Enhanced muscle by myostatin propeptide increases adipose tissue adiponectin, PPAR-α, and PPAR-γ expressions

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Abstract

Muscle tissue utilizes a large portion of metabolic energy for its growth and maintenance. Previously, we demonstrated that transgenic over-expression of myostatin propeptide in mice fed a high-fat diet enhanced muscle mass and circulating adiponectin while the wild-type mice developed obesity and insulin resistance. To understand the effects of enhanced muscle growth on adipose tissue metabolism, we analyzed adiponectin, PPAR-α, and PPAR-γ mRNA expressions in several fat tissues. Results indicated muscled transgenic mice fed a high-fat diet displayed increased epididymal adiponectin mRNA expression by 12 times over wild-type littermates. These transgenic mice fed either a high or normal fat diet also displayed significantly high levels of PPAR-α and PPAR-γ expressions above their wild-type littermates in epididymal fat while their expressions in mesenteric fats were not significantly different between transgenic mice and their littermates. This study demonstrates that enhanced muscle growth has positive effects on fat metabolisms through increasing adiponectin expression and its regulations.

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The prevalence of adult obesity is steadily increasing throughout the country as reported by the Behavioral Risk Factor Surveillance System (BRFSS) and the Centers for Disease Control (CDC). The most recent data indicates that among the American population surveyed 60.5% were overweight, 23.9% were obese, and 3% were extremely obese [1]. Obesity is a risk factor for development of several different chronic diseases such as type 2 diabetes, cardiovascular disease and cancer [2]. Adipose tissue was originally believed to be a relatively inert storage depot for energy. Now, adipocytokines or adipokines produced by the adipose tissue is known to play a critical role in energy regulation, glucose and lipid homeostasis [3]. Modification in expression of the adipocytokines may lead to the development of obesity and obesity-related disorders [4]. Adiponectin is a 30-kDa adipocytokine hormone secreted primarily by the adipose tissue. It is a relatively abundant serum protein and accounts for between 0.01% and 0.03% of total serum protein in humans [5]. Increased serum levels of adiponectin are associated with increased insulin sensitivity, increased fatty acid oxidation, and decreased hepatic glucose production [6]. Obesity tends to increase the plasma concentrations of most proteins produced by adipose tissue because of the increase in total fat mass, but plasma levels of adiponectin were found to be much lower in obesity [6]. Plasma adiponectin concentration was reported to be significantly lower in individuals with essential hypertension, coronary artery disease (CAD), and type 2 diabetes [7]. Significantly lower levels of adiponectin were also observed in fat tissues from obese mice and obese humans [8,9]. Adiponectin can ameliorate insulin resistance by decreasing hepatic glucose production through lowering activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) [10]. Along with its ability to decrease the activity of hepatic gluconeogenic enzymes, adiponectin improves insulin sensitivity by

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decreasing the concentration of circulating triglycerides and free fatty acids [6].

The molecular mechanisms that regulate the expression of the adiponectin gene are still under investigation and poorly understood. A PPAR-γ response element has been found in the promoter region of adiponectin and TZDs, PPAR-γ agonists, which can increase adiponectin expression [10]. PPARs are ligand-activated transcription factors that bind to DNA elements such as PPAR response elements (PPREs) and form a heterodimer with 9-cis-retinoic acid receptors (RXRs) in the promoter region of many genes. Both PPAR-α and PPAR-γ are able to lower circulating lipid concentrations and are therefore able to ameliorate insulin resistance via different mechanisms [10]. The activation of PPAR-α stimulates uptake, binding, and oxidation of fatty acids in mitochondria, peroxisomes, and microsomes resulting in decreased tissue content of free fatty acids. PPAR-γ is an important regulator of gene expression and fat cell differentiation, triggering conversion of pre-adipocytes to adipocytes and regulating activation of lipoprotein lipase, acyl-CoA synthase, fatty acid translocase (CD36), and fatty acid transport protein. PPAR-γ also increases the number of small adipocytes with increased insulin sensitivity over large adipocytes [11].

Previously, we generated transgenic mice through muscle-specific expression of the cDNA sequence (5′-region 886 nucleotides) encoding for the propeptide of myostatin [12–14]. The active or mature form of myostatin is generated by cleavage of the precursor protein at the tetrapeptide (RSSR) site. The remaining N-terminal peptide is termed the propeptide. The myostatin propeptide transgenic mice were heavier than the wild-type animals on the normal diet. In contrast, high-fat diet induced wild-type mice with 170–214% more fat mass than transgenic mice, and developed impaired glucose tolerance and insulin resistance. Interestingly, high-fat diet significantly increased adiponectin secretion while blood insulin, resistin, and leptin levels remained normal in the transgenic mice [13]. We theorized that the combination of the myostatin propeptide transgene and a high-fat diet would demonstrate significant differences in adiponectin, PPAR-α, and PPAR-γ mRNA expression among fat depots. An increase in adiponectin mRNA expression would correlate to an increase in mRNA expressions of PPAR-α and PPAR-γ. To determine which fat tissue responsible for the increase in serum adiponectin level, and possible relationships with PPAR-α and PPAR-γ expression, we analyzed expression level of adiponectin, PPAR-α and PPAR-γ mRNAs in tissue samples from the transgenic and wild-type mice fed either a normal or high-fat diet in this study.

Materials and methods

Animals. Transgenic mice expressing myostatin propeptide were maintained and sampled as previously described [13]. The animals were weaned at 4 weeks of age and given un-restricted access to a normal fat diet (10% kcal fat) until 9 weeks of age. Then the male mice were randomly assigned to one of two types of diet (normal fat, 10% kcal fat, Laboratory Rodent Diet 5001; or high fat, 45% kcal fat, Research Diets) based on genotype (transgenic and wild-type). There were four groups of animals with four animals per group, wild-type/normal-fat diet (WT/NF), transgenic/normal-fat diet (TG/NF), wild-type/high-fat diet (WT/HF), and transgenic/high-fat diet (TG/HF).

Tissue sampling, RNA extraction, and cDNA preparation. Subcutaneous, epididymal, and mesenteric adipose tissue fat pads were dissected and weighed when mice were sacrificed at 18 weeks of age. Total RNA was isolated from tissues mentioned above using Trizol reagent (Invitrogen, Carlsbad, CA) and chloroform, as previously described [12]. Prior to the reverse transcriptase reaction, total RNA was treated with 1 μL deoxyribonuclease I (Invitrogen) and the appropriate buffers according to protocol to remove any residual genomic DNA. cDNA was then synthesized using 2 μg total RNA, 1 μL amplification grade SuperScript II Reverse Transcriptase (Invitrogen), and the appropriate reaction buffers to a final volume of 20 μL per reaction tube according to the protocol set by Invitrogen. The cDNA was stored at −80 °C until use.

Quantitative real-time PCR. The relative expressions of adiponectin, PPAR-α, and PPAR-γ were determined by quantitative real-time PCR in an ABI 7300 Sequence Detection System (Applied Biosystems, Forrest City, CA). Primers were designed using OligoPerfect Designer (Invitrogen), and summarized in Table 1. Each well of the 96-well reaction plate contained a total volume of 50 μL. cDNA (0.5 μL) solution was combined with 1.5 μL each of forward and reverse primers (10 pmol/μL), 21.5 μL distilled water, and 25 μL SYBR Green PCR Master Mix (Applied Biosystems). Optimal annealing temperatures for the primers used was determined to be 60 °C and 45 cycles were run. The abundance of each mRNA transcript was measured and expressed in comparison to steady-state glyceraldehyde 3-phosphate dehydrogenase (GAPDH). To validate real-time PCR quantification of mRNA levels in adipose tissue, we first ran reverse-transcription PCR, followed by agarose gel electrophoresis, demonstrated the PCR product of adiponectin, PPAR-α, PPAR-γ, and GAPDH matched the expected sizes of each pair of primers. Then we tested different amounts of total RNA for amplification of target genes. An optimal condition was achieved using 0.05 μg of total RNA, which is in the middle of the linear response range of real-time PCR reaction to the amount of the total RNA used. We used the expression of GAPDH as an internal control to normalize the expression of target genes. Real-time PCR was duplicated for each cDNA sample. Each gene mRNA level in arbitrary unit was acquired from the value of the threshold cycle (C_t) of the real-time PCR as related to that of GAPDH using the comparative C_t method through the formula $2^{\Delta C_t}$ ($C_t = C_{t\ GAPDH} - C_{t\ gene\ of\ interest}$) [15].

Statistical analysis. The data for mean comparisons were analyzed by using the JMP program. (SAS Inst., Cary, NC). Significant differences between the mass of the main muscles and adipose tissue mass were analyzed by ANOVA and Student's t-test. The effects of genotype, diet, and fat pad location on the mRNA expression levels of adiponectin, PPAR-α, and PPAR-γ were analyzed by ANOVA, two-tailed Student's t-test, and Tukey's HSD test. Least square means and their standard errors are reported. Significance was determined at $P < 0.05$.

Results

The weights of individual fat pad from the experimental mice were summarized in Table 2. The weights of epididymal and subcutaneous fat pad of transgenic mice fed a high-fat diet were not significantly different from transgenic mice and wild-type mice fed normal fat diet ($P > 0.05$) while wild-type mice fed a high-fat diet had significantly increased both fat pads ($P < 0.01$). Transgenic mice fed a high-fat diet also showed a reduced amount of mesenteric fat pad (Table 2).

To investigate the changes of gene expressions of adiponectin, PPAR-α, and PPAR-γ in adipose tissue, we ana-