

Somatotropic function: The somatomedin hypothesis revisited¹

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ABSTRACT: The discovery in 1922 that an alkaline extract of the anterior pituitary can increase growth and change carcass composition of rats led to the discovery of growth hormone (somatotropin, ST). Since the early studies, much has been learned about the biological effects of ST. The advent of recombinant DNA technology has led to the commercial development of ST-based products for animal agriculture. Administration of porcine ST (pST) at maximally effective doses (approximately 100 $\mu\text{g}\cdot\text{kg BW}^{-1}\cdot\text{d}^{-1}$) to growing pigs for 30 to 77 d increases average daily gain approximately 10 to 20%, improves productive efficiency (i.e., the ratio of body weight gain to feed consumed) 13 to 33%, decreases lipid accretion rates by as much as approxi-

mately 80%, and stimulates protein deposition (muscle growth) by as much as 70%. These responses are associated with a decrease in feed intake of approximately 10 to 15%. The effects of ST are mediated directly and indirectly. The indirect effects of ST are mediated by the somatomedin (insulin-like growth factor-I). The discovery of somatomedin led to the introduction of the somatomedin hypothesis, which explained the basis of ST action. Since the discovery of the somatomedins, there have been several modifications of the hypothesis developed to accommodate the evolution in understanding of how ST and IGF-I affect a diverse array of biological events. This review will summarize the history of ST and the evolution of the somatomedin hypothesis.

Key Words: Growth Hormone, Insulin-Like Growth Factor-I, Somatomedin Hypothesis, Somatotropin

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Introduction

Breakthroughs in biotechnology in the 1970s enabled ST to be produced by recombinant DNA technology. The advent of recombinant DNA techniques greatly facilitated studies assessing the effects of recombinant bovine ST (bST) and porcine ST (pST) on lactation and growth (reviewed by Etherton and Bauman [1998]). These studies in conjunction with results from mechanistic studies markedly increased the understanding of underlying biological mechanisms that account for the diverse effects of both bST and pST. These results and those from studies with cell culture and lab animal models led to the recognition that somatotropin had both direct and indirect effects on growth and metabolism. The indirect effects are mediated by the somatomedins, notably IGF-I. This review will provide a brief perspective of the history of somatotropin and the evolution of somatomedin research.

The History of Somatotropin

Evans and associates were the first to demonstrate the presence of a substance in the anterior pituitary gland that increased the growth rate of rats (Evans and Long, 1922a,b; Evans and Simpson, 1931). Downs (1930) and Bierring and Nielsen (1932) made the initial discovery that an alkaline extract of the anterior pituitary gland decreased carcass fat in rats. This was later verified by Lee and Schaffer (1934), who found that pair-fed rats injected with a crude alkaline extract of bovine pituitaries not only grew faster but also contained proportionally more muscle and less fat. These findings led to the conclusion that a substance was present in the pituitary gland that stimulated growth and affected carcass composition. It was not until 1945, however, that the GH of the anterior pituitary was first isolated from bovine anterior pituitaries (Li et al., 1945). This enabled Li et al. (1948) to conduct the first experiment showing that crude preparations of GH mimicked the effects of the alkaline pituitary extract on carcass fat in rats (rats were treated 6 d/wk for 437 d with a graded injection regimen increasing from 0.4 to 2.0 mg/d; carcass fat was decreased by 47%). By today's protein purification standards, this GH preparation was not pure. Importantly, however, this study not only established that GH accounted for the effects of the pituitary alkaline extract on growth and body

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composition, but also provided the foundation on which subsequent, improved purification schemes for the hormone were based.

The early findings that GH preparations could decrease the carcass fat of rats prompted a number of studies to evaluate the effects of pituitary preparations of porcine GH (pGH) on growth and carcass composition of pigs (Giles, 1942; Turman and Andrews, 1955; Henricson and Ullberg, 1960). These studies were inconclusive likely because the GH preparations were not pure. In 1972, Machlin established that treating pigs with pituitary-derived porcine GH significantly improved weight gain and feed efficiency. Prior to the 1980s, few studies were conducted that investigated the effects of GH in domestic livestock. There was little interest in commercial application of GH to animal agriculture because at the time there was no feasible means for economically producing large quantities of pituitary-derived GH. Consequently, the GH supply was limited to that extracted with varying purity from the pituitary glands of slaughtered animals. Landmark discoveries, however, in biotechnology research in the mid-1970s made it possible to produce GH by recombinant DNA technology. The availability of large quantities of recombinantly derived pGH permitted extensive investigations to be conducted that conclusively demonstrated that chronic administration of recombinant GH markedly improved growth rate, carcass composition, and productive efficiency of pigs (reviewed in Etherton and Bauman [1998]). Collectively, these studies established that daily administration of a maximally effective dose of pGH (approximately 100 $\mu\text{g}/\text{kg}$ BW daily) to growing pigs for 30 to 77 d increased average daily gain approximately 10 to 20%, improved productive efficiency (i.e., the ratio of body weight gain to feed consumed) 13 to 33%, decreased lipid accretion rates by as much as approximately 80%, and stimulated protein deposition (muscle growth) by as much as 70%. These responses were associated with a decrease in feed intake of approximately 10 to 15%.

Research with farm animals over the past 30 yr has shown that GH has a multitude of biological effects that extend beyond increasing growth (see Table 1). Because of this, many scientists have elected to use *somatotropin* rather than “growth hormone” as the preferred name for the hormone. For ST to cause these remarkable changes in growing animals, such as pigs, it is evident that GH affects many physiological processes in numerous tissues and organs so that more nutrients can be used for lean tissue accretion and fewer nutrients are deposited in adipose tissue (reviewed in Etherton and Bauman [1998]; Etherton [2000, 2001]). The effects that ST has in target tissues are highly coordinated to effect the marked changes observed in nutrient partitioning in growing pigs. The alterations in metabolism occur for all nutrient classes—carbohydrate, lipid, protein, and minerals.

The Somatomedin Hypothesis: A Historical Evolution

The original somatomedin hypothesis developed from early efforts to learn more about how ST increased growth. The pioneering studies demonstrating that the pituitary gland produced ST, which, in turn, increased growth and reduced body fat, launched the early studies designed to learn more about how the anterior pituitary increased linear growth. Since linear growth was increased by administration of alkaline extracts of the pituitary, one question concerned how the alkaline extract increased bone growth. Kibrick et al. (1941), realizing that bone growth involved the proliferation of cartilage cells in the epiphyseal plate of long bones, found that epiphyseal plates of ST-deficient rats (hypophysectomized, or hypox) increased in width following administration of growth hormone. It was subsequently demonstrated that cartilage metabolism could be assessed by quantifying incorporation of $^{35}\text{SO}_4$ into chondroitin sulfate of epiphyseal cartilage (Ellis et al., 1953; Denko and Bergenstal, 1955; Murphy et al., 1956) and that ST could increase sulfate incorporation into cartilage from hypox rats (Murphy et al., 1956).

These observations led to the landmark study by Salmon and Daughaday (1957), which suggested that a factor was present in serum, which they termed a “sulfation factor,” that mediated the growth-promoting effects of ST. This paper demonstrated that sulfate incorporation by the cartilage from hypox rats was not stimulated in vitro by added ST or serum from hypox rats (see Table 2). Sulfate incorporation, however, was stimulated by serum from normal rats or ST-treated rats. Salmon and Daughaday (1957) hypothesized that ST stimulated somatic growth indirectly via circulating sulfation factors. Thus, the original somatomedin hypothesis was proposed. The word *somatomedin* was developed (Daughaday et al., 1972) to indicate a circulating substance that is increased by ST and mediates the effects of ST on sulfate incorporation into cartilage. A central precept of the somatomedin hypothesis was that somatomedin was synthesized in the liver in response to ST and subsequently transported to peripheral target tissues. Somatomedin-C (Sm-C) was the name ascribed to the somatomedin that was ST-responsive.

Over the time Daughaday and his colleagues were making their discoveries, another line of research was evolving that would lead to the discovery of the same family of hormones, albeit based on a different biological system. Subsequent to the development of a radioimmunoassay for insulin, Froesch et al. (1963) and Burgi et al. (1966) observed that the vast majority of the insulin-like activity measured by bioassay in serum could not be suppressed by the addition of anti-insulin serum. This was puzzling and raised the question of what accounted for this bioactivity. They referred to this activity as nonsuppressible insulin-like activity (NSILA) and launched a robust effort to identify NSILA. Nonsuppressible insulin-like activity in human serum was

Table 1. Biological effects of somatotropin in farm animals during growth and lactation^a

Tissue	Physiological process affected
Skeletal muscle (growth)	Protein accretion ↑ Protein synthesis ↑ Amino acid and glucose uptake ↑ Partial efficiency of lysine utilization
Bone (growth)	↑ Mineral accretion paralleling tissue growth
Mammary tissue (lactation)	↑ Synthesis of milk with normal composition ↑ Uptake of nutrients used for milk synthesis ↑ Activity per secretory cell ↑ Maintenance of secretory cells ↑ Blood flow consistent with change in milk synthesis
Adipose tissue	↓ Glucose uptake and glucose oxidation ↓ Lipid synthesis if in positive energy balance ↑ Basal lipolysis if in negative energy balance ↓ Insulin stimulation of glucose metabolism and lipid synthesis ↑ Catecholamine-stimulated lipolysis ↓ Ability of insulin to inhibit lipolysis ↓ glucose transporter-4 translocation (?) ↓ Transcription of fatty acid synthase gene ↓ Adipocyte hypertrophy ↑ IGF-I mRNA abundance
Liver	↑ Glucose output ↓ Ability of insulin to inhibit gluconeogenesis
Intestine	↑ Absorption of Ca, P required for milk (lactation) or bone (growth) ↑ Ability of 1,25 vit. D ₃ to stimulate calcium-binding protein ↑ Calcium-binding protein
Systemic effects	↑ IGF-I and IGFBP-3 ↓ IGFBP-2 ↑ Acid-labile subunit ↓ Amino acid oxidation and blood urea nitrogen ↓ Glucose clearance ↓ Glucose oxidation ↓ Response to insulin tolerance test ↑ Free fatty acid oxidation if in negative energy balance ↑ Cardiac output consistent with increases in milk output (lactation) ↑ Enhanced immune response

^aAdapted from Etherton and Bauman (1998).

present as a large-molecular weight (100 to 150 kDa) acid-insoluble fraction, and as a smaller acid-soluble fraction, termed NSILA-P and NSILA-S, respectively (Burgi et al., 1966; Jakob et al., 1968). Rinderknecht and Humbel (1976, 1978a,b) isolated two forms of insulin-like factors from NSILA-S and determined the amino acid sequence of these peptides. They were subsequently named *insulin-like growth factor-I* (IGF-I) and *II*.

Recognition of the existence of two protein families (Sm-C/IGF-I and IGF-II) involved in the ST axis that shared remarkable biological similarities resulted in considerable debate about whether these were related or different protein families. Further research established that IGF-I mediated the effects of ST on cell proliferation and sulfate incorporation into cartilage (Froesch et al., 1985). Subsequent research by a number of research groups revealed that IGF-I and Sm-C had

Table 2. Sulfate incorporation by rat costal cartilage in vitro (a summary of the results of Salmon and Daughaday, 1957)

Cartilage source	Added substrate	Sulfate uptake ^a
Normal rat	–	+
Hypophysectomized rat	–	–
Hypophysectomized rat	ST	–
Hypophysectomized rat	Hypox rat serum ^b	–
Hypophysectomized rat	Serum from ST-treated hypox rat	+
Hypophysectomized rat	Normal rat serum	+

^aThe plus sign denotes that added substrate stimulated sulfate uptake. The minus sign denotes no stimulatory effect of added substrate.

^bHypox = hypophysectomized.

Table 3. Some biological effects of IGF-I (adapted from Le Roith et al., 2001)

Increases glucose uptake (in vitro, in absence of IGF-binding proteins)
Promotes wound healing
Stimulates myogenesis
Inhibits apoptosis
Chemotactic
Activation of cell cycle genes
Enhances steroidogenic responsiveness to LH/hCG in Leydig cells
Stimulate progesterone production in granulosa cells
Promotes wound healing
Increases lipid synthesis (in vitro, in absence of IGF-binding proteins)
Stimulates DNA synthesis
Stimulates protein synthesis
Stimulates RNA synthesis
Stimulates cell proliferation

very similar biological properties (Van Wyk et al., 1980). In 1983, the question of whether Sm-C and IGF-I were identical molecules was resolved when Klapper et al. (1983) established that they had the same amino acid sequence. Insulin-like growth factor-I is a polypeptide of 70 amino acids and IGF-II has 67 amino acids. Thus, scientists conducting research on Sm-C or IGF had been studying the same growth factor, albeit on the basis of different biological properties. To harmonize the field and to avoid confusion, Daughaday et al. (1987) suggested the adoption of a single nomenclature system, namely IGF-I and IGF-II. During the past 20 yr, IGF-I has been shown to have a remarkable diversity of biological effects (see Table 3).

The Remodeling and Evolution of the Somatomedin Hypothesis

The evolution in understanding the biology of insulin-like growth factors was occurring in parallel with discoveries being made in animal agriculture that pST and bovine ST had remarkable effects on growth and lactation (reviewed by Etherton and Bauman [1998]). With respect to research on the biology of ST action in pigs, it became clear that not all the effects of ST were mediated in a way that was consonant with the somatomedin hypothesis. First, the effect that ST treatment had on adipose tissue growth was not an insulin-like effect. Studies conducted in our lab and others showed that treatment of pigs with ST remarkably reduced lipid synthesis, glucose uptake, and insulin sensitivity and that this accounted for the reduction in lipid deposition (adipose tissue growth: see Etherton and Bauman [1998] for review). Studies we conducted with IGF-I in vitro demonstrated that free IGF-I was an insulin mimic (Walton et al., 1989) and that this could be blocked by the addition of IGF-binding protein-3 (IGFBP-3). Since $\geq 95\%$ of circulating IGF-I in plasma is transported by a family of IGFBP, it was difficult to envision a model that would accommodate the effects

of ST being mediated by IGF-I, at least in adipose tissue. Thus, we concluded that the effects of ST on adipose tissue metabolism were direct effects and not mediated by IGF-I.

The first suggestion that the somatomedin hypothesis did accurately depict the ST axis was based on studies that revealed the presence of IGF-I in cultured explants of fetal mouse tissue maintained in serum-free medium (D'Ercole et al., 1980). Subsequently, it was shown that ST stimulated IGF-I synthesis in many tissues (D'Ercole et al., 1984; Daughaday and Rotwein, 1989). As soon as cDNA clones for IGF-I were available, it became clear that ST regulated the expression of the IGF-I gene in many extrahepatic tissues (Lund et al., 1986; Murphy et al., 1987; Roberts et al., 1987). These observations led to the conclusion that the locally produced IGF-I—that is, synthesized locally in target tissues, but not the IGF-I in the circulation—mediated the effects of ST in an autocrine or paracrine manner (see Le Roith et al. [2001] for review).

During this time, several studies were done in which IGF-I was administered systemically to rats and mice to determine whether growth could be stimulated via an endocrine mechanism (i.e., via circulating IGF-I) (Schoenle et al., 1982; Skottner et al., 1987). In general, these studies revealed that systemic administration of large doses of free IGF-I (i.e., not associated with IGF-binding proteins) increased growth; however, the extent of the effect was small compared with ST. Subsequently, it was demonstrated that injection of ST into the tibial epiphyseal cartilage plate of hypox rats stimulated growth of the epiphyseal plate only in the injected growth plate but not in the contralateral plate (not treated with ST) (Isaksson et al., 1982; Russell and Spencer, 1985). Evidence was also presented showing that co-infusion of IGF-I antibodies blocked the effect of ST (Schlechter et al., 1986). These results persuasively established that the effect of ST treatment was localized to the treatment site. These findings reaffirmed the idea that ST has effects locally that are mediated by IGF-I.

The observation that IGF-I is present both in the circulation and is produced locally has raised questions about the function of circulating IGF-I (Le Roith et al., 2001). Mice that lack IGF-I have retarded growth and most die after birth (reviewed in Le Roith et al. [2001]). Data from a mouse model in which the liver IGF-I gene was specifically deleted (Yakar et al., 1999) indicate that these mice grow and develop normally even though serum IGF-I levels are reduced by approximately 75%. Based on this study, hepatic IGF-I is unimportant for sustaining normal growth. This study also confirms that liver is a major source of IGF-I, at least in mice; however, it is not important for normal postnatal growth. The findings by Yakar et al. (1999) also provide direct evidence for locally produced IGF-I. More recently, it has been shown that double gene disruption (of the liver IGF-I gene and the acid-labile subunit gene) leads to a significant decrease in linear growth of mice

(Yakar et al., 2002). In these mice, IGF-I levels were reduced to about 10 to 15% of normal. Thus, circulating IGF-I seems to play a key role in growth.

Implications

In science, new discoveries bring clarity to ideas and concepts that, in some instances, are quite entrenched. The somatomedin hypothesis has evolved substantially and is now better framed because we have an improved understanding of the somatotrophic axis and how the diversity of biological effects of somatotropin are mediated. With respect to the somatomedin hypothesis, there are effects of somatotropin that are mediated by insulin-like growth factor-I, both circulating (endocrine) and locally produced. In addition, there is compelling evidence that somatotropin has effects that are independent of insulin-like growth factor-I. With respect to animal agriculture, a better understanding of somatotropin and insulin-like growth factor-I biology has led to commercially approved products of biotechnology, such as recombinant bovine somatotropin, that have benefited producers who have adopted them. As is always the case, future research will lead to new biotechnological products that will benefit animal agriculture in ways that are presently not envisioned.

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