

New pharmacological approaches to modulate skeletal muscle metaflammation in mice models of diabetes

F. Chiazza¹, E. Benetti¹, R. Mastrocola², D. Nigro², M. Rogazzo¹, J.C. Cutrin^{3,4}, G. D'Antona⁵, M. Aragno², M.A. Minetto⁶, C. Thiemermann⁷, R. Fantozzi¹, M. Collino¹

¹Dept. of Drug Science and Technology, University of Turin, Turin, Italy

²Dept. of Clinical and Biological Sciences, University of Turin, Turin, Italy

³Dept. of Biotechnology and Sciences for the Health, University of Turin, Italy

⁴ININCA-CONICET, Buenos Aires, Argentina

⁵Dept. of Molecular Medicine University of Pavia, Italy

⁶Division of Endocrinology, Diabetology and Metabolism, Dept. of Medical Sciences, University of Turin, Italy

⁷Queen Mary University of London, Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, London, UK

The main aims of my PhD project were (I) the development of *in vivo* models of obesity associated with type-2 diabetes (diabetes) and (II) the testing of innovative pharmacological approaches dealing with the molecular modulation of the chronic inflammation involved in the development of metabolic disorders (metaflammation) [1]. We focused on skeletal muscles, which account for almost 80% of the insulin-stimulated glucose uptake, thus, exerting a key role in regulating whole body glucose homeostasis.

As drug targets, we investigated Peroxisome Proliferator Activated Receptor (PPAR)-delta, and the heterocomplex between the Erythropoietin Receptor (EpoR) and the β -common Receptor (β cR), which mediates the tissue-protective effects of Erythropoietin (EPO).

Male mice were chronically fed a diet enriched in fats (Protocol 1, 45% High Fat diet, HF), or in sugars (Protocol 2, 15% High-Fructose Corn Syrup, HFCS), or in both sugars and fats (Protocol 3, 40% High Fat and 30% High Sucrose diet, HFHS).

A subgroup of HFCS mice (Protocol 2) was treated with a highly potent and selective PPAR-delta agonist, GW0742 (1 mg/kg/day for 16 weeks). A subgroup of HFHS mice (Protocol 3) received an EPO derivative devoid of haematopoietic effects, which selectively binds the EpoR- β cR heterocomplex, the pyroglutamate Helix B Surface Peptide (pHBSP, 30 μ g/kg s.c. for 11 weeks). Both the drugs have been previously tested in our lab at the doses here reported in models of cardiovascular diseases [2, 3].

Our results show that the three different dietary manipulations caused a significant increase in body weight, dyslipidemia and hyperinsulinemia and evoked insulin resistance due to impaired insulin signaling within the skeletal muscle. The effects of dietary manipulation were comparatively evaluated in two different muscles characterized by high dependence on either oxidative or glycolytic metabolism, the soleus and the tibialis, respectively. Results showed that the soleus stores more triglycerides (TG) than the tibialis in control animals, while the TG content of the two muscles was comparable in mice exposed to the HF or HFHS diets. Moreover, the chronic exposure to high caloric diets evoked a massive skeletal muscle accumulation of advanced glycation end-products (AGEs) that was preferential for the lipid-accumulating cells and increased expression of the lipogenic pathway SCAP/SREBP.

When drugs effects were evaluated, either GW0742 or pHBSP attenuated the systemic metabolic anomalies caused by diet. To elucidate the potential mechanisms underlying their beneficial effects, we proved that GW0742 induced PPAR-delta upregulation in skeletal muscle and this effect was accompanied by marked inhibition of nuclear factor-kB activation and muscular expression of inducible-nitric-oxide-synthase, intercellular-adhesion-molecule-1 and the myokine Fibroblast Growth Factor-21 (FGF-21). Similarly, diet-induced overproduction of IL-6 and FGF-21 were attenuated by pHBSP and, most importantly, pHBSP markedly enhanced mitochondrial biogenesis. Besides, both GW0742 and pHBSP stimulate glucose transport in skeletal muscle by increasing the translocation of the sugar transporter GLUT-4 to the plasma membrane.

Overall, the models of dietary manipulation here described can be proposed as suitable experimental tools for studying 'diabetes' and its potential pharmacological modulation. Our results demonstrated that activation of either PPAR-delta or EpoR/ β cR protects the skeletal muscle against the diet-induced metabolic abnormalities by affecting multiple levels of the insulin and inflammatory cascades. These local protective effects may support their potential role as innovative pharmacological tools in metabolic disorders.

[1] Osborn, Olivia *et al.*, Nat Med. 2012;18(3):363-74

[2] Collino M, Benetti E, *et al.*, Free Radic Biol Med. 2011;50(2):345-53.

[3] Patel NS, Collino M, *et al.*, Mol Med. 2012;18:719-27.