

Effect of Chronic Growth Hormone Administration on Skeletal Muscle in Dogs*

SYLVAIN MOLON-NOBLOT,¹ PHILIPPE LAROQUE,¹ SRINIVASA PRAHALADA,² LEA G. STABINSKI,² CHAO-MIN HOE,³ CHENNEKATU P. PETER,² PIERRE DUPRAT,¹ AND MATTHEW J. VAN ZWIETEN²

¹Merck Sharp & Dohme-Chibret Laboratories, Research Center, Department of Safety Assessment, BP 134, Route de Marsat, 63203 Riom, France,

²Merck Research Laboratories, Department of Safety Assessment, West Point, Pennsylvania 19486, USA, and

³Merck Research Laboratories, Department of Biometrics, West Point, Pennsylvania 19486, USA

ABSTRACT

Administration of growth hormone (GH) results in increased body weight gain in dogs. Increased body weight gain is believed to be a result of the trophic effect of GH on the musculoskeletal system. However, edema is one of the side effects described in man following exogenous GH administration. Thus, the objective of this study was to determine if the expected increased weight gain in GH-treated dogs is a result of increased muscle mass. Porcine growth hormone (pGH), administered subcutaneously to beagle dogs at doses of 0.025, 0.1, and 1 IU/kg/day for 14 wk, resulted in elevated serum GH and insulin-like growth factor-1 (IGF-1) levels (see accompanying paper, Prahalada et al). This was associated with a significant increase in body weight gain and weights of the cranial tibialis muscle in both male and female dogs. The increased muscle mass likely contributed to the significant increase in body weight gain seen in both sexes. Quantitative analysis of skeletal muscle sections stained for ATPase activity showed increases in type I (slow twitch) and type II (fast twitch) myofiber sizes in mid- and high-dose males and in high-dose females. The ratio of type I and type II muscle fibers remained unchanged. Hypertrophic myofibers were enlarged but had a normal histologic and ultrastructural organization when observed by light and transmission electron microscopy. The results of this study have demonstrated that increased muscle mass in pGH-treated dogs is related to hypertrophy of muscle fibers and not due to edema. Exogenous GH administration has an anabolic effect on skeletal muscle in dogs.

Keywords. Muscle hypertrophy; experimental; pharmacological effect; morphometry; electron microscopy

INTRODUCTION

In mammals, pituitary growth hormone (GH) is essential for postnatal growth of most somatic tissues (28, 35, 40, 44). GH has both intrinsic anabolic and catabolic activities. Its catabolic (direct) activity results in enhanced lipolysis and restricted glucose transport caused by insulin resistance (15, 37, 38); its anabolic (indirect) effects, mediated by insulin-like growth factors (IGFs), or somatomedins (3, 12, 13), result in protein synthesis and growth. Chronically elevated serum levels of GH in humans results in gigantism or acromegaly (23, 50), and in GH-deficient children, human GH is a potent anabolic agent known to stimulate growth (21, 34, 45). Although the response of tissues to GH is not uniform among species studied, the skeletal muscle is responsive in species such as rat, pig, and man to GH administration (8, 19, 48). Transgenic mice expressing high levels of serum GH have dramatic body growth and skeletal muscle growth (30), and in hypopituitary dwarfs there is a remarkable increase in muscle mass and strength following chronic injection of human GH (7).

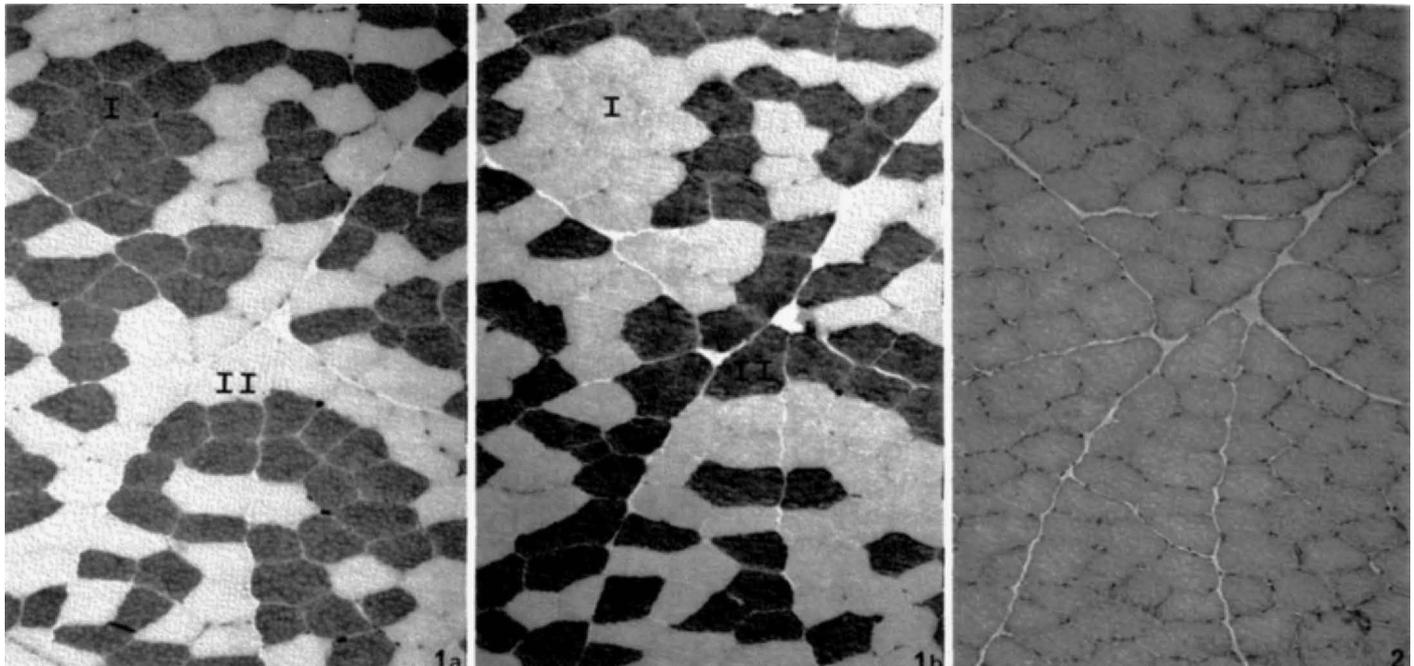
The dog is a predictive model for human GH secretion and metabolism (15, 43). Congenital GH deficiency in German shepherds leads to dwarfism, and short-term ad-

ministration of GH to dwarf dogs results in increased size, weight, and skeletal muscle mass (14). Because the porcine and dog GHs have a similar amino acid composition (49) and complete immunologic cross-reactivity (2, 46), they are thought to have similar physiologic and pharmacologic properties (16). Although the skeletal muscle composition is well known in normal dogs (1, 5, 24), the long-term effect of GH administration on striated muscle fibers has not been precisely described. The purpose of this study was to characterize the effects of chronic administration of porcine growth hormone (pGH) on the dog skeletal muscle fibers using quantitative histochemistry and transmission electron microscopy (TEM).

METHODS

Experimental Design. The study used 16 male and 16 female beagle dogs, obtained from Hazelton Research Products, Inc. (Kalamazoo, MI) and Marshall Farms (North Rose, NY). The animals were 54–69 wk old and weighed from 6.9 to 15.7 kg at study initiation. They were housed in individual stainless steel pens, in an environmentally controlled room with a 12-hr light cycle. Approximately 350 g of Purina Certified Canine Chow biscuits were provided once daily until week 10 of the study, when the ration was increased to 550 g daily for all dogs; drinking water was available *ad libitum*. The test compound was injected subcutaneously to 3 treat-

* Address correspondence to: Dr. Srinivasa Prahalada, Merck Research Laboratories, Merck and Co., WP 45-232, Sumneytown Pike, West Point, Pennsylvania 19486, USA.



FIGS. 1, 2.—1) Frozen sections of CRT muscle stained for ATPase activity. Type I fibers (slow twitch) have dark staining at pH 4.3 (a) and type II fibers (fast twitch) have dark staining at pH 9.8 (b). LM. $\times 130$. 2) General view of muscle fibers and intercellular connective tissue in a cross section of the CRT in a high-dose pGH-treated dog. LM. H&E. $\times 130$.

ment groups of 4 males and 4 females at doses of 0.025, 0.1, and 1.0 IU/kg/day. Porcine GH (~ 1.80 IU/mg; Dr. Parlow, Research and Education Institute, Inc., Torrance, CA) was injected as a sterile solution in 0.03 M NaHCO_3 in 0.15 M NaCl at a final pH of 9.5. The control group was injected with 0.2 ml/kg of the vehicle subcutaneously. A total of 95 (males) or 96 (females) daily doses of pGH were given. Additional details are provided in the accompanying paper (32). Body weights were recorded during the pretest period and once per week during the study. General care and maintenance of animals as well as additional observations are described elsewhere (9).

Muscle Processing. At necropsy, the left and right cranial tibialis (CRT) muscles were removed and weighed together. In the center of each muscle, 3 adjacent transverse samples ($5 \times 5 \times 5$ mm) were cut and frozen in isopentane/liquid nitrogen and 2 coronal slices ($5 \times 5 \times 2$ mm) were taken and immersed in a 2.5% glutaraldehyde phosphate-buffered fixative solution. An additional cross section of each muscle was taken for routine hematoxylin and eosin (H&E) staining.

Quantitative Histochemistry. Histochemical staining and quantitative evaluation of the cranial tibialis (CRT) muscle were done on all males and females from the control, mid-, and high-dose groups and on males from the low-dose group. Nine-micron transverse sections were obtained from the frozen left skeletal muscle samples with a Bright cryostat and stained for myofibrillar ATPase activity. Staining for ATPase activity allows simultaneous identification of type I fibers (slow twitch) and type II fibers (fast twitch) in muscle sections (4).

Type I fibers are characterized by dark staining at pH 4.3 and 4.5 and by light staining at pH 9.8 (Fig. 1a), while type II fibers are characterized by dark staining at pH 9.8 and light staining at pH 4.3 and 4.5 (Fig. 1b). In this study, staining for ATPase activity was done at pH 4.3, 4.5, and 9.8 on transverse sections of skeletal muscle originating from 2 adjacent portions of the muscle. On each stained section, the mean fiber area and the volume density of type I and type II fibers were determined using an image analyzer (Imagenia 2000, Biocom S.A., Lyon, France).

Electron Microscopy. Blocks approximately $2 \times 2 \times 1$ mm were prepared from glutaraldehyde-fixed skeletal muscle samples, postfixed for 1 hr at room temperature in a 1% osmium tetroxide solution, and embedded in epon following graded dehydration in alcohol. Semithin and ultrathin sections were obtained using a Reichert Ultracut E ultramicrotome. Toluidine-blue-stained semithin sections from all control and high-dose animals were examined by light microscopy and uranyl acetate-lead citrate-contrasted ultrathin sections from all control and high-dose animals were examined at 80 keV using a Jeol 1200 EX TEM.

Statistical Analysis. Body weight gains (combined sexes) were analyzed for normality using the Wilk and Shapiro statistics and for homogeneity using the Levene test; analysis of variance was done by trend test at $p < 0.05$. Statistical analysis was done separately for each gender to evaluate pGH effect on CRT muscle weight, type I and type II fibers area, and the ratio of type I/II volume density (%); the NOSTASOT (no statistical sig-

TABLE I.—Percent increase in body weight gain and CRT muscle weight in dogs given pGH.

	Dose groups (IU/kg/day)			
	0.0	0.025	0.1	1.0
Males				
Body weight ^a	+2	+8	+23 ^c	+37 ^c
Muscle weight ^b		+20	+50 ^c	+45 ^c
Females				
Body weight ^a	+2	+6	+26 ^c	+45 ^c
Muscle weight ^b		+6	+16	+25 ^c

^a Percent change from pretest.

^b Percent increase over controls.

^c Statistically significant trend through indicated dose ($p < 0.05$).

nificance of trend) method (47) was used to test an increase or decrease in response over the dose range.

RESULTS

Body Weight and Skeletal Muscle Weight

Injection of pGH caused an increased body weight gain and increased CRT muscle weight (Table I). There were statistically significant ($p < 0.05$) increases in body weight gain in the mid- and high-dose groups (combined sexes). The mid- and high-dose dogs gained an average of 2.8 kg and 4.7 kg, respectively, over the course of the study; control dogs gained an average of 0.4 kg and the low-dose group gained 0.8 kg. CRT muscle weights were increased in all pGH-treated groups in both sexes; they reached statistical significance ($p < 0.05$) in the mid- and high-dose males (+50% and +45% from controls, respectively) and in high-dose females (+25% from controls).

Microscopic Examination

Light microscopic (LM) examination of H&E-stained sections of CRT muscles showed no histopathological changes and no evidence of edema in pGH-treated dogs (Fig. 2). Muscle fibers of pGH-treated dogs were indistinguishable from those of controls by light microscopy. Muscle cells and adjacent connective tissue had a normal ultrastructural organization when observed by TEM. In particular, spatial organization of thin and thick myofilaments was normal when examined in longitudinal and transverse sections, and the distribution of fine structures such as mitochondria, glycogen particles, and endoplasmic reticulum was also similar to that observed in control dogs (Fig. 3a, b). The intercellular connective tissue surrounding the muscle fibers had a normal ultrastructural appearance, consisting of a thin layer of collagenous fibrils containing a rich network of blood capillaries.

Quantitative Histochemistry

The results of the morphometry performed on the frozen sections of CRT muscles stained for ATPase activity are summarized in Table II. Administration of pGH resulted in increases in areas of type I and II fibers as compared to controls that were statistically significant ($p < 0.05$) through the high dose in both sexes. In males, the increases above control values for type I fibers were 36% and 19% and the increases for type II fibers were 21%

and 30% in the high- and mid-dose groups, respectively. In high-dose females, the increases above control values for type I and type II fibers were 30% and 17%, respectively. The increased fiber size was interpreted as hypertrophy of myofibers. In both sexes, there were no differences in the type I/II ratios following repeated pGH administration. In the control group, the mean values of areas for type I and type II muscle fibers and the type I/II ratio values were comparable to those reported in the literature for CRT muscles in beagle dogs (24).

DISCUSSION

The CRT was selected to evaluate the effect of pGH because the structure and function of this muscle in normal dogs is documented in literature (24). As seen in the present study in dogs, an increase in muscle mass and body weight gain following exogenous GH administration has been reported in several species (18, 20, 42), including man (11, 34, 45). In the present study, repeated subcutaneous administration of pGH to dogs resulted in hypertrophied type I and type II skeletal muscle fibers, without changes in fiber distribution. No edema was observed by LM and TEM, and the CRT muscles in pGH-treated dogs had a normal tissular and cellular organization. Similar to dogs, repeated pGH administration to pigs resulted in increased muscle mass and myofiber size with no effect on fiber distribution (29, 42, 48). The pGH treatment was associated with the production of pale, soft exudative muscle in pigs (41); however, this observation needs further confirmation. In contrast to observations in dogs, rats implanted with GH-secreting tumor cells had variations in muscle fiber response; the size of type I myofibers was increased, whereas the size of type II myofibers was unaffected (33).

Skeletal muscle hypertrophy in dogs following chronic pGH treatment is likely secondary to an increase in protein content as reported in other species. In hypophysectomized animals, GH stimulates both RNA and protein synthesis in skeletal muscle (20, 48); these studies demonstrated that muscle fiber hypertrophy results from stimulated synthesis and slightly reduced degradation of muscle proteins. Administration of GH to growing pigs enhanced lean body mass and decreased fat mass (6). In man, recombinant GH (rGH) therapy leads to a positive nitrogen balance and protein anabolism in both GH-deficient children (7) and in normal adults (10). In nutritionally depleted subjects, mRNA levels in muscles were significantly elevated after rGH administration (19), and in healthy subjects, rGH induced an increase in skeletal muscle protein content, namely heavy-chain myosin (22).

In the present study, skeletal muscle changes correlated with increased serum GH and IGF-1 levels (32). Serum IGF-1, the major GH-dependent somatomedin, is essential in the control of somatic growth (25–27, 50). In the hypophysectomized rats, IGF-1 stimulates somatic growth following GH administration (38). In dogs and humans, IGF-1 levels are controlled by GH; acromegalic dogs have high serum IGF-1 concentrations (17) and dwarfism in German shepherds, primarily caused by GH deficiency, results in low circulating levels of IGF-1 (18).

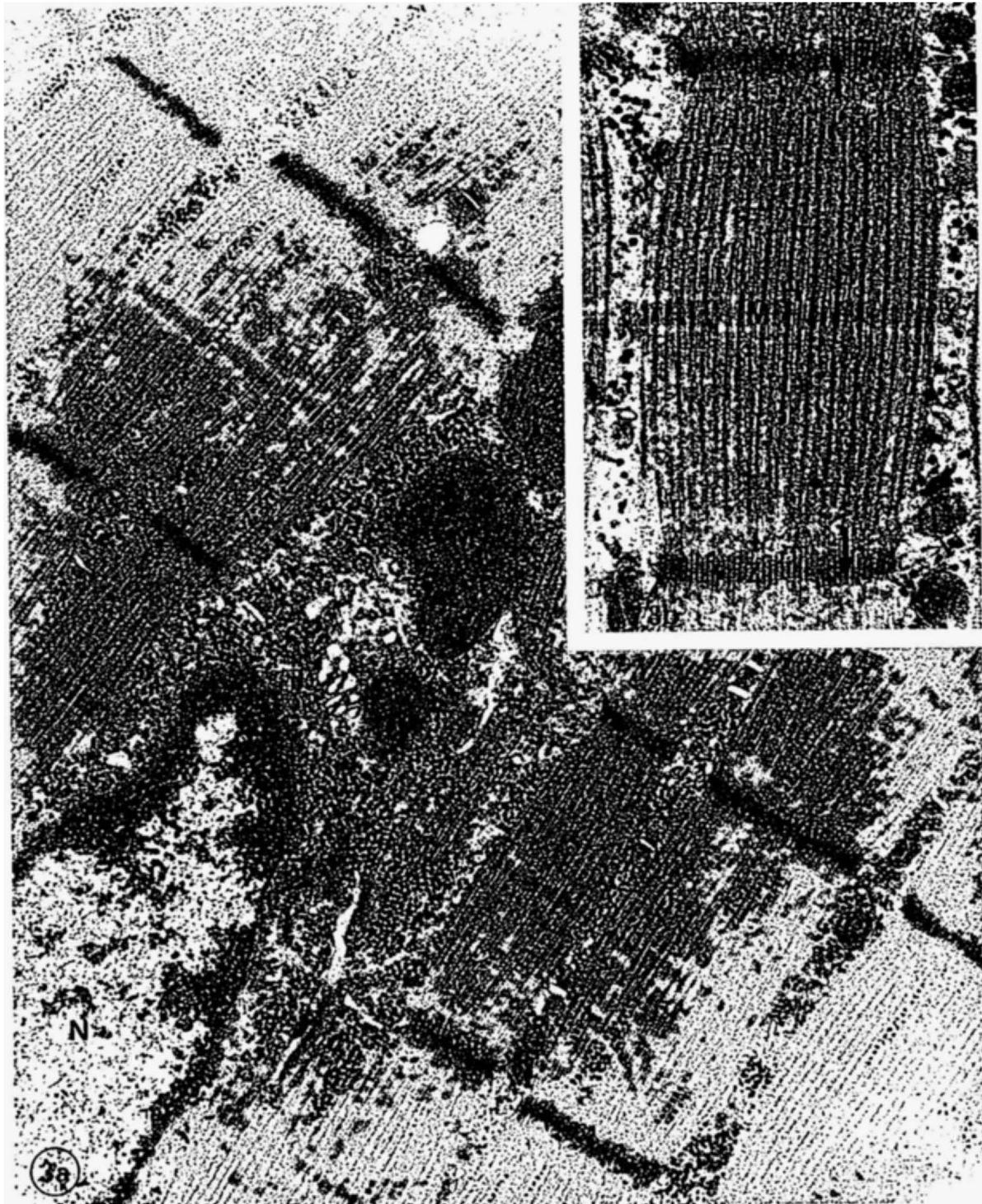


FIG. 3.—(a) General orientation and organization of myofibrils in a skeletal muscle cell in longitudinal section, from a dog given pGH (f = fibril; M = mitochondria; N = nucleus). $\times 15,000$. (b) High-power magnification of a muscle fibril in longitudinal section (Z = Z line; I = I band with thin filaments; H = H band with thick filaments; M = M band with cross-linking filaments). $\times 25,000$.

The primary effect of elevated serum GH and IGF-1 levels in transgenic mice expressing human GH is to increase overall growth (30, 31, 39). Furthermore, it has been shown that IGF-1 enhances the differentiation of muscle cells *in vitro* (36).

In summary, chronic injection of pGH to dogs caused a dose-related increase in CRT muscle weight as a result of hypertrophy of type I and II myofibers. Hypertrophy of the skeletal muscle in pGH-treated dogs contributed to the increase in body weight gain.

TABLE II.—Morphometry of CRT muscle in dogs given pGH.

Sex	Fibers	Dose groups (IU/kg/day)							
		0		0.025		0.1		1.0	
		Area ^a	Fraction ^b	Area	Fraction	Area	Fraction	Area	Fraction
Males	Type I	2,481 ± 406	44	2,463 ± 274	41	2,945 ± 391	41	3,388 ± 404 ^d	47
Males	Type II	2,978 ± 245	56	3,246 ± 194	59	3,864 ± 168 ^d	59	3,615 ± 488 ^d	53
Females	Type I	2,451 ± 144	35	NE ^c	NE	2,617 ± 406	39	3,196 ± 222 ^d	40
Females	Type II	3,391 ± 187	65	NE	NE	3,304 ± 439	61	3,969 ± 204 ^d	60

^a Mean value (μm^2) of 120 measures of the fiber areas.

^b Mean value (%) of 40 measures of the area fraction occupied by each type of fibers.

^c NE = not evaluated as no changes observed in females at dose 0.025 IU/kg/day.

^d Statistically significant trend through indicated dose ($p < 0.05$).

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