



HSP72 preserves insulin sensitivity and prevents peripheral tissue lipotoxicity by increasing oxidative metabolism in skeletal muscle



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Introduction

Heat shock proteins (HSPs) are highly conserved proteins that play an important role in maintaining cellular homeostasis. Interestingly, HSP72 (the inducible form of the 70kDa family of HSPs) gene expression is lower in patients with type 2 diabetes compared with healthy controls and expression levels strongly correlate with insulin sensitivity¹. Furthermore, induction of HSP72 achieved by heat-shock therapy, transgenic overexpression, or pharmacologic means provides protection against diet- or obesity-induced glucose intolerance and insulin resistance². Here we show that HSP72 expression plays a pivotal role in regulating skeletal muscle oxidative capacity.

Methods

Skeletal muscle specific HSP72 transgenic (HSP72Tg) and HSP72 knockout (HSP72KO) (mice with a global null mutation in *Hspa1a* / *Hspa1b*, genes responsible for transcribing HSP72) & their respective controls were studied with the focus on oxidative capacity.

Results - HSP72Tg

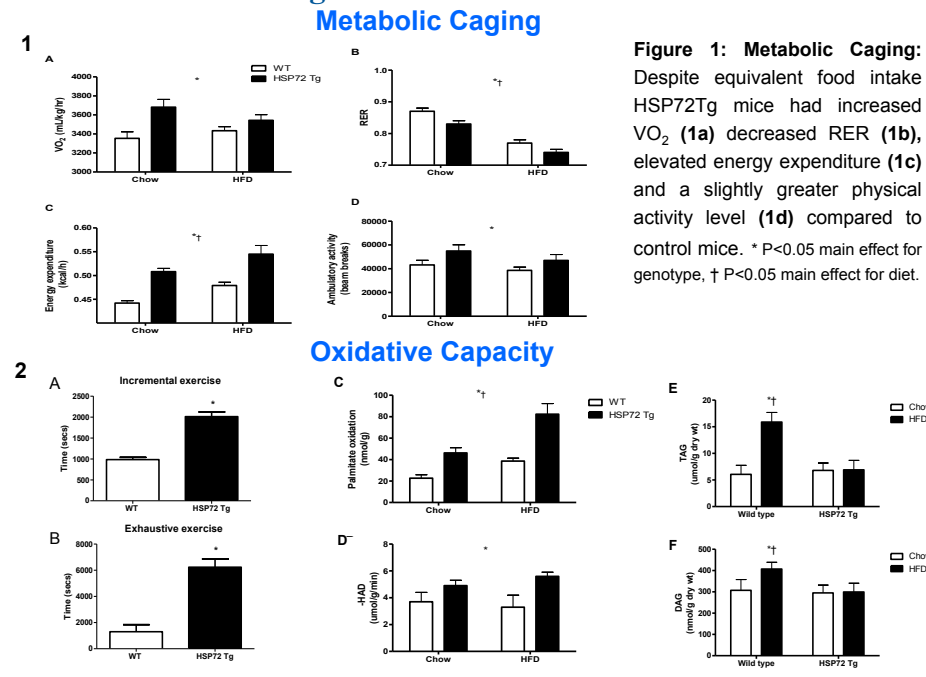


Figure 1: Metabolic Caging: Despite equivalent food intake HSP72Tg mice had increased VO₂ (1a) decreased RER (1b), elevated energy expenditure (1c) and a slightly greater physical activity level (1d) compared to control mice. * P<0.05 main effect for genotype, † P<0.05 main effect for diet.

Figure 2: HSP72Tg animals display increased oxidative capacity: Running time during both incremental fatigue testing and endurance exercise testing was increased in the HSP72Tg animals (2a&b) ex vivo skeletal muscle fatty acid oxidation rates (2c) and -HAD enzyme activity (2d) were elevated in the HSP72Tg muscle while the accumulation of intramuscular TAGs (2e) and DAGs (2f) were significantly reduced on a HFD. * P<0.05 main effect for genotype, † P<0.05 main effect for diet.

Respiration, fibre typing & mitochondrial biogenesis

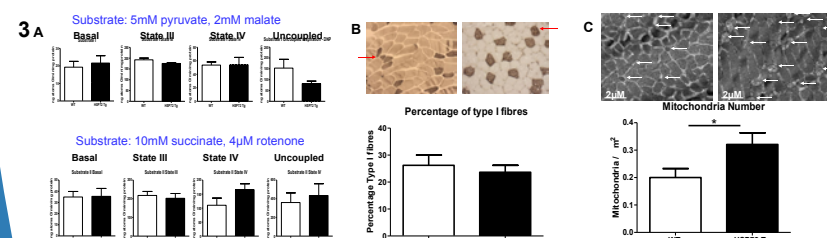


Figure 3: HSP72Tg mice have increase mitochondria number: There was no difference in oxygen consumption in isolated mitochondria under basal, ADP stimulated or uncoupled conditions (3a) nor was there are difference in the amount of slow twitch fibres (2b), however there was a 50% increase in mitochondria number in the HSP72Tg muscle (2c) * P<0.05.

Results - HSP72KO

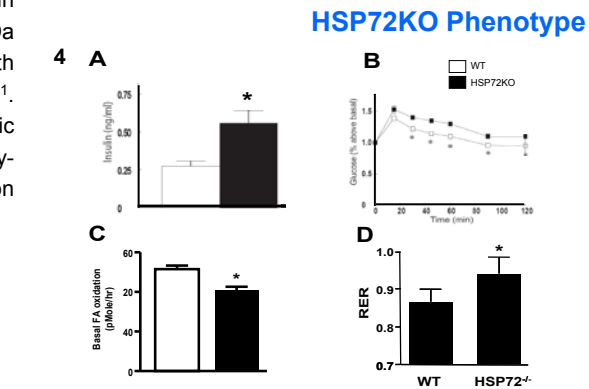


Figure 4: HSP72KO mice get hyperinsulinemia on a normal chow diet (4a) display glucose intolerance compared with WT animals (AUC; P<0.05) (4b), and have higher RER values (4c). Primary skeletal muscle myocytes were cultured from the animals. In culture, these muscle cells display a decrease in basal fatty acid oxidation compared to control (4d). *P<0.05.

Muscle lipids & oxygen consumption

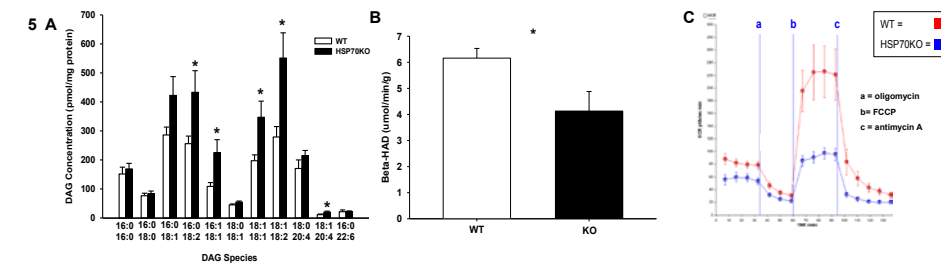


Figure 5: Lipidomic analysis revealed HSP72KO animals have increased skeletal muscle DAG species accumulation (5a) In addition, the activity of the -oxidation enzyme, -hydroxyacyl CoA dehydrogenase, was decreased in skeletal muscle from HSP72 KO mice (5b). Experiments using a Seahorse XF Extracellular Flux analyser on the primary skeletal muscle cell lines indicated that there was a defect in basal and uncoupled respiration in the HSP72KO line compared with a control cell line (5c). * P = <0.05.

DBC1/SIRT1

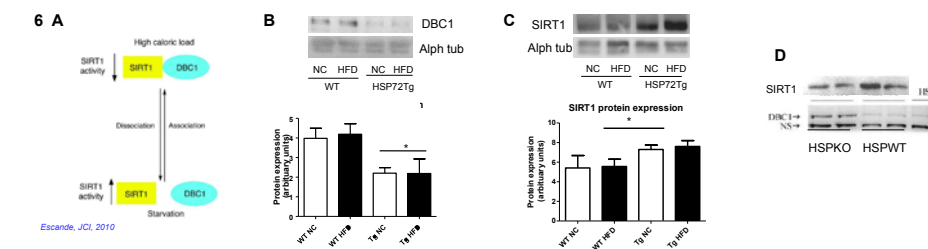


Figure 6: Recently published in JCI, Escande *et al.* (3) describe the process by which DBC1 complexes with SIRT1 acting as a brake on SIRT1's activity (6a). We demonstrate that HSP72Tg mice have decreased skeletal muscle DBC1 protein expression levels (6b) and increased SIRT1 protein expression (6c). Conversely HSP72-/- mice have increased DBC1 levels corresponding with decreased SIRT1 protein levels (6d).

Conclusion

Together, these data indicate that HSP72 prevents obesity and insulin resistance by increasing oxidative metabolism in skeletal muscle possibly via a mechanism that involves DBC1 & SIRT1.

References

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