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Evidence of altered mitochondrial function in glycolytic, but not oxidative skeletal muscle in mice after one month of high fat, high sucrose feeding

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Introduction

Rates of obesity and the metabolic syndrome, which are strongly associated with type 2 diabetes (T2D) and cardiovascular disease, continue to rise worldwide. Skeletal muscle is an important tissue in the development of these disorders, as it serves as the major "glucose sink" in the body, and skeletal muscle mitochondrial dysfunction occurs in both metabolic syndrome and T2D [1]. Understanding of the early processes involved in skeletal muscle dysfunction, including changes in mitochondrial function, is currently lacking. It has been proposed that greater mitochondrial oxidation capacity, as is the case in oxidative muscles such as the soleus, can allay the onset of mitochondrial dysfunction induced by high fat, high sugar (HFHS) feeding. Previous studies in fructose fed rats appear to support this view [2]. If this is the case, soleus muscle may be less likely to develop mitochondrial dysfunction than a more glycolytic muscle, such as the gastrocnemius, when exposed to short term HFHS feeding. Here we investigated effect of HFHS feeding over one month on skeletal muscle oxidative capacity, comparing respiration in soleus and gastrocnemius fibres.

Methods

Sixteen male C57Bl/6J mice were fed either a normal chow (n=8), or a high fat, high sucrose diet (n=8, dietary composition: 42% fat, 42.7% carbohydrates, 15.2% protein) for four weeks. High resolution respirometry was carried out on saponin-permeabilised soleus (weighing 1-3 mg) and gastrocnemius muscle fibre bundles (weighing 3-10 mg) using Oxygraph-2k respirometers. A previously published [3] SUIT protocol shown in Figure 1 was used to probe mitochondrial metabolism. Fatty acid oxidation supported OXPHOS coupling efficiency (OCE_{FAO}) was defined as the difference between octanoyl carnitine supported OXPHOS ($OctM_P$) and LEAK respiration ($OctM_L$) divided by $OctM_P$. Flux control ratios (ratios of oxygen flux supported by a given substrate relative to the maximal oxygen flux rate in the same coupling state) were calculated for octanoyl carnitine supported OXPHOS (FCR_{FA}), N-pathway substrate supported OXPHOS (FCR_N) and S-pathway substrate supported electron transfer system (ETS) capacity (FCR_S). Additionally, the ratio of octanoyl carnitine supported OXPHOS to pyruvate supported OXPHOS ($FCR_{FA/P}$) was determined to provide a measure of the relative capacity for fatty acid or pyruvate oxidation by mitochondria. Finally, the P/E ratio (maximal OXPHOS respiration (GMS_P) divided by ETS capacity (GMS_E)) was calculated as a measure of the limitation of OXPHOS capacity by the ETS.

Results

High-resolution respirometry revealed that soleus fibres had higher oxidative capacity than gastrocnemius fibres in both chow (96% higher) and HFHS fed mice (134% higher) (p=0.001). There was no evidence of mitochondrial dysfunction in the soleus, with all wet mass adjusted rates measured, and all flux control ratios unchanged with HFHS feeding. In gastrocnemius, mass adjusted rates were also not changed, but analysis of flux control ratios revealed a reduction in the proportion of oxidation phosphorylation (OXPHOS) that could be supported by fatty acid oxidation (35% lower, p=0.006) relative to maximal OXPHOS respiration in the gastrocnemius of HFHS fed mice compared with chow fed controls (see Figure 2). The fatty acid oxidation supported OXPHOS coupling efficiency showed a trend towards being lower in the gastrocnemius of HFHS fed mice compared with chow fed mice (by 45%, p=0.075). Additionally, there were trends towards reduced Complex I supported OXPHOS respiration relative to maximal OXPHOS (p=0.064). The P/E ratio (GMS_P, the maximal OXPHOS rate, divided by GMS_E, the ETS capacity), a measure of how limited OXPHOS respiration is by the phosphorylation machinery, showed a trend (p=0.095) towards being increased in gastrocnemius fibres of HFHS fed mice compared with controls, which could suggest OXPHOS respiration was less limited by the phosphorylation machinery in HFHS fed mouse gastrocnemius than in controls.

Conclusion

This study provides support for the hypothesis that a greater oxidative capacity is protective against short-term diet induced mitochondrial dysfunction, as soleus fibres did not show any evidence of dysfunction, while gastrocnemius fibres showed defects in respiratory processes supported by fatty acid oxidation in HFHS fed animals, as well as potential evidence of dysfunction of Complex I supported respiration. Further work is needed to investigate the mechanism(s) leading to mitochondrial dysfunction, and the effects this has on other cellular processes.

References

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Figures

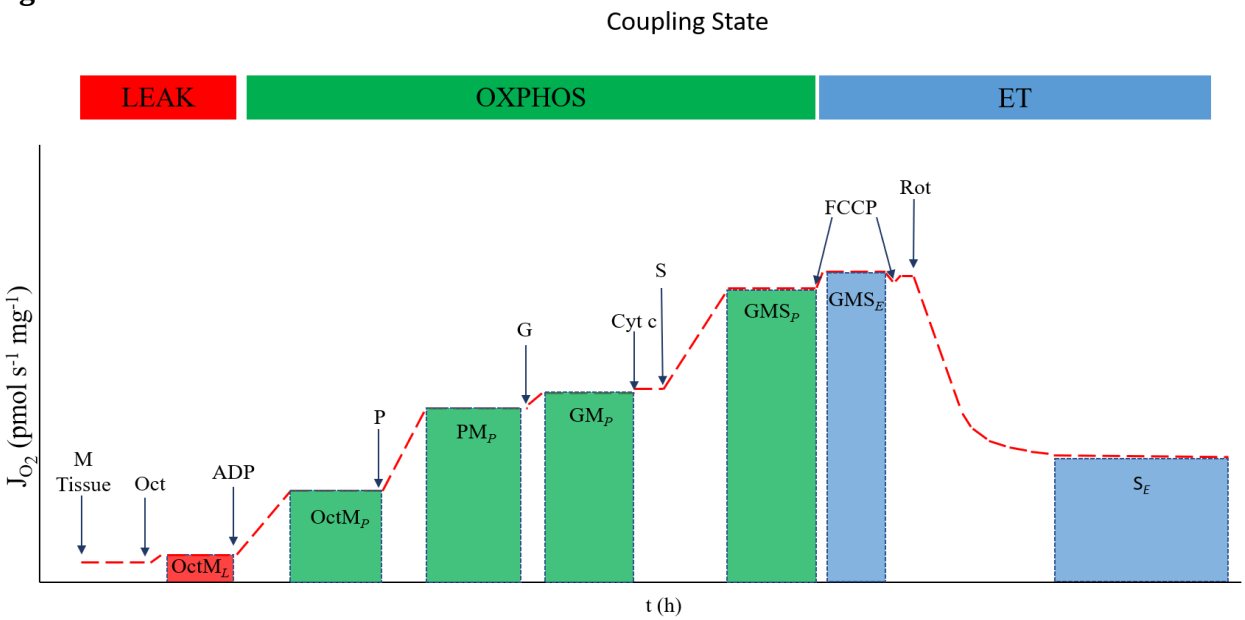

Figure 1 – The SUIT protocol employed in this study. Additions are denoted by arrows, coupling states are colour coded and shown at the top, the red dotted line shows the oxygen consumption rate. Shorthand for measured rates based on substrates present and coupling state are shown in the corresponding part of the graph. M – malate, Oct – octanoyl carnitine, ADP – adenosine diphosphate, P – pyruvate, G – glutamate, Cyt c – cytochrome C, S – succinate, FCCP - carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, Rot – rotenone, ET – non-coupled electron transfer.

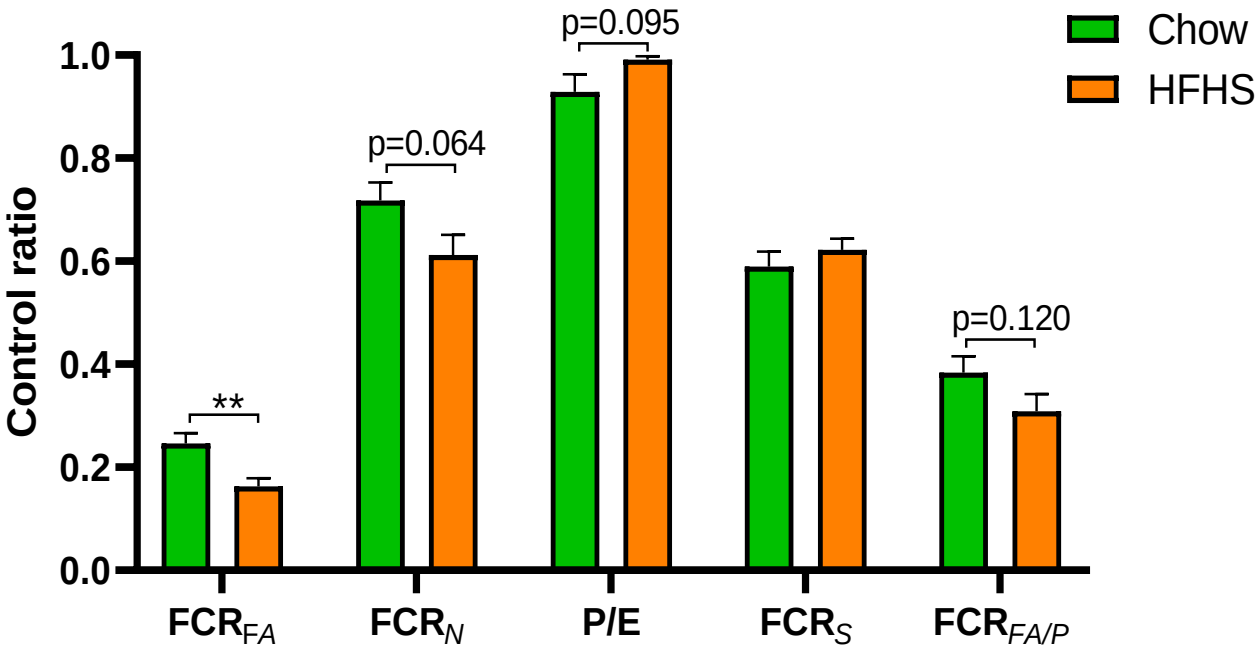

Figure 2 – Flux control ratios of chow and HFHS fed mice assessed in gastrocnemius fibres. Definitions of the control ratios can be found in the Methods and Materials section. N=8 per group. Bars show mean + SEM. Flux control ratios were compared by an unpaired two-tailed Student's t-test; * $p<0.05$, ** $p<0.01$