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IN VIVO TOXICITY EVALUATION OF TROGLITAZONE, ROSIGLITAZONE AND PIOGLITAZONE IN CD-1 MICE

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ABSTRACT
The objective of this research work was comparative evaluation of in vivo toxicity of troglitazone, rosiglitazone and pioglitazone in CD-1 mice. Briefly, the animals were treated with the three drugs (troglitazone, rosiglitazone and pioglitazone) for 3 consecutive weeks at different dose levels. During the study, the animals were evaluated for mortality/morbidity, clinical signs of toxicity, body weight/body weight gain, food consumption and clinical pathology (hematology, clinical chemistry and urinalysis). At the end of treatment period, the animals were necropsied, gross pathology observations were recorded, and selected organs were weighed and subjected to histopathology evaluation. The results indicated that, the repeated administration of troglitazone, rosiglitazone and pioglitazone for 3 consecutive weeks to CD-1 mice resulted in microscopic histopathological changes in liver, bone marrow (femur and sternum) and thymus (only rosiglitazone and pioglitazone). There were no other toxicologically significant changes noted in any of the in-life phase parameters or terminal clinical pathology parameters. The changes noted in this study correlated well with the expression pattern of peroxisome proliferator-activated receptor gamma (PPAR-γ) receptors and literature findings.

KEY WORDS
PPAR-γ, Troglitazone, Rosiglitazone, Pioglitazone, Toxicity, Mice.

INTRODUCTION
Type 2 diabetes constitutes majority of the diabetic cases in the world. Over a period of time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. In 2014, 9% of adults (18 years and older) had diabetes. In 2012, diabetes was the direct cause of 1.5 million deaths. WHO projects that diabetes will be the 7th leading cause of death in 2030 [1]. India has the highest number of diabetic patients in the world becoming the diabetes capital of the world. Approximately, 41 million Indians are having diabetes and every fifth diabetic in the world is an Indian [2]. The treatment of Type 2 diabetes mainly involves the lifestyle changes (such as regular exercising, maintaining a balanced diet, avoiding smoking and drinking alcohol or soft drinks, and drinking plenty of water) and treatment with oral hypoglycemic agents (small chemical molecules) of different classes such as biguanides, sulphonylureas, thiozolidinediones, DPP IV inhibitors etc. along with Insulin. Among these different classes of drugs, thiazolidinediones (TZD) form an important class of drugs for targeting muscular insulin resistance. These insulin sensitizers are expected to improve blood glucose control in patients with type 2 diabetes mellitus [3]. These drugs directly target insulin resistance in the skeletal muscle, one of the principal underlying metabolic defects in type 2 diabetes mellitus. Drugs of this class act as ligands for the peroxisome proliferator-activated receptor gamma subtype (PPAR-γ), which is directly involved in the regulation of genes controlling glucose homeostasis and lipid metabolism [4,5]. There are three main agents of the thiazolidinediones family - troglitazone, rosiglitazone and pioglitazone. Troglitazone, the first agent of this class to be approved, was effective in controlling glycemia but was removed from the market because of serious liver toxicity. Both rosiglitazone and pioglitazone were considered to be relatively safe and were indicated either as monotherapy or in combination with a sulfonylurea, metformin, or insulin when diet, exercise, and a single agent do not result in adequate glycemic control. This class of drugs has mainly been associated with the hepatotoxicity [6] and

cardiovascular effects [7] as the major adverse toxicities.

The repeat dose toxicity studies form the most important part of the overall safety evaluation of the medicinal products to ensure the safety of human subjects enrolled in clinical studies as well as in the post marketing setting. There are multiple national and international regulatory guidelines which provide the requirements and guidance for the conduct of such repeat dose toxicity studies. Repeated dose toxicity study comprises the evaluation of adverse general toxicological effects occurring as a result of repeated daily dosing with a substance for a specified period. The studies yield information on general characteristics of the toxicity, the target organs of toxicity, the dose–response (curve) for each toxicity endpoint, responses to toxic metabolites formed in the organism, delayed responses, cumulative effects, the margin between toxic/non-toxic dose, information on reversibility/irreversibility of the effect, and NOAEL (No Observed Adverse Effect Level), NOEL (No Observed Effect Level) for toxicity.

The objective of this study was comparative *in vivo* toxicity evaluation of troglitazone, rosiglitazone and pioglitazone in CD-1 mice after repeated administration through oral (gavage) route for 3 consecutive weeks.

MATERIALS AND METHODS

Test substances and vehicle items

Test substances troglitazone, rosiglitazone and pioglitazone were procured from vendors as indicated: Troglitazone (Ramidus AB, Sweden), rosiglitazone (Tokyo chemical industry co. ltd, Japan), pioglitazone hydrochloride (Glenmark Pharmaceuticals ltd, Mumbai). The vehicle items Methyl cellulose and Tween 80 were procured from Merck Specialties Pvt Ltd.

Animals

CD-1 mice were obtained from the animal facility of Glenmark Research Centre. The study was conducted in accordance with an Institutional Animal Ethics Committee (IAEC) approved protocol and the experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Mice were approximately 7-9 weeks old at study initiation. Up to 5 animals of same sex were housed together in an individually ventilated polysulphone cages containing corn cob as bedding material in an environmentally monitored air-conditioned room maintained at a temperature of 22 $\pm$ 3 ^{0}C, relative humidity of 40 to 70 % and lighting cycle of 12 hrs light / 12 hrs dark. Commercial pellet diet and community tap-water passed through a reverse osmosis system were given *ad libitum.*

Experimental design

Five mice per sex per group were given daily oral (gavage) doses of troglitazone, rosiglitazone and pioglitazone for 3 consecutive weeks at the dose levels summarized in Table 1. A vehicle control group was given vehicle (0.5 % w/v Methyl Cellulose (99.75 %) + Tween 80 (0.25%)). Animals were observed for mortality, morbidity and clinical signs of toxicity twice daily. Individual body weights and food consumption were recorded twice weekly throughout the study. The clinical pathology (hematology, clinical chemistry and urinalysis) evaluations were performed at the end of treatment period as per the list of parameters summarized in Table 2. On the scheduled days of necropsy, all the animals were fasted, weighed, euthanized and then necropsied. The animals were euthanized by carbon dioxide asphyxiation followed by exsanguinations. The gross pathological changes were recorded for each animal. The organ weights and histopathology evaluation of selected the organs/tissues was carried as detailed in Table 2.

Statistical analysis

Statistical analysis was performed on body weights, body weight gain, hematology, clinical chemistry, organ weights and organ to body/brain weight percentages. Males and females were considered separately for analysis. All the comparisons were made between treatment groups and vehicle control group. The data was evaluated using one-way ANOVA with Dunnett's post test (multiple comparison test). A $p \leq 0.05$ was considered significant in all evaluations. The complete analysis was performed using GraphPad Prism statistical software version 5.02 for Windows, GraphPad Software, San Diego California USA.

Table 1. Summary of dose levels, dose volume and formulation concentrations

Drug	Dose level (mg/kg/day)	Dose volume (mL/kg)	Concentration (mg/mL)
Vehicle	0	10	0
Troglitazone	80	10	8
	800	10	80
Rosiglitazone	2	10	0.2
	20	10	2
Pioglitazone	10	10	1
	100	10	10

Table 2. Summary clinical pathology parameters evaluated in the study

Hematology	Clinical chemistry	Urinalysis	Organ weights	Histopathology*
Hemoglobin	Aspartate aminotransferase	Volume	Brain	Brain
Red blood cell counts	Alanine aminotransferase	Color	Liver	Liver
Hematocrit	Alkaline Phosphatase	Appearance	Heart	Heart
Mean corpuscular hemoglobin	Total protein	Microscopy of urine sediment	Kidneys	Kidneys
Mean corpuscular hemoglobin concentration	Glutamate Dehydrogenase	Glucose	Adrenals	Adrenals
Mean corpuscular volume	Urea	Bilirubin	Thymus	Thymus
Platelet count	Creatinine	Ketone	Spleen	Spleen
White blood cell counts				
Differential WBC count (Neutrophils, Lymphocytes, Basophils, Eosinophils, Monocytes	Bilirubin total	Specific gravity	Testes/ Ovaries	Testes/ Ovaries
		Blood	Epididymes	Epididymes
		pH		Brown adipose tissues
		Proteins		White adipose tissues
		Urobilinogen		Bone marrow - Femur
		Nitrite		Bone marrow - Sternum

Note; *Only for control and high dose groups for troglitazone, rosiglitazone and pioglitazone

Table 3. Summary of histopathological findings

Dose (mg/kg/day)	**Male**				**Female**			
	CON	**TRO**	**ROS**	**PIO**	**CON**	**TRO**	**ROS**	**PIO**
	0	800	20	100	0	800	20	100
Animals per group	5	5	5	5	5	5	5	5
Organs/tissues with lesions	**Incidence**							
Bone, femur with joint								
Tissues examined	5	5	5	5	5	5	5	5
No abnormality detected	5	5	1	0	5	2	0	0
Minimal to mild/moderate adipocyte infiltration	0	0	4	5	0	3	5	5
Bone, sternum								
Tissues examined	5	5	5	5	5	5	5	5
No abnormality detected	5	5	5	0	5	5	4	0
Minimal to moderate adipocyte infiltration	0	0	0	5	0	0	1	5
Liver								
Tissues examined	5	5	5	5	5	5	5	5
No abnormality detected	4	3	3	5	3	3	0	0
Minimal to mild hepatocellular necrosis	0	2	0	0	0	1	0	0
Minimal to mild inflammatory cell infiltration	0	1	0	0	0	0	0	0
Minimal to mild hepatocellular degeneration	0	0	0	0	0	0	4	5
Thymus								
Tissues examined	5	5	5	5	5	5	5	5
No abnormality detected	5	4	5	1	4	5	2	2
Mild lymphoid depletion	0	0	0	2	0	0	0	1
Mild apoptosis in cortex	0	0	0	1	0	0	2	2

CON: Vehicle control, TRO: Troglitazone, ROS: Rosiglitazone, PIO: Pioglitazone

RESULTS AND DISCUSSION

The treatment with troglitazone, rosiglitazone and pioglitazone in CD-1 mice for 3 consecutive weeks did not result in the treatment related toxicologically significant findings in any of the in-life phase parameters such as clinical signs, body weight, body weight gain and food consumption. The terminal clinical pathology evaluations such as hematology, clinical chemistry and urine analysis parameters did not show any toxicologically meaningful changes. There were no changes noted in absolute and relative organ weights and no gross pathological abnormalities were noticed in any of the animals at the time of necropsy examination. The only treatment related changes noticed in this study were restricted to the histopathology evaluation of bone marrow sternum, bone marrow femur, liver and thymus as described below in the Table 3. There were no treatment related toxicologically significant changes noticed in the remaining organs evaluated in this study.

Liver:

The treatment with troglitazone, rosiglitazone as well as pioglitazone resulted into the histopathological changes in liver (Figure 1). The changes in the troglitazone treated animals were minimal to mild hepatocellular necrosis (in both genders) and inflammatory cell infiltration (only in males) whereas the animals treated with rosiglitazone and pioglitazone showed minimal to mild hepatocellular degeneration. There were no other correlative changes noted in the clinical pathology parameters.

The information in literature suggests that, the TZDs have been associated with hepatic adverse effects in some treated patients although the mechanism of hepatotoxicity still remains equivocal. Although PPAR*γ* is expressed at a much lower level in liver than in adipose tissue (hepatic PPAR*γ* represents only 10–30% of the level in adipose tissue), PPAR*γ* agonists

exert various PPARγ-dependent effects in liver in addition to PPARγ-independent effects [8]. TZDs of the first generation were found to be highly hepatotoxic e.g. the first TZD ciglitazone was abandoned after clinical trials and the second, troglitazone, was rapidly withdrawn from the market after reports of severe liver failure and death. The second generation of PPARγ agonists, rosiglitazone and pioglitazone, were approved by the United States Food and Drug Administration (USFDA) in 1999. Hepatic failures have also been observed after administration of these two TZDs but they were less frequent and severe [9].

There are multiple *in vitro* hepatotoxicity studies which have been reported for these drugs. Most of the studies indicated that troglitazone has higher potential to cause hepatotoxicity compared to rosiglitazone and pioglitazone. Troglitazone has been reported to cause *in vitro* hepatotoxicity in HepG2 cells at the concentrations of 25 µM or more [10] while maximum plasma concentrations reached 3 to 6 µM in humans, making the extrapolation of *in vitro* data to the *in vivo* situation questionable. Further, the daily dose necessary for troglitazone therapeutic efficacy was 600 mg/day while it was only 8 mg/day for rosiglitazone and 45 mg/day for pioglitazone indicating that patients were exposed to quite different doses between the first and second generations of TZDs [11]. This difference could also be due to the variation between their relative potencies PPARγ activation.

Bone marrow (femur and sternum):

The microscopic changes in bone marrow femur (Figure 2) were restricted to the minimal to moderate adipocyte infiltration in troglitazone (only females), rosiglitazone and pioglitazone treated animals. The similar changes were noticed in bone marrow sternum (Figure 3) in all pioglitazone treated animals and one rosiglitazone treated female. There were no correlative changes noted in the hematological parameters.

This finding correlates well with the reports in the literature indicating PPARγ activation by TZDs leading to adipocytic transformation and induced adipogenesis of mesenchymal cells in bone marrow [12,13]. It has also been shown that pharmacological inhibition of PPARγ reduces bone marrow adiposity [14].

Thymus:

The microscopic evaluation of thymus (Figure 4) indicated mild lymphoid depletion and mild apoptosis in cortex in pioglitazone treated animals. The mild apoptosis in cortex was also noted in rosiglitazone treated females. The changes in thymus did not correlate with any changes in hematological parameters or any microscopic changes in spleen. It is possible that the longer term administration of thiazolidinones at higher doses may lead to severe effects in the thymus which can lead to significant hematological changes. There are reports in literature indicating expression of PPARγ in thymus, the effect of activation of PPARγ on naive T cell production and acceleration of age-related thymic involution [15].

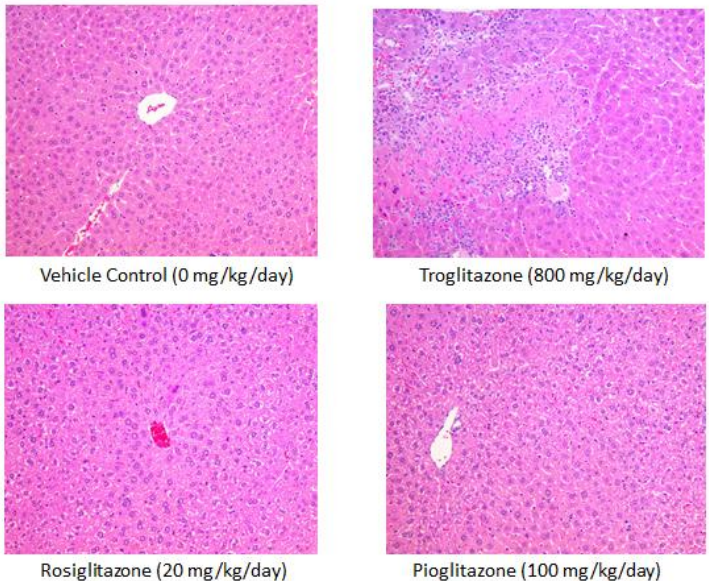

Figure 1. Representative images (20x) indicating the microscopic changes in liver

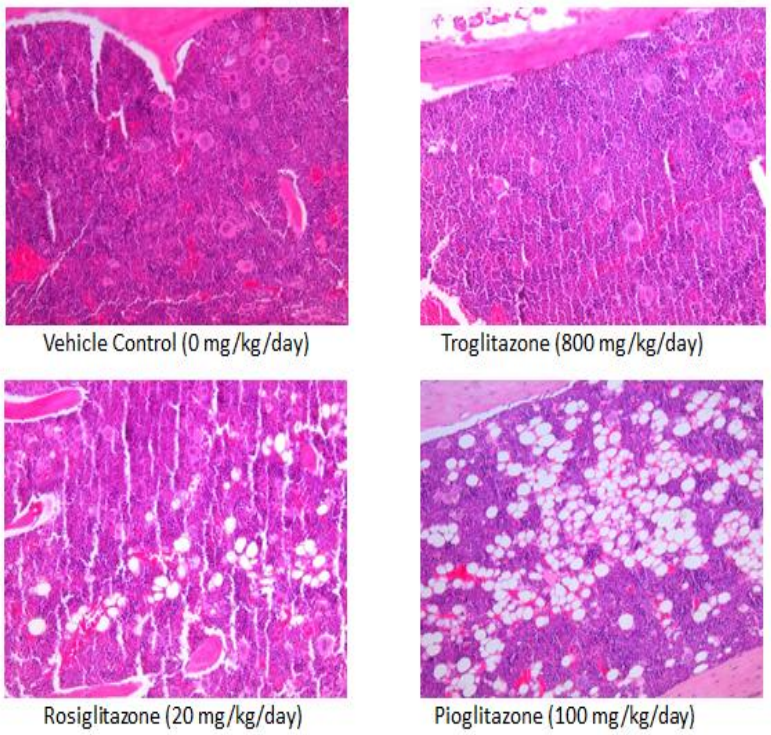

Figure 2. Representative images (20x) indicating the microscopic changes in bone marrow sternum

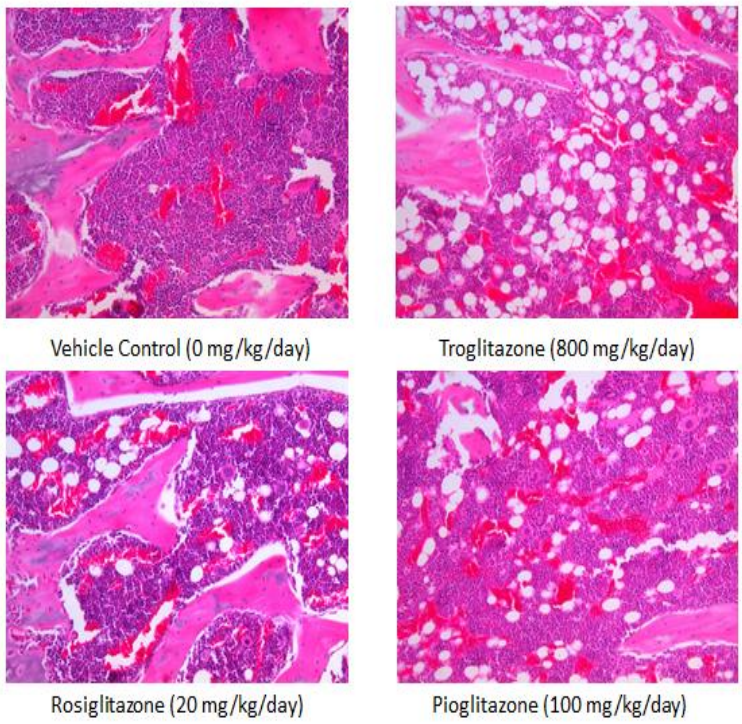

Figure 3. Representative images (20x) indicating the microscopic changes in bone marrow femur

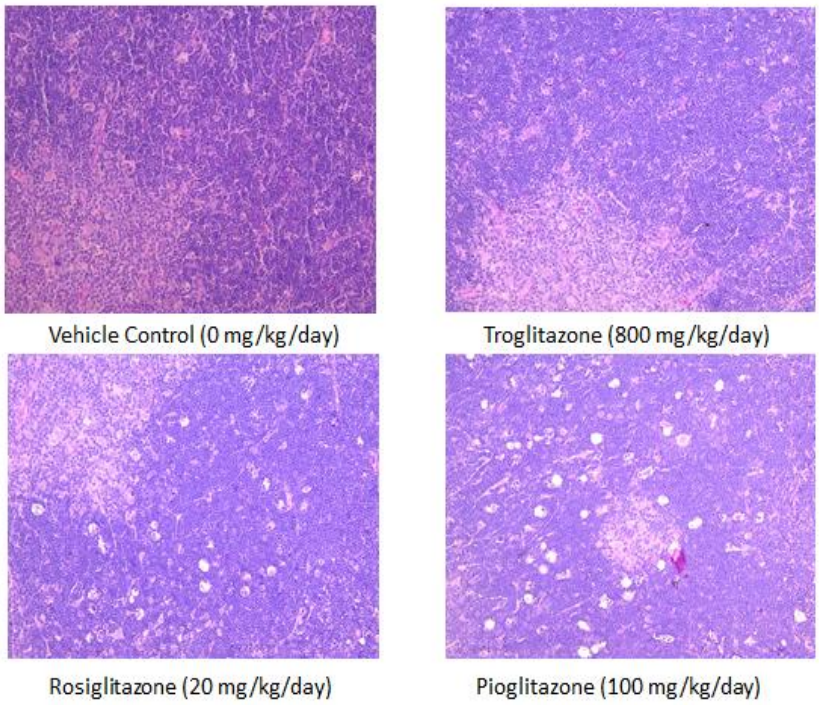

Figure 4. Representative images (20x) indicating the microscopic changes in thymus

CONCLUSION

The repeated administration of troglitazone, rosiglitazone and pioglitazone for 3 consecutive weeks to CD-1 mice resulted in microscopic histopathological changes in liver, thymus and bone marrow (femur as well as sternum). There were no other toxicologically significant changes noted in any of the in-life phase parameters or terminal clinical pathology parameters. The changes noted in this study correlated well with the expression pattern of peroxisome proliferator-activated receptor gamma (PPAR-γ) receptors and literature findings.

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REFERENCES

1. WHO, 2016. Website: http://www.who.int/mediacentre/factsheets/fs312/en accessed on February 24, 2016.
2. Joshi SR, Parikh RM, India-Diabetes capital of the world: Now heading towards hypertension. Journal of association of physicians of India, 55:323-324 (2007)
3. Scheen AJ, Lefèbvre PJ, Oral antidiabetic agents: a guide to selection. Drugs, 55(2):225-236 (1998)
4. Saltiel AR, Olefsky JM, Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes, 45(12):1661-1669 (1996)
5. Spiegelman BM, PPAR-g adipogenic regulator and thiazolidinedione receptor. Diabetes, 47: 507-514 (1998)
6. Scheen AJ, Hepatotoxicity with Thiazolidinediones. Is it a Class Effect? Drugs, 24(12):873-888 (2001)
7. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, Le Winter M, Porte D, Semenkovich CF, Smith S, Young LH, Kahn R, Thiazolidinedione Use, Fluid Retention, and Congestive Heart Failure: A Consensus Statement from the American Heart Association and American Diabetes Association. Circulation, 108:2941-2948 (2003)
8. Fajas L, Auboeuf D, Raspé E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J, The organization, promoter analysis, and expression of the human PPARγ gene. Journal of Biological Chemistry, 272(30):18779–18789 (1997).
9. Guo L, Zhang L, Sun Y, Muskhelishvili L, Blann E, Dial S, Shi L, Schroth G, Dragan YP, Differences in hepatotoxicity and gene expression profiles by anti-diabetic PPAR γ agonists on rat primary hepatocytes and human HepG2 cells. Molecular Diversity, 10 (3):349–360 (2006)
10. Sancheti PM, Pawar SP, In Vitro Cytotoxicity Evaluation of Troglitazone, Rosiglitazone and Pioglitazone in HepG2 Cells. Am J Pharm Health Res, 4(1):61-73 (2016).

11. Rogue A, Spire C, Brun M, Claude N, Guillouzo A, Gene Expression Changes Induced by PPAR Gamma Agonists in Animal and Human Liver. PPAR Research, 2010 (2010)
12. McDonough AK, Rosenthal RS, Cao X, Saag KG, The effect of thiazolidinediones on BMD and Osteoporosis. Nat Clin Pract Endocrinol Metab, 4:507–513 (2008)
13. Gimble JM, Robinson CE, Wu X, Kelly KA, Rodriguez BR, Kliewer SA, Lehmann JM, Morris DC, Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. Mol Pharmacol, 50(5):1087-1094 (1996)
14. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ, Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature, 460:259–263 (2009)
15. Youm YH, Yang H, Amin R, Smith SR, Leff T, Dixit VD, Thiazolidinedione treatment and constitutive-PPARγ activation induces ectopic adipogenesis and promotes age-related thymic involution. Aging Cell, 9(4): 478–489 (2010)

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