PPARδ Agonist Treatment Increases Skeletal Muscle Lipid Metabolism Without Altering Mitochondrial Coupling: An *In Vivo* Magnetic Resonance Spectroscopy Study

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Introduction

Peroxisome Proliferator Activated Receptor-δ (PPARδ) belongs to the family of PPARs (α, γ, and δ) and is expressed in a number of tissues including the heart and skeletal muscle. PPARδ activation leads to an up-regulation of energy expenditure by regulating genes involved in skeletal muscle fatty acid oxidation (1) and mitochondrial uncoupling (2). Recently it was reported that PPARδ transgenic mice have increased exercise tolerance and increased thermogenesis as a result of increased recruitment of type 1 muscle fibers and lipid metabolism (3). However direct, non-invasive assessment of lipid metabolism and mitochondrial coupling in skeletal muscle following PPARδ stimulation has not been examined. Therefore in this study, we examined the temporal and dose dependent response of selective PPAR-δ treatment on intramyocellular lipid content and mitochondrial energy coupling efficiency in rats by *in vivo* magnetic resonance spectroscopy.

Methods

Sprague Dawley rats were treated with a selective PPARδ agonist, GW610742X (5 or 100 mg/kg uid for 7 days, n=24) via oral gavage. All *in vivo* MR spectroscopy/imaging experiments were performed using a triple tune (^{1}H, ^{31}P, ^{13}C) concentric surface coil setup on a 4.7T/40 cm Bruker Biospec system. IMCL (intramyocellular), EMCL (extramyocellular), and total creatine (tCr) measurements were performed on the soleus muscle using ^{1}H PRESS spectroscopy (TE=22 ms, TR=1000 ms, 3x3x3 mm voxel) on days 0, 3, and 7. On day 8 following the *in vivo* MR experiments, a 1-^{13}C glucose clamp (120 min) was initiated in order to assess relative glucose vs fat oxidation in the soleus muscle. A POCE ^{1}H NMR measurement of metabolite ^{13}C enrichment in muscle extracts was performed at 9.4T. Gene expression analysis of key metabolic genes was performed on the soleus muscle.

In separate group of animals, skeletal muscle mitochondrial coupling was assessed after 7 days of dosing (4). Briefly, *in vivo* ^{31}P MRS was used to measure hindlimb muscle unidirectional ATP synthesis by selectively saturating the γ-ATP resonance (TR=4.3 s, NS=208, SW=5 kHz, 2 k data). ^{13}C MRS was used to measure the hindlimb TCA cycle flux by examining glutamate turnover during 2-^{13}C acetate precursor administration (TR=0.5s, NS= 1455, SW=10 kHz, 4 k data) (Fig. 1). Mitochondrial coupling efficiency was measured as the ratio of unidirectional ATP synthesis to TCA cycle flux.

Results

There were no differences in the soleus IMCL/tCr ratio throughout the 7 day treatment period in the vehicle group. However, while there was a slight, non-significant decrease in the soleus IMCL/tCr ratio by day 7 in the low GW610742X dose group, there was a significant temporal decrease in the IMCL/tCr ratio in the soleus muscle by day 7 (↓49%, $P<0.05$) in the high dose group (Fig. 2). The relative fat oxidation rate in soleus muscle increased in a dose dependent manner (↑52% and 93% in the 5 and 100 mg/kg/day groups respectively, $P<0.05$). However there was no alteration, at either dose, in hindlimb mitochondrial coupling efficiency measured as the ratio of unidirectional ATP synthesis to TCA cycle flux

Expression of both glucose and lipid metabolism/thermogenesis genes were examined in soleus muscle. GLUT4 expression was decreased by 2 fold while CPT1, UCP2, and UCP3 were increased by 2.8, 2.1, and 2.2 fold respectively at the high dose.

Fig 1

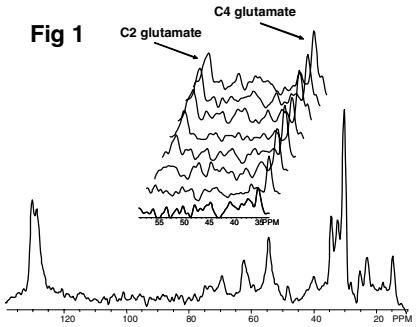
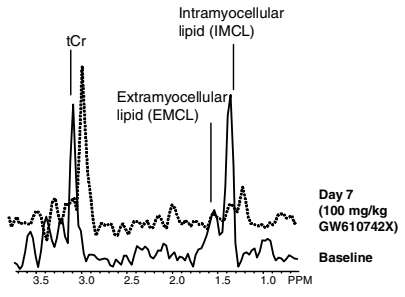


Fig 2



Summary

In summary, these are the first non-invasive measurements illustrating a PPARδ mediated decrease in muscle lipid content which was consistent with a shift in metabolic substrate consumption from glucose to lipid. However, the mitochondrial coupling efficiency was not altered in the presence of increased uncoupling protein expression.

References

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