Muscle membrane permeability, damage and atrophy in Zucker obese rats

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Type 2 Diabetes (T2D) has a heterogeneous pathophysiology. Development of the disease is often related to the effects of an increase in fatty acid (FA) content. These include: 1) increased production of reactive oxygen species (ROS), and reduced antioxidant defences available (Johansen *et al.* 2005); 2) increase in inflammatory mediators leading to insulin resistance (Dandona, Aljada & Bandyopadhyay (2004) and apoptosis/necrosis (Shoelson, Herrero & Naaz, 2004); 3) reduced membrane fluidity which inhibits mitochondrial function (Lamson & Plaza, 2002); and 4) altered sarcoplasmic reticulum function (Eibschutz *et al.*, 1984; Ganguly *et al.*, 1986). These defects can be damaging to muscle thereby reducing muscle mass, function and morphology. The amount of muscle damage is examined with the use of a fluorescent dye known as Evans Blue Dye (EBD), which permeates any damaged or leaky tissue, thus exhibiting higher fluorescence. As such, the aim of this study was to determine if there is more skeletal and cardiac muscle damage and atrophy in T2D muscles at rest compared to normal muscles, and whether this damage is associated with increased lipase and protease activity.

Twenty eight male Zucker rats (a widely-used model of T2D) at 14 weeks of age, comprising of 14 Zucker Obese rats (OBESE) and 14 Zucker Lean (LEAN) rats, were injected with 1% EBD 24 hours prior to sampling to allow absorption into muscle tissue. On the following day, animals were anaesthetised (Nembutal, 60 $mg.kg^{-1}$) and a portion of EDL (fast-twitch), *soleus* (slow-twitch), and cardiac muscles were removed, covered in O.C.T. compound and frozen in isopentane cooled with liquid nitrogen, for later histological analysis of muscle damage and atrophy. The remainder of the muscles were immediately snap frozen in liquid nitrogen and stored at −80˚C for later isoprostane (marker lipase-induced membrane breakdown) and calpain (marker of proteolysis) analyses.

Overall, OBESE rats had a significantly higher amount of muscle exhibiting EBD fluorescence than LEAN littermates in EDL and *soleus* ($p<0.01$), and cardiac ($p<0.05$) muscles. In addition, the fluorescent intensity was also shown to be significantly higher in EDL and cardiac muscle of OBESE rats ($p<0.05$), with a trend in soleus muscle of OBESE rats ($p=0.06$). Absolute and relative (per body weight) muscle masses were significantly lower in EDL and soleus ($p<0.05$) of OBESE animals, concomitant with lower fibre area ($p<0.05$). Cardiac muscle mass was significantly higher in OBESE rats ($p<0.05$), but when taken as a percentage of total body weight, it was significantly lower than the LEAN group ($p<0.01$). Despite the lower mass and higher muscle damage and/or membrane leakiness as shown by EBD fluorescence, no differences were observed in markers of necrosis as indicated by the presence of non-membrane bound nuclei, isoprostane production, or in μ-calpain, and calpain-3. Interestingly, total arachidonic acid content (a key component of plasma membranes) was found to be significantly lower in the EDL and soleus muscles of the OBESE animals ($p<0.05$), but not in cardiac muscle.

In conclusion, this study showed that resting muscles from T2D rats exhibit higher skeletal and cardiac muscle damage and atrophy, which cannot be attributed to isoprostane production, inflammation or calpain activation.

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