Alterations of Skeletal Muscular Bioenergetics in a Model of Insulin Resistance

P-W. So[1], G. Hamilton[2], J. D. Bell[1]

[1]Molecular Imaging Group, Imaging Sciences department, MRC Clinical Sciences Centre, Hammersmith Hospital, Imperial College London, London, United Kingdom, [2]MRI Unit, Imaging Sciences Department, MRC Clinical Sciences Centre, Hammersmith Hospital, Imperial College London, London, United Kingdom

INTRODUCTION

The obese obob$^{(-/-)}$ mouse is commonly used as a model of insulin resistance. Insulin resistance may be triggered by the abnormal accumulation of fatty acids and their breakdown products in non-adipose tissue, especially muscle. Fatty acids may accrue in muscle as a result of mitochondrial dysfunction, mitochondria being the site of cellular fatty acid metabolism [1]. Adenosine triphosphate (ATP) and phosphocreatine (PCr) levels are maintained by mitochondria and these levels may provide an indicator of mitochondrial function. In this study, intracellular lipid content in skeletal muscle was assessed by ^{1}H MRS in both obob$^{(-/-)}$ mice and their lean counterparts, C57BL/6. Muscle levels of PCr and ATP were also measured by ^{31}P MRS. The effect of supplementation with omega-3-fatty acids (ω3-fa), abundant in fish oil, was also studied to determine if the presence of such lipids in the diet might influence muscular bioenergetics.

METHODS

Animals and Treatment: Eight control (C57BL/6, 4 weeks old) mice were subdivided into equal groups of four and placed on a fish-oil enriched diet (12% fat containing ω3-fa) or a normal chow diet (3-4% fat, <1% ω3-fa) for a period of 20 weeks. Eight obese (obob$^{(-/-)}$, 4 weeks old) mice were also subdivided into equal groups of four and placed on either the fish oil or the chow diet for the same period of time.

MR Scanning: At 20 weeks on the respective diets, ^{31}P-^{1}H MR spectra were collected from the hind leg of mice on a 9.4T Inova MR scanner (Varian Inc., USA). Anaesthesia was induced and maintained by inhalation of 1-2% isoflurane/oxygen mix. A ^{1}H^{31}P double tuned surface coil was placed on the muscle of the hind limb of the mouse and spectra obtained using TR=1s, 90^{o} and 1024 averages, and data analysed using the AMARES algorithm [2] included in MRUI [3]: metabolite levels were expressed as a percentage of total phosphorous. Localised ^{1}H MR spectra was also collected from a 2x2x2mm voxel placed in the hind limb but on a 4.7T Inova MR scanner (Varian Inc., USA) using a PRESS sequence (SW=4000Hz, TE=14ms, TR=10s, 64 transients). The ratio of the water to lipid peak in the ^{1}H MR spectrum was calculated to estimate intramyocellular lipid content.

All values are quoted as means±SEM. Statistical significance was tested using ANOVA and Bonferroni correction.

RESULTS

Table: Skeletal muscle levels of intramyocellular lipid, ATP and PCr in the obob$^{(-/-)}$ and C57BL/6 mice on either the normal rodent chow or the 12% supplemented fish oil diet.

*, ** and *** are significant at p<0.05, 0.01 and 0.001, respectively, for comparing within diet but different strain and # is significant at p<0.05 when comparing between the same strain but different diet.

	Diet	Intramycellular lipid	ATP	PCr
C57BL/6	fish oil	71.75±19.47***	14.44±0.31	42.76±1.08**
obob$^{(-/-)}$	fish oil	666.8±132.9$^{\#}$	13.02±0.89	31.88±2.74
C57BL/6	chow	38.75±2.87	14.78±0.18*	40.23±1.10***
obob$^{(-/-)}$	chow	300.5±44.8	12.30±0.43	26.54±1.80

Intramyocellular lipid levels were significantly higher in the obob$^{(-/-)}$ mice compared to their lean counterparts (Table). Obese mice fed the ω3-fa enriched diet showed significantly higher amounts of intramyocellular lipid than those on the lower fat, normal chow diet. Thus, the obob$^{(-/-)}$ mice readily accumulated lipids in skeletal muscle compared to its lean counterpart and supplementation of increased amounts of dietary fat resulted in increased intramyocellular lipid content.

ATP levels were significantly higher in the C57BL/6 mice compared to the obob$^{(-/-)}$ mice on the chow diet (p<0.05). ATP levels were similar in the C57BL/6 mice regardless of diet (Table) whereas obob$^{(-/-)}$ mice on the fish oil diet showed generally higher ATP levels than those on the normal chow diet although significance was not reached (Table 1). Significantly higher levels of PCr was observed in the C57BL/6 mice compared to the obob$^{(-/-)}$ mice (Table 1). Further, PCr levels were generally lower in the obese mice on the chow than on the fish oil diet.

PCr is synthesised from ATP in the intermembrane space of mitochondria catalyzed by creatine kinase, and considered to act as a transport molecule between sites of ATP production and consumption in muscle. The equilibrium PCr concentration is therefore dependent on the rate of mitochondrial ATP production and thus, our data suggests that ATP production may be reduced in the obese mice compared to their lean counterparts and/or ATP consumption is increased. An increased ATP consumption in the obob$^{(-/-)}$ mice may reflect augmented proton leak in the muscle mitochondria similar to that observed in isolated liver mitochondria from obob$^{(-/-)}$ mice [4]. Furthermore, muscular levels of PCr and ATP were generally higher, although not significantly, in the fish oil fed mice, suggesting possible modulation of mitochondrial function by ω-3fa.

CONCLUSION

Significant differences in muscular bioenergetics were observed between the obob$^{(-/-)}$ and their lean counterparts by ^{31}P MRS, and these effected appear to be modulated by ω-3fa.

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