EFFECTS OF ENHANCED MUSCLE GROWTH ON FATTY ACID UPTAKE IN SKELETAL MUSCLE

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Tissue samples from transgenic and wild-type mice fed on both high-fat and normal diets were analyzed for the expression of lipoprotein lipase (LPL) by reverse transcription–polymerase chain reaction (RT-PCR). Our results showed lower levels of LPL expression in muscle tissue of transgenic mice compared to that of wild-type mice. These results suggest that extra dietary fat was not stored in adipose tissue and did not enter skeletal muscle. Further investigations are necessary to determine alternative pathways for extra dietary fat in transgenic mice.

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INTRODUCTION

Obesity affects nearly a third of the American population. One strategy to control obesity would be to enhance muscle growth and restrict growth of adipose tissue. Control of obesity can be easily studied by using mice as a model system. Transgenic mice can be used to examine the role of specific proteins and hormones in the growth of muscles and adipose tissue. Muscle growth can be enhanced by inhibiting myostatin, a negative regulator of muscle growth. Yang et al[1] developed transgenic mice in which myostatin was suppressed by overexpressing a segment of the myostatin protein known as the prodomain. The resulting transgenic mice showed a 44% increase in muscle mass and a 20% faster growth rate compared to the non-transgenic control mice. Even when fed a high-fat diet, transgenic mice did not display increased adipose tissue weight.

Weight gain in adipose tissue may be due to high uptake and low oxidation of fat. One of the enzymes involved in the uptake of fatty acids is lipoprotein lipase (LPL). In separate studies of humans, muscle LPL has been shown to be inversely correlated with percentage body fat.[2]

We used myostatin-suppressed transgenic and wild-type mice to determine whether enhanced muscle growth increases fatty acid uptake in skeletal muscle. These mice were fed two types of diet: normal and high fat. We hypothesized that myostatin-suppressed transgenic mice would display elevated levels of LPL in skeletal muscle. The expression levels of LPL in the skeletal muscle of transgenic and control mice were determined with reverse transcription–polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Transgenic and wild-type mice were housed in cages in a constant temperature (22°C) room with a 12-hour light/dark cycle. Mice were weaned at four weeks of age and given free access to a normal-fat diet (10% kcal fat, D12450, Research Diets, New Brunswick, NJ) until nine weeks of age. Based on the genotypes (transgenic and wild-type), male mice were randomly assigned to two types of diet: normal-fat and high-fat diet (45% kcal fat, D12451, Research Diets) from 9 to 18 weeks. All animal experiments were approved by the institutional animal care and use committee at the University of Hawaii and conducted in accordance with National Institutes of Health guidelines for animal care.

Mice were killed at 18 weeks of age after an overnight fast. Individual muscles were dissected and weighed. Three different fat pads were dissected and weighed: subcutaneous, epididymal, and retroperitoneal adipose tissue fat pads.

Total RNA was isolated from the gastrocnemius muscle (100 mg) from 18-week-old mice with TRIzol reagent (Invitrogen, Carlsbad, Calif) and quantified by using a spectrophotometer.

Reverse transcription was carried out with 3 μg total RNA with Superscript II

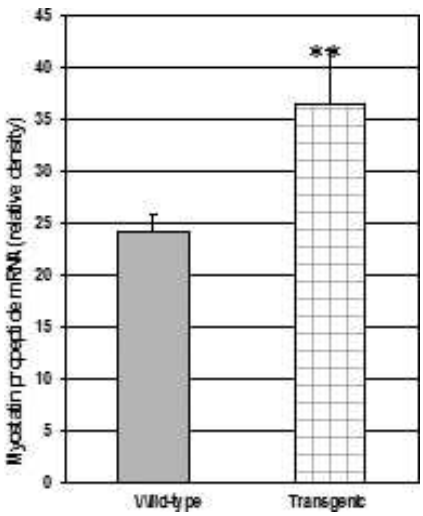

Fig 1. Expression levels of myostatin propeptide transgene by RT-PCR. The density of the RT-PCR product was quantified, and least square means and standard error of the relative densities from transgenic and littermate control mice (n=8 per group) are shown. Bars identified by ** differ at P <.01.

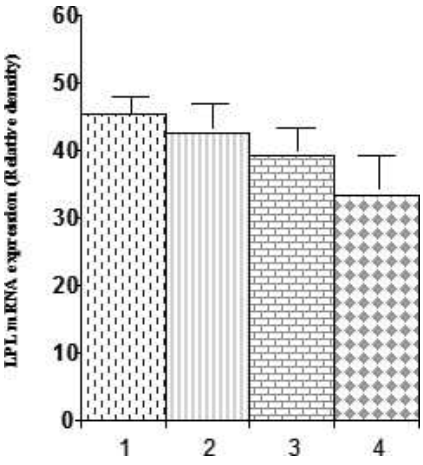

Fig 2. Expression levels of LPL in myostatin prodomain transgenic mice. The density of the RT-PCR product for LPP was quantified, and least square means and standard error of the relative densities from transgenic mice fed a normal fat (NF) and high-fat (HF) diet (n=4 per group) is shown. 1=control, NF; 2=transgenic, NF; 3=control, HF; 4=transgenic, HF.

Reverse Transcriptase at 42°C for four hours; cDNA samples were amplified by using a Thermocycler BioRad for 35 cycles. The PCR reaction contained 200 μM dNTP, 50 mM MgCl, and .2 units DNA Taq polymerase. The reaction conditions included a 95°C hot start for five minutes, denaturing at 95°C for 30 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 30 seconds, and final term at 72°C for five minutes. Samples were analyzed by running a 2% agarose gel. DNA bands were visualized after staining with ethidium bromide and photographed under ultraviolet light with a digital camera. The following primers were used: myostatin prodomain primer forward: 5′-GCTCTTGGAAGATGACGATT-3′ and reverse: 5′-CATTTGGGCTTGCCATCC-3′; LPL primer forward: 5′-CAGCAAGACCTTCGTGGTGA-3′ and reverse: 5′-GTACAGGGCGGCCACAAGT-3′ (Integrated DNA Technologies).

RESULTS

The first set of experiments was run to ascertain experimental conditions. The reagent and DNase-treated controls did not show any gel bands. The contaminating DNA evident in lane 2 did not appear in lane 3 where DNase was applied. These results indicate that reagents were clean and DNase treatment was effective. The experiment was run with varying amounts of total RNA. The relationship between the amount of RNA used and the intensity of the gel bands was linear. A second gel examined the expression of the myostatin prodomain in all 16 samples. Transgenic mice displayed elevated levels of the myostatin prodomain (Figure 1). Control mice on a high-fat diet showed significantly less LPL expression than did control mice on a normal diet. Transgenic mice showed less LPL expression than control mice. The transgenic mice on a high-fat diet showed the least LPL expression (Figure 2).

DISCUSSION

The control mice on a high-fat diet showed significantly less LPL expression than did the control on a normal diet. This finding was expected because the control mice on a high-fat diet had twice the amount of normal adipose tissue weight, confirming earlier findings that muscle LPL inversely correlates with percent body fat. Transgenic mice did not show elevated levels of LPL in skeletal muscle. Although they had similar adipose tissue weight, transgenic mice on a high-fat diet expressed significantly less LPL than did transgenic mice on a normal diet. This finding suggests that extra dietary fat that was not stored in the adipose tissue did not enter skeletal muscle. Fat may have been used in the liver in an alternative pathway. Another possibility is that LPL enzyme activity in transgenic mice may be higher than in control mice, although this difference is not detectable at the mRNA level. Further study is needed to examine protein expression and enzyme activity of LPL in skeletal muscle to confirm these results. Future investigations should include examining fatty acid uptake in the liver, which might be a preferable site for fat utilization.

ACKNOWLEDGMENTS

This work was supported by a grant from Ingeborg V. F. McKee Fund of the Hawaii Community Foundation, and the NIDDK/Drew National High School Student Summer Research Apprenticeship Program. Special thanks to Dr. George Hui for his comments and advice on the project.

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