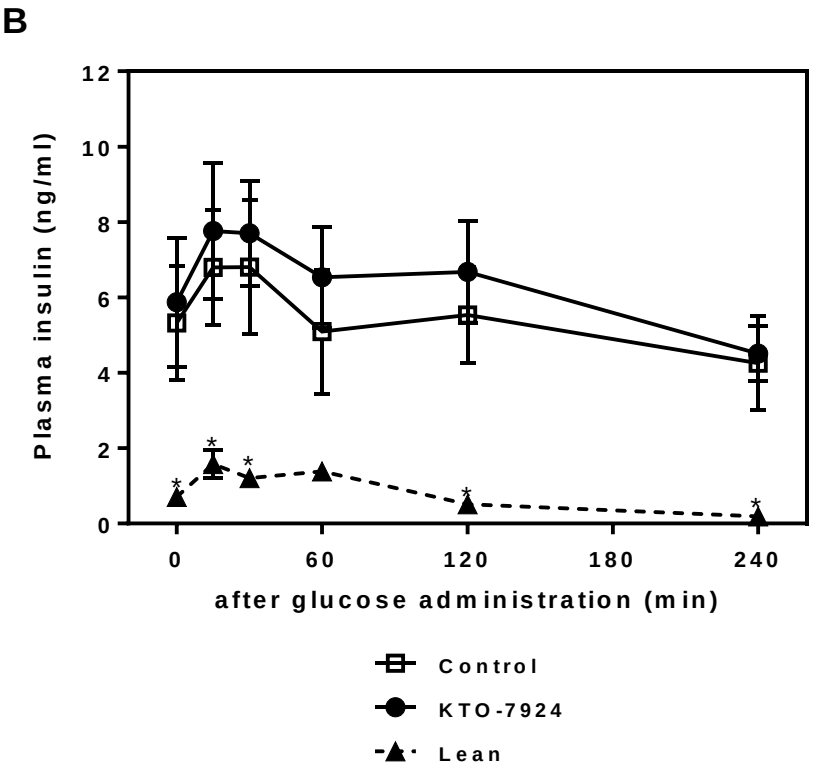


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Fig. 4

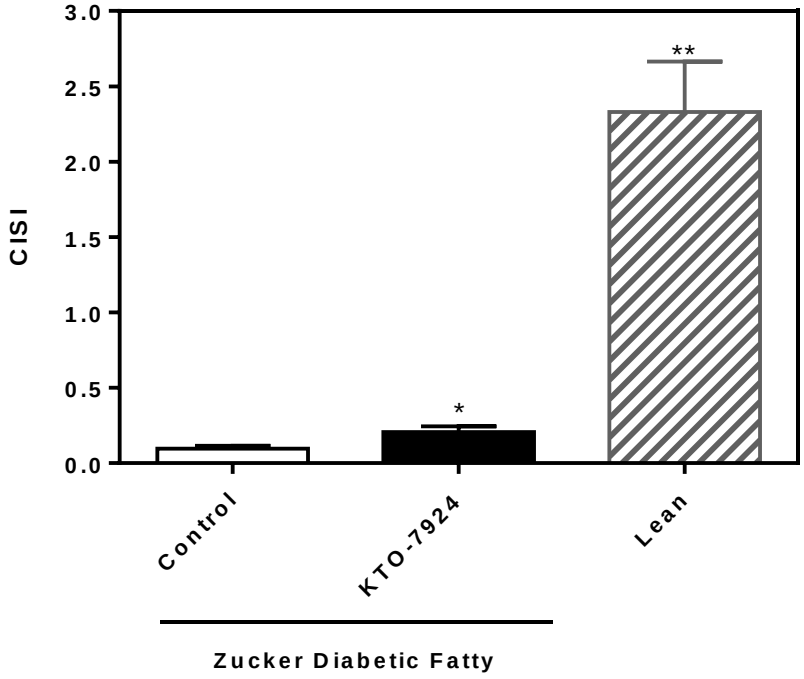

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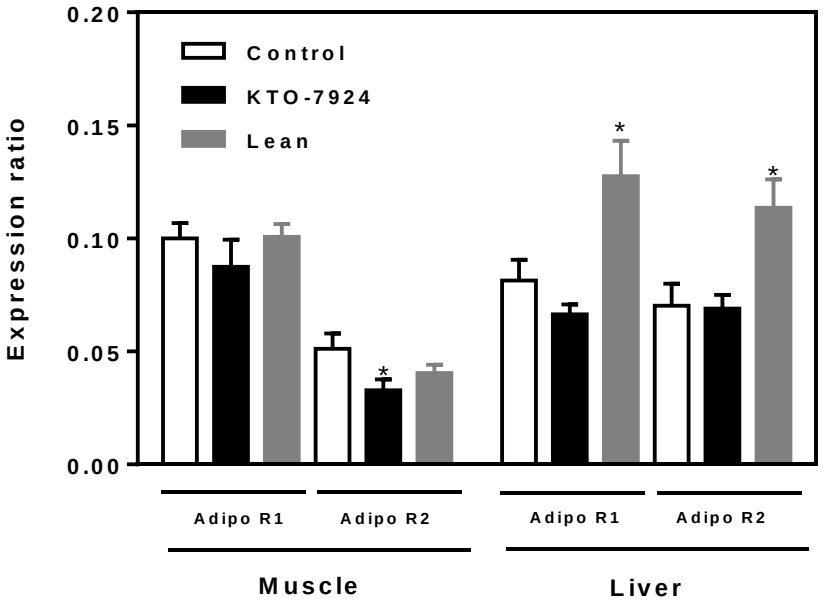
Fig. 5



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