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αB-crystallin and HspB2 deficiency is protective from diet-induced glucose intolerance

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ABSTRACT

Emerging evidence suggests molecular chaperones have a role in the pathogenesis of obesity and diabetes. As αB-crystallin and HspB2 are molecular chaperones and data suggests their expression is elevated in the skeletal muscle of diabetic and obese animals, we sought to determine if αB-crystallin and HspB2 collectively play a functional role in the metabolic phenotype of diet-induced obesity. Using αB-crystallin/HspB2 knockout and littermate wild-type controls, it was observed that mice on the high fat diet gained more weight as compared to the normal chow group and genotype did not impact this weight gain. To test if the genotype and/or diet influenced glucose homeostasis, intraperitoneal glucose challenge was performed. While similar on normal chow diet, wild-type mice on the high fat diet exhibited higher glucose levels during the glucose challenge compared to the αB-crystallin/HspB2 knockout mice. Although wild-type mice had higher glucose levels, insulin levels were similar for both genotypes. Insulin tolerance testing revealed that αB-crystallin/HspB2 knockout mice were more sensitive to insulin, leading to lower glucose levels over time, which is indicative of a difference in insulin sensitivity between the genotypes on a high fat diet. Transcriptome analyses of skeletal muscle in αB-crystallin/HspB2 knockout and wild-type mice on a normal or high fat diet revealed reductions in cytokine pathway genes in αB-crystallin/HspB2 knockout mice, which may contribute to their improved insulin sensitivity. Collectively, these data reveal that αB-crystallin/HspB2 plays a role in development of insulin resistance during a high fat diet challenge.

1. Introduction

The αB-crystallin and HspB2 genes reside in a head-to-head orientation encoding small heat shock proteins [1]. An orientation dependent intragenic promoter permits the differential expression of the two genes [1–4]. αB-crystallin is highly expressed in the crystalline lens and muscle [5]. The αB-crystallin gene (Cryab) encodes a protein that acts as molecular chaperone and cell death antagonist that inhibits caspase-3 activation [6–8]. In humans, mutations resulting in altered αB-crystallin sequence results in eye diseases including cataracts, as well as in muscle diseases such as myopathies [9–11]. Not surprisingly, in these diseases, there is evidence of abnormal protein aggregation, likely secondary to diminished chaperone activity of αB-crystallin. HspB2 has been shown to also have chaperone activity [12,13] and anti-apoptotic activity [14]. p53 has been shown to regulate the expression of both αB-crystallin and HspB2, and silencing expression of αB-crystallin, but not HspB2, in MCF-7 breast cancer cells leads to higher intracellular glucose levels [15]. A genetic knockout (KO) mouse model exists deleting both αB-crystallin and HspB2 genes; these mice develop a progressive myopathy as they age [16].

While its role in cataracts and myopathies is well established, αB-crystallin and HspB2 may also play a pathogenic role in other diseases. Indeed, αB-crystallin transcripts are upregulated in the muscle of diabetic animals [17–19] and in humans with obesity [18,20–22], suggesting that αB-crystallin may contribute to the etiology of these disorders. These observations are particularly interesting because other heat shock proteins have been reported to have a role in obesity and type 2 diabetes, which may reflect their ability to modulate the inflammatory process that occurs with insulin resistance. Hsp72 has been shown by one group to be reduced in skeletal muscle from diabetic subjects with genetic overexpression of Hsp72 improving glucose tolerance in mice [23,24]. Other studies have found no change in Hsp72 levels in skeletal muscle from diabetic patients but instead found Hsp90 to be overexpressed [25]. Taken together, these data led us to investigate if αB-crystallin/HspB2 may have a role in obesity-related metabolic disorders.

To evaluate the functional role of αB-crystallin/HspB2 in diet-induced obesity and diabetes, we placed αB-crystallin/HspB2-deficient

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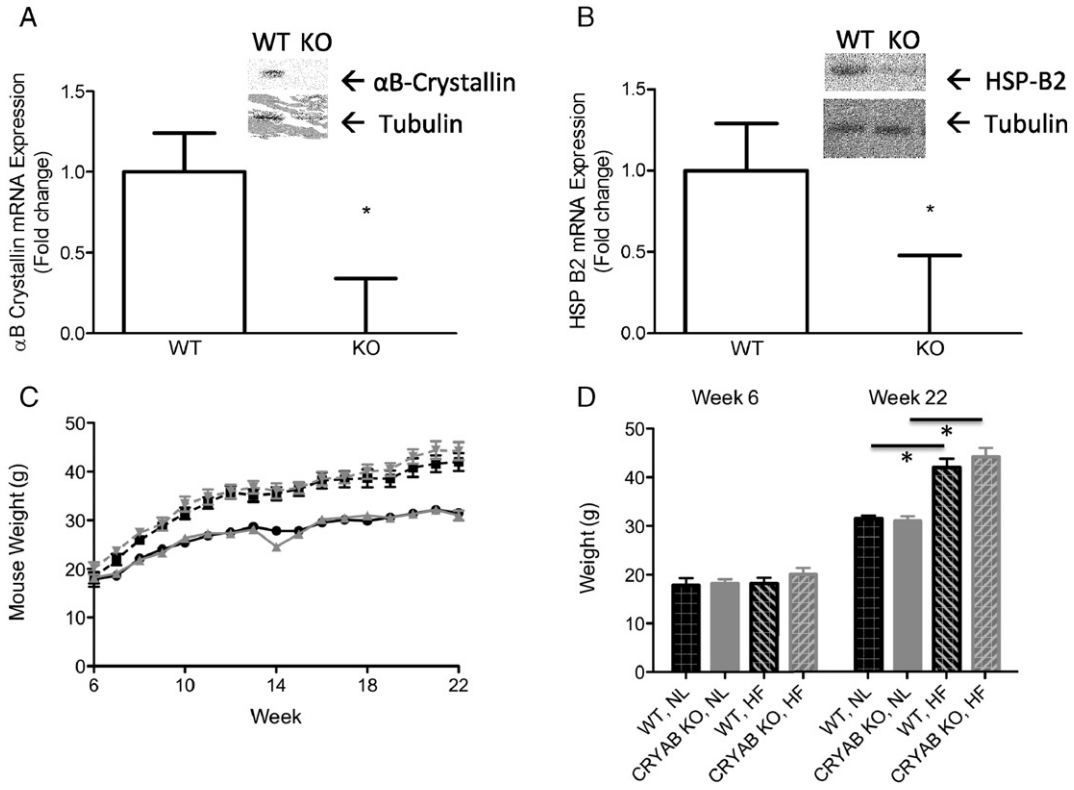

Fig. 1. Weight changes to αB-crystallin/HspB2 KO and WT mice. A–B) Real-time PCR ($n = 3$ from independent replicates, mean ± SEM) and Western blot (similar results for 3 independent replicates) evidence of genetic deletion of αB-crystallin and HspB2 is shown, respectively. C) Weekly weights are shown for mice, starting at 6 weeks of age, were followed to week 22, while on normal chow or high fat diet. Shown is mean ± SEM, $n = 9$–11. Black circle-WT mice on normal chow, black square-WT mice on high fat, grey triangle-KO mice on normal chow, and grey-upside triangle on high fat. D) Weights at the start (week 6) and end (week 22) of the experimental study between the diet groups (normal chow *vs.* high fat) and genotypes (αB-crystallin/HspB2 KO *vs.* WT mice). Shown is mean ± SEM, $n = 9$–11. Black bar - WT mice on normal chow, black bar with lines - WT mice on high fat, grey bar - KO mice on normal chow, and grey bar with lines upside triangle on high fat. *, $p < 0.05$ determined by Student's *t*-test.

and littermate controls (wild-type, WT) on normal chow or high fat diets and monitored weight and metabolic parameters over time. We rigorously tested their glucose homeostasis *via* glucose tolerance tests and their insulin resistance by insulin tolerance tests throughout these dietary interventions. The outcome revealed that αB-crystallin/HspB2 has a role in the development of glucose intolerance, likely by increasing insulin resistance. Because of this, we profiled the skeletal muscle transcriptome between the genotypes and under each dietary condition. Taken together, these data reveal that αB-crystallin/HspB2 is involved in the genesis of insulin resistance on a high fat diet, and we provide extensive RNA profiling to illuminate potential mechanistic insights into the muscle-specific role of αB-crystallin/HspB2.

2. Materials and methods

2.1. Animals

αB-crystallin/HspB2 knockout mice [16] in the FVB/N background were kindly provided by Dr. Xuejun Wang (University of South Dakota). By a heterozygous breeding approach, WT and KO male mice were generated. All mice were group housed with a maximum of 5 animals per cage in a temperature controlled facility with 12 hour light-dark cycle and *ad libitum* access to normal chow (LM-485, Harlan Laboratories, Indianapolis, IN) and water. For high fat diet experiments, mice were given *ad libitum* access to high fat chow (TD.06414, Harlan Laboratories, Indianapolis, IN) beginning at 6 weeks of age. Energy density of normal chow diet was 3.1 kcal/g, and 5.1 kcal/g for high fat chow (with approximately 60% of calories from fat). All experiments described herein and this specific study (protocol 2011-2561) were approved by the Institutional Animal Care and Use Committee at Northwestern University. The primary method of euthanasia for the mice was carbon dioxide; once all signs of life were absent cervical dislocation followed as the secondary euthanasia method.

2.2. Glucose tolerance test

Blood was obtained from tail veins for glucose determination (measured with a One-Touch Ultra Glucometer) and insulin assays (by ELISA assay, ALPCO). Both oral (OGTT) and intraperitoneal glucose tolerance tests (IPGTT) were done on mice fasted overnight and glucose delivery occurred either by intraperitoneal injection (2.0 g/kg body weight) or oral gavage (2 g/kg body weight). Glucose and/or insulin levels were measured at multiple time points (0–180 min) during the OGTT and IPGTT. For both the IPGTT and OGTT, area under the curve was calculated by standard approaches using the trapezoidal rule.

2.3. Insulin tolerance tests

Insulin tolerance tests (ITTs) were conducted on mice that were fasted for 6 h with insulin delivered by intraperitoneal injection at 0.75 U/kg. Glucose was measured at multiple time points from blood obtained over 0–120 min and plotted as percentage of the glucose at 0 min.

2.4. Transcriptome analyses

For reasons related to the metabolic phenotype, we explored the transcriptome in skeletal muscle, where αB-crystallin/HspB2 knockout male mice (at 22 weeks of age) were either on normal chow or high fat diet and three independent samples per genotype were used in the analysis. First, the hind leg muscle was isolated at the time the mice were euthanized and immediately frozen in liquid N_2. Next, the total RNA was extracted with Rneasy Mini Kit (Qiagen) assessed for quality