The Role of Growth Hormone/Insulin-Like Growth Factors in Adipocyte Differentiation

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Growth of the adipose tissue results from both the enlargement of mature adipocytes and the formation of new adipocytes from adipocyte precursor cells. The differentiation process of adipocyte precursor cells is controlled by a variety of hormones. Clinical observations indicate that growth hormone (GH) and insulin-like growth factor I (IGF-I) are able to influence the growth of the adipose organ. Recent in vitro studies using cultures of clonal and primary adipocyte precursor cells have elucidated the role of GH and IGF-I in adipocyte differentiation. From these studies it can be concluded that GH is able to enlarge the pool of adipocyte precursor cells capable of differentiating into mature adipocytes, which occurs under the control of other adipogenic hormones. However, due to its metabolic action, GH is also able to reduce the volume of mature adipocytes and thus the net result of its biological action is aimed at reducing body fat. IGF-I stimulates the differentiation process by inducing critical cell divisions of adipocyte precursor cells necessary for their differentiation. IGF-I, which is known to be regulated by GH and several nutritional factors, may exert its effects in the adipose tissue in an autocrine/paracrine and endocrine way. This review summarizes the results of recent studies investigating the role of GH and IGF-I in adipocyte differentiation.

GROWTH HORMONE (GH) and insulin-like growth factors (IGFs) are important regulators of tissue growth and differentiation.[1-3] The growth- and differentiation-promoting activity of GH may result from a direct action of the hormone and an indirect one, which is mediated by IGF-I. GH regulates IGF-I production in liver cells and in cells of peripheral tissues and thereby influences the amount of IGF-I in the circulation and the extracellular space.[2,3] However, up to now, direct and indirect actions of GH in the living body can hardly be distinguished from each other.[4]

IGF-I and IGF-II have been shown to directly stimulate differentiation of myoblasts, osteoblasts, and other cells of mesodermal origin.[2,5] In blood, IGFs are bound to specific binding proteins, which have been shown to induce both inhibitory and stimulatory effects on IGF action.[6,7] IGF-I and some of its binding proteins are sensitive to nutritional alterations. Serum concentrations of IGF-I decrease with fasting and protein restriction and return to normal with refeeding or increasing dietary protein.[8,9]

In addition to their growth-promoting activity, both GH and IGF-I have several metabolic effects, especially on glucose and lipid metabolism.[2,10]

Clinical observations and in vitro studies have suggested that adipose tissue is a target tissue for the action of IGF-I and GH. IGF-I treatment of patients with Laron's syndrome results in a significant decrease in subcutaneous fat.[11] Children with GH deficiency are generally moderately obese. At the cellular level, they have an increased mean adipocyte volume but a reduced number of fat cells as compared with healthy children. After GH substitution, these changes are shifted toward normal.[12] GH treatment of obese adults also leads to a significant reduction of body fat.[13] GH excess, as found in acromegaly, results in a decrease in body fat.[14] These clinical findings are not only the result of effects of GH and IGF-I on adipose tissue growth, but also of their ability to regulate fat cell metabolism. During the last few years, several in vitro studies have been performed to elucidate the cellular effects of GH and IGF-I in adipose tissue. This review summarizes the results of these studies, as well as data from the authors' laboratories.

ADIPOSE TISSUE GROWTH AND DEVELOPMENT

Body fat increases during childhood and adolescence, and also to a varying degree during adulthood. At the cellular level, growth of the adipose tissue organ results from both enlargement of the existing mature fat cell and proliferation and differentiation of adipocyte precursor cells, particularly during sensitive periods.[15,16] Earlier studies suggested that the number of fat cells becomes fixed during childhood, with only minor changes during later life, and that a close relationship exists between the onset of obesity and the cellular pattern of the enlarged body fat mass.[17,18] However, subsequent studies have extended this observation by showing that adults with severe obesity exhibit an increased total fat cell number independent of the onset of obesity.[19] In support of the latter concept, we have recently demonstrated that adipose tissue of adults contains a remarkable number of specific precursor cells that are able to differentiate into mature fat cells under appropriate conditions.[20]

The characteristic alterations of adipose tissue cellularity in GH deficiency observed 21 years ago[12] have suggested that GH and IGF-I may play an important role in adipocyte differentiation and metabolism. Recent studies of the effects of GH and IGF-I have been facilitated by development of suitable in vitro models. Clonal cell lines such as 3T3 cells, derived from the mesenchymal tissue of embryonic Swiss mice,[21] or ob17 cells, established from epididymal fat pads of *ob/ob* mice,[22] are extensively used for studying adipocyte differentiation, with the advantage of an

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almost unlimited availability of homogenous cells. Primary cultures of adipocyte precursor cells obtained from mammalian adipose tissue samples represent a more physiological model to study adipocyte differentiation. In both cell-culture models, adipocyte precursor cells resemble fibroblasts in the undifferentiated state. These cells are able to proliferate upon stimulation by mitogenic agents, but after growth arrest and depending on the adipogenic activity of the culture medium, they acquire a round shape and accumulate triglycerides. Finally, the cells develop the morphological appearance of adipocytes. After differentiation, the former adipocyte precursor cells exhibit all biochemical characteristics of mature adipocytes.[23]

EFFECTS OF GH ON THE DIFFERENTIATION OF ADIPOCYTE PRECURSOR CELLS

In the clonal cell lines, GH exerted a stimulatory action on fat cell formation.[24-27] According to results obtained in early studies with 3T3-F442A cells, Green et al[28] proposed a "dual-effector model" of GH action. In a first step GH triggers precursor cells to enter the differentiation program, and in a second step IGF-I acts as a mitogen under the control of GH to stimulate the clonal growth of these committed cells. More recent studies with 3T3-F442A cells support this hypothesis by showing that during the first step of GH's action, the cells enter an antimitogenic state in which they are refractory to mitogenic agents.[29] After this arrest of cellular growth, the differentiation program can be expressed under the control of insulin. Other consequences of this GH-induced antimitogenic state are alterations of cytoskeletal protein expression.[30,31]

The molecular mechanisms associated with stimulation of adipocyte differentiation by GH have been intensively studied in ob17 cells. After treatment of these cells with GH, a rapid and transient stimulation of c-*fos* gene transcription can be observed, which seems to be mediated by protein kinase C.[32] Interestingly, this intracellular signaling pathway was also activated in rat adipocytes in response to GH incubation.[33] Other important effects of GH in clonal adipocyte precursor cells include the induction of IGF-I gene expression[34] and an increase in sensitivity of the cells to the action of IGF-I.[35]

In contrast to the finding in clonal preadipocytes, a stimulatory effect of GH on fat cell formation was not observed in primary cultures of adipocyte precursor cells. Hausman and Martin[36] reported a reduction of both size and number of fat cell clusters in cultured pig adipose precursor cells in the presence of GH. In addition, studies in our laboratory have shown that GH inhibits the differentiation of both rat[37] and human (M. Wabitsch, unpublished observation, June 1994) adipocyte precursor cells in a dose-dependent manner. In addition, GH was found to stimulate the production of IGF-I in adipocyte precursor cells.[37] IGF-I, in turn, stimulates cell proliferation in an autocrine/paracrine manner, leading to an enlarged pool of precursor cells.[37] As shown in previous experiments, the factors required for differentiation of human adipocyte precursor cells under serum-free culture conditions include insulin, triiodothyronine, and cortisol.[20] Under these conditions, no cell divisions occur.

The contradictory results for the GH effect on adipose differentiation in cell lines and in primary cultures of adipocyte precursor cells point to substantial differences between the two models. The most likely explanation for this discrepancy is that the two models represent two different stages of the adipocyte lineage. It is likely that the presence of GH is a prerequisite for an early stage of adipose differentiation, as observed in clonal cell lines. In contrast, adipocyte precursor cells obtained from adipose tissue may be in a later stage of the differentiation program. They have already been exposed to GH in vivo and are capable of undergoing terminal differentiation in the absence of GH.

EFFECTS OF IGF-I ON THE DIFFERENTIATION OF ADIPOCYTE PRECURSOR CELLS

There are only a few studies that have investigated the effect of IGF-I on the differentiation of adipocyte precursor cells. In 3T3L1 preadipose cells, IGF-I was found to contribute to fat cell formation. Under both serum-containing and serum-free culture conditions, IGF-I had a dose-dependent stimulatory action on adipose differentiation.[38,39] An adipogenic effect of IGF-I was also reported in adipocyte precursor cells obtained from rat,[40] pig,[41] and rabbit[42] adipose tissue in primary culture. Although the mechanism of action of IGF-I on adipose differentiation is not well understood, it is assumed that this growth factor leads to a clonal expansion of cells susceptible to developing into adipocytes.

Several recent studies suggest that the adipose tissue organ produces large amounts of IGFs and IGF-binding proteins. Peter et al[43] found considerably higher levels of IGF-I mRNA and IGF-I peptide in rat white adipose tissue than in most other tissues, in the same range as those in liver. They concluded that IGF-I produced in adipose tissue may significantly contribute to IGF-I levels in the circulation. IGF-I production was also measured in cultured stromal-vascular cells from rabbit and pig adipose tissue,[42-45] which was under the control of GH[44,45] and glucocorticoids.[42] Kern et al[46] have demonstrated IGF-I production in cultured endothelial cells derived from human adipose tissue. In addition to this observation, we were recently able to measure significant IGF-I and IGF-binding protein-3 production in cultures of human adipocyte precursor cells (M. Wabitsch, unpublished observation, December 1994). The results of these studies suggest that locally produced IGF-I plays an important role in the regulation of adipose tissue growth. Although the physiological function and mechanism of action of IGF-I in adipose tissue is far from being understood, it is tempting to assume that the locally synthesized IGF-I and its binding proteins act in a autocrine or paracrine manner. However, IGF-I levels in the circulation may additionally influence fat cell formation. IGF-I serum levels, which are known to be sensitive to nutritional variations,[8,9] may thereby represent a possible link between nutrition and adipose tissue growth and metabolism.

METABOLIC EFFECTS OF GH AND IGF-I IN FAT CELLS

Besides its modulation of proliferation and differentiation of adipocyte precursor cells, GH has several metabolic effects on mature adipocytes. Whereas the mitogenic action of the hormone is mediated by IGF-I and can be entirely blocked by an IGF-I antibody, the metabolic effects of GH are unrelated to IGF-I synthesis. Clinical observations indicate a strong diabetogenic action of GH by inducing a state of insulin resistance.[47,48] Several in vitro studies that have been summarized recently[49] have elucidated the effects of GH on key metabolic pathways. According to these data, GH reduces glucose uptake, glucose oxidation, and lipogenesis and stimulates release of glycerol from adipocytes.[49] In differentiated 3T3-F442A cells, in which reduction of glucose uptake and lipogenesis by GH can be readily observed,[50] it has been recently shown that GH reduces the number of GLUT1 transporters and GLUT1 mRNA, with no change in GLUT4 protein or mRNA levels.[51] Therefore, the inhibitory effect of GH on the differentiation of adipocyte precursor cells in primary culture seems to be closely related to the decrease in glucose transport and lipogenesis induced by this hormone.[37]

These metabolic effects of GH generally lead to a reduction of body fat by decreasing the mean fat cell volume. The long-term diabetogenic effects of GH, which can be found in vivo and in vitro, are in contrast to the short-term effects of GH.[49] Addition of GH for 1 to 3 hours to adipocytes that have been preincubated without GH results in a transient stimulation of glucose uptake and lipogenesis and an inhibition of lipolysis. The physiological relevance of this insulin-like effect of GH is unclear, since it is not correlated with any clinical observations. However, the pulsatile secretion of GH, which induces constant periods without detectable GH concentrations in serum, could suggest that insulin-like effects of GH may also occur in vivo.

Due to its structural similarity to insulin, IGF-I exerts classic insulin-like effects in all insulin target tissues, including adipose tissue.[3] The stimulatory effect of IGF-I on cellular glucose uptake in preadipocytes and adipocytes seems to be mediated by both the insulin receptor and the IGF-I receptor, depending on the concentration used.[52] However, stimulation of lipoprotein lipase activity by IGF-I in human adipocytes can be totally blocked by an antibody against the IGF-I receptor,[46] indicating that this stimulation is fully mediated through the IGF-I receptor. The effects of IGF-I on lipolysis seem to depend on the concentration used. IGF-I is antilipolytic when used in sufficiently high concentrations to activate the insulin receptor.[53] However, IGF-I and IGF-II have a lipolytic effect at low concentrations.[54] The mechanisms responsible for the beneficial effect of IGF-I on glucose metabolism in diabetic patients during IGF-I treatment,[55-57] which also comprises a normalization of insulin-stimulated glucose transport in adipocytes from these patients,[58] are still unknown and require further investigation.

Table 1. Effects of GH and IGF-I on Adipocyte Precursor Cells and Mature Adipocytes in Primary Culture

	Adipocyte Precursor Cells	Mature Adipocytes
GH	Stimulation of IGF-I production	Decrease in glucose transport
	Inhibition of differentiation	Decrease in lipogenesis
		Increase in lipolysis
IGF-I	Stimulation of proliferation	Increase in glucose transport
		Increase in lipogenesis
		Inhibition of lipolysis

Table 1 shows a summary of the effects of GH and IGF-I on adipocyte precursor cells and mature adipocytes in primary culture.

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