Influence of obesity on myosin heavy chain isoform expression in mouse hindlimb and diaphragm muscle

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Abstract

The myosin heavy chain (MyHC) isoform mRNA and protein expression in young adult (14 weeks) genetically obese mice ($Lepr^{db-lb}$) was evaluated. Electrophoretic analysis of the diaphragm (Dia) muscle revealed that MyHC-2a was increased at the protein level from ~36% in wild type to ~46% in obese mice. MyHC-2b protein in the Dia of obese mice was decreased to just 32% of wild type levels. MyHC isoform mRNA levels, as quantified by real-time reverse transcriptase-polymerase chain reaction analysis, revealed that MyHC-2a mRNA was increased in the Dia of obese mouse by a factor of 3.3. Immunohistochemical analysis of MyHC isoforms in Dia single fibers revealed a non-statistically significant tendency for an increase in MyHC-2a fibers. The Gastrocnemius-plantaris muscle complex showed no significant changes at either the protein or mRNA level. In conclusion, the Dia in obese mice shows signs of compensatory adaptation to increased load-bearing, by increasing MyHC-2a expression at the expense of MyHC-2b. This suggests that the Dia in obese mice experiences some level of increased contractile demand, likely in response to elevated intra-abdominal fat, even at a relatively young age.

Hypothesis

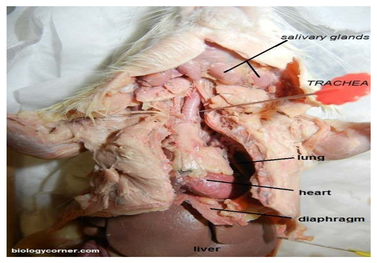
1. With increased adiposity the Dia muscle will become stressed and fiber types will change.
2. Obesity induces elevated Dia loading and subsequent compensatory adaptation

Introduction

Previous studies have shown that obesity is a major health problem worldwide. In the USA, the Centers for Disease Control (CDC) estimates that ~60% of adult Californians are classified as overweight. The percentage of individuals reported to be severely obese in the USA increased by 75% from 2000 to 2005.

Obesity is associated with various diseases, most commonly shown in the metabolic syndrome, a combination of health complications that are associated with an increased risk for cardiovascular diseases and diabetes mellitus type 2. Theses health complications include: obesity; and high triglycerides levels, high blood cholesterol, high blood pressure, high plasma glucose.

Obesity is also associated with increased fat accumulation in the thoracic wall and the abdominal cavity, thus, reducing chest wall compliance and elevating intra-abdominal resistance to thoracic cavity expansion. This results in an increased contractile load on the Dia.



There are two types of skeletal muscle fibers: type I and type 2. According to their twitch speed properties, type I fibers are slow, and type 2 fibers are fast. Type 2 fibers are further classified into 2a, 2b and 2x. Dia skeletal muscle fibers contain four MyHC isoforms, which are MyHC-I, MyHC-2a, MyHC-2b, and MyHC-2x. The order of these four isoforms based on increasing twitch speed is: MyHC-I, MyHC-2a, MyHC-2x, and MyHC-2b. Therefore, MyHC-2b has the fastest rate of fatigue.

Materials and Methods

Experimental animals: 1) 14 week-old female genetically obese mice ($Lepr^{db-lb}$); 2) wild type mice.

SDS-PAGE: MyHC proteins were separated by high resolution SDS-PAGE. The SDS-PAGE gels were stained, photographed, and scanned for the quantification of MyHC protein isoforms.

Immunohistochemistry: Analysis of MyHC content in single fibers. The muscle tissue of obese mice and wild type were cross sectioned. The cross-sections were stained using a series of monoclonal antibodies specific for mouse MyHC isoforms and typed for MyHC content according to their pattern of mAb reactivity.

Results

The body weights of the 14-week old genetically obese $Lepr^{db-lb}$ female mice were significantly greater than wild type by ~2.5 times (Figure 1).

The MyHC isoform content of the gastrocnemius-plantaris muscle complex in $Lepr^{db-lb}$ mice was not significantly different from wild type (Figures 2 and 3). In contrast, the Dia muscle of $Lepr^{db-lb}$ mice showed a significant increase in the proportion of MyHC-2a (~35% increase) and a decrease in the proportion of MyHC-2b (~3 fold reduction) (Figures 2 and 3). However, MyHC isoforms I and 2x were unchanged in $Lepr^{db-lb}$ Dia relative to wild type (Figures 2 and 3).

Analysis of MyHC isoform mRNA levels revealed that MyHC-2a mRNA was significantly elevated (~3x) in $Lepr^{db-lb}$ mouse Dia relative to wild type (Figure 4). MyHC isoforms-I, 2b, 2x were not significantly different in $Lepr^{db-lb}$ relative to wild type (Figure 4).

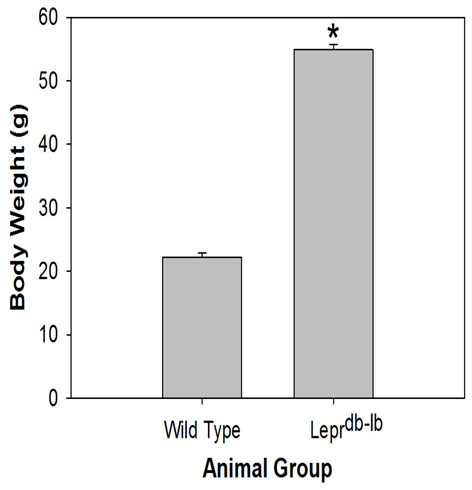


Figure 1. Mean body weights of wild type and obese ($Lepr^{db-lb}$) mice. Values are mean values ± the standard error of the mean (n = 5/group). The asterisk denotes significantly different from wild type ($p \leq 0.05$).

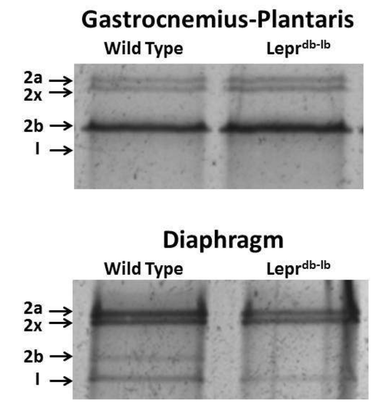


Figure 2. Representative high resolution SDS-PAGE showing the myosin heavy chain (MyHC) isoforms in wild type and $Lepr^{db-lb}$ mouse gastrocnemius-plantaris complex (top) and diaphragm (bottom) muscles. MyHC isoforms I, 2a, 2x, and 2b are designated.

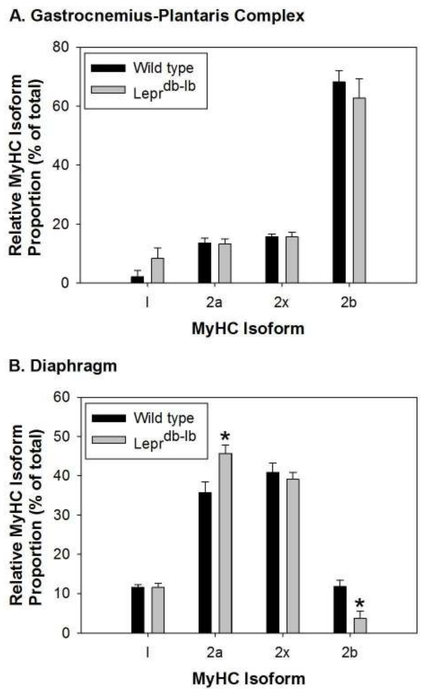


Figure 3. Relative MyHC protein isoform proportions of Gastrocnemius-Plantaris complex and Diaphragm as determined from SDS-PAGE gels (see Figure 2).
A: Wild type and $Lepr^{db-lb}$ MyHC isoform proportion from the Gastrocnemius-Plantaris muscle complex.
B: Wild type and $Lepr^{db-lb}$ error of the mean (n = 5/group). The asterMyHC isoform proportion from Diaphragm muscle. Values are mean values ± the standard isk denotes significantly different from wild type ($p \leq 0.05$)

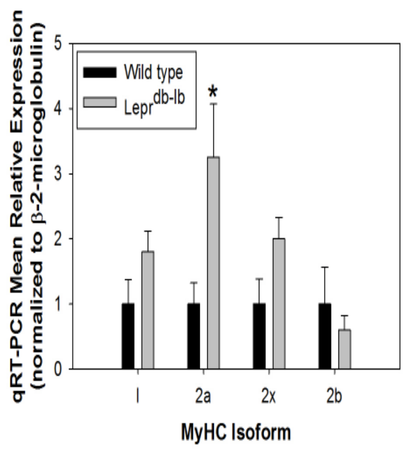


Figure 4. Mean relative MyHC isoform mRNA expression of Diaphragm muscle (qRT-PCR, normalized to beta-2-microglobulin). Values are mean values ± the standard error of the mean (n = 5/group). The asterisk denotes significantly different from wild type ($p \leq 0.05$).

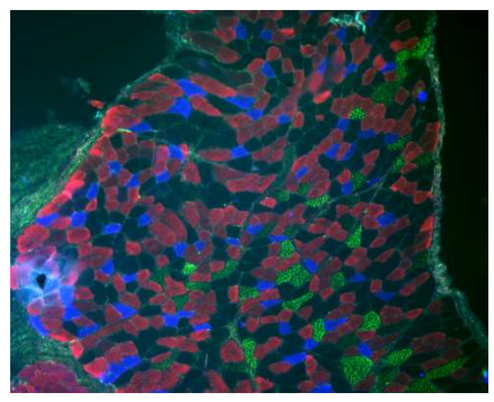


Figure 5. Representative Dia muscle Cross-sections stained immunohistochemically for the MyHC isoforms. MyHC-I fibers are blue, MyHC-2a fibers are red, MyHC-2x fibers are black, and MyHC-2b fibers are green.

Discussion and Conclusion

Our results supported our hypothesis that with increased adiposity the Dia muscle became stressed and fiber types changed.

We studied the genetically obese female mice. The mice lacking functional leptin receptors were almost twice the body weight of wild type mice. Leptin is the primary adipose hormone that conveys an adiposity signal to the brain. Leptin receptor functions as a receptor for leptin. Lacking of leptin receptor prevents the response to leptin and leads to over eating and fat storage. Therefore, there are greater amount of adipose tissue in obese mice, in other words, obese mice have more weight than wild type mice.

Figure 3B showed MyHC-2a of Dia muscle was increased at the protein level from ~ 36% in wild type to ~46% in obese mice. At mRNA level, MyHC-2a of obese mice was also increased by a factor of 3.3 (**Figure 4**). Since MyHC-2a is slower than MyHC-2b, these date are consistent with the slowing of the muscle due to elevated contractile demand.

References

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