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The modulator effect of GH on skeletal muscle lysosomal enzymes is dietary protein dependent

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Abstract

Objective: The purpose of this work is to determine whether changes in dietary protein level could alter the modulator effect that GH has on the muscle lysosomal system by influencing the hydrolytic activities of cathepsin D, acid RNase and DNase II and the participation of these enzymes in muscle growth.
Design: BALB/c female mice were fed a diet containing 20% (HP) or 12% (MP) protein ad libitum and were treated with either saline (s) or rhGH (GH) (74 ng/g) for 29 days. Body weight and feed intake were recorded daily. At 25, 30, 35, 40, 45 and 50 days of age, five mice from each group were slaughtered and nucleic acids and protein concentrations and cathepsin D, acid RNase and DNase II activities in gastrocnemius muscle were analysed. Correlation coefficients were used to analyse the links between the activity of each enzyme with its substrate.
Results: GH-treatment induced a depletion–recovery response in muscle growth through a compensatory mechanism. Changes in protein content, DNA and RNA concentrations were related to changes in lysosomal enzyme activities. Muscle cathepsin D activity in saline mice fell as the dietary protein concentration increased. GH-treatment reversed this effect by enhancing the proteolytic activity in muscle of well-fed mice and inhibiting it in mice fed a 12% protein diet. This inversion appears to be related to the different mechanism elicited by GH-treatment on skeletal muscle protein growth in each dietary group. An opposite trend was observed in muscle acid nuclease activities. Acid RNase and DNase II increased according to the dietary protein concentration, since a 12% protein diet induced a lower catabolism, especially on muscle DNA of saline mice. In contrast, GH-treatment decreased acid RNase and DNase II activities, but only in mice fed a 20% protein diet, perhaps leading to spare muscle RNA for protein synthesis, as well as to the inhibition of DNA degradation during catch-up growth. A lower dietary protein concentration appeared to reverse the GH protective effect on nucleic acids.
Conclusions: GH seems to act as a dietary protein-dependent modulator of the skeletal muscle lysosomal enzyme activity. These lysosomal enzymes play a role during muscle growth in GH-treated post-weaning mice by modifying muscle protein and DNA and RNA degradation.

Keywords: RhGH; Dietary protein; Lysosomal enzymes; Muscle growth

1. Introduction

The administration of GH to growing animals enhances growth rate, thus improving rates of protein accretion and decreasing rates of lipid deposition [1], although the effect of GH on tissue growth seems to vary between different phases of growth. Thus, GH administration at a dose of 120 μg kg^{-1} BW on pigs during the 10–25 kg growth phase [2] does not improve the rate or efficiency of gain during the first half of the study, but it tends to increase growth rate and improve feed utilisation during the second half. Part of the growth

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regulation by the GH/IGF-I system affects amino acid oxidation and the efficiency of amino acid use [3]. GH challenge decreases plasma urea nitrogen levels at all ages but the magnitude of the response is age-dependent, with greater reductions occurring in older pigs [4], indicating that protein accretion response to GH treatment is enhanced in later growth phases. These effects on amino acid metabolism parallel age-dependent changes in the GH/IGF-I axis, resulting in only 20–30% increases in circulating IGF-1 in young pigs, compared to a 300% increase in older pigs [4]. Thus, somatotropin appears to have minimal effects during the prenatal and early post-weaning growth phase, but it has substantial effects in later stages of development.

It is assumed that proper nutrition is essential for optimal function of GH. In young, rapidly growing animals in the fully fed state, GH treatment improves protein balance by reducing protein degradation rather than by stimulating protein synthesis, whereas in fasting conditions GH treatment of young 20 kg swine improves protein balance by increasing the basal rate of whole protein synthesis with no change in the rate of proteolysis in the post-absorptive state [5]. Thus, GH treatment in young growing swine enhances metabolic efficiency by minimising the loss of protein during fasting and maximising the protein gained during meal absorption. There is also evidence that the anabolic response to GH is only effective provided an adequate minimum level of protein intake is achieved [6].

Previously, we found that exogenous administration of recombinant human growth hormone (rhGH) to weaned BALB/c male and female mice induces a biphasic response on feed intake and body weight, resulting in a delay in the growth process from 25 to 30 days of age [7], and causing decreases in skeletal muscle weight and myonuclei number. During the second stage (from 35 to 50 days of age), hyperphagic behaviour allows mice fed a 20% protein diet, and not mice fed a 12% protein diet, to recover their body and muscle weight to control values through compensatory muscle hypertrophy [8,9], indicating the need to intake an adequate level of dietary protein for GH action.

Muscle protein degradation plays an important role in the rate of muscle growth. Lysosomes are implicated in the turnover of cytoplasmic soluble constituents and of cellular organelles, including even nuclei [10]. Degradation of proteins, DNA and RNA in mammalian cells is mediated by a wide variety of lysosomal enzymes. The endopeptidase, aspartic protease (cathepsin D) [11], and the endonucleases: acid deoxyribonuclease (DNase II) [12] and acid ribonuclease (RNase) [13], are known to be involved in intracellular protein, DNA and RNA degradation respectively, within the lysosomal compartment in various organic tissues including muscle. Cathepsin D may degrade mainly endocytosed and autophagocytosed cell constitutive proteins and act over hydrophobic residues of the polypeptide chain [14], while DNase II cleaves deoxyribonucleotide linkages in native and denatured DNA yielding products with 3′-phosphates. Similarly, acid RNase cleaves preferentially to phosphodiester linkages adjacent to a pyrimidine and purine nucleotide of RNA, via a 2,3′-cyclic phosphate intermediate, to form oligo or mononucleotides with a terminal 3′ phosphate [15]. It has recently been reported that cathepsin D [16] and DNase II [17] may play a part in apoptotic cell death. The lysosomal pathway is regarded to be a minor contribution to the proteolytic pathway for bulk skeletal muscle compared to the ubiquitin–proteasome (UB)-dependent system [18]. One of the main reasons for considering that the autophagic lysosomal system plays a minor role in skeletal muscle has been that typical lysosome structures have rarely been seen in normal muscle tissues. Nevertheless, the characterisation of cathepsin D [19] as well as the existence of a lysosomal apparatus with a full complement of acid hydrolases [20], and the identification of LC3 [21], a mammalian homologue of yeast Apg8p, which is defined as one of the autophagy-specific proteins associated with the autophagosomal membranes [22] have been reported recently in skeletal muscle.

Extrinsic influences exercise a primary function during muscle development to modify expression of hydrolytic enzymes such as proteinases [23]. Amino acids are known to be regulators of proteolysis [24]. Moreover, IGF-I has been postulated as an endogenous regulator of tissue proteolysis [25] but data on the direct effects of GH on lysosomal enzymes and their possible role in modulating skeletal muscle protein degradation are controversial. Whereas no significant effect of GH treatment on enzyme proteolytic activity in muscle of GH-treated pigs [26] or on the UB pathway mRNAs [27] has been reported; GH appears to act on lysosomal enzymes of proliferative chondrocytes in the growth plate [28], and on lysosomal enzymes of skeletal muscle of female weaning BALB/c mice during the metabolic response to rhGH administration, as we have previously found [29].

The purpose of this work is to determine whether changes in dietary protein level can alter the modulator effect that GH administration has on the lysosomal system of the gastrocnemius muscle of post-weaning rhGH-treated female BALB/c mice, when they are fed with two dietary protein concentrations (12% or 20%) by influencing the hydrolytic activities of cathepsin D, acid RNase and DNase II. A further objective of this work is to determine the possible participation of these enzymes (through the degradation of skeletal muscle protein, DNA and RNA) in the mechanism of muscle growth during the growth lag and subsequent recovery of the gastrocnemius muscle weight described above.