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Activation of IGF-1 System During Adipogenesis: Examination In Vitro and In Vivo
(Under the Direction of ROY JOSEPH MARTIN)

Studies were designed to examine the local IGF-1 system in adipose tissue development. The first experiment gave direct evidence of IGF-1 receptors on preadipocytes in primary cultures using fluorescence double-label immunocytochemistry verifying that IGF-1 could directly act on preadipocytes. The second study compared IGF-1 and IGF-1 receptor mRNA expression in white adipose tissues of 12-wk-old genetically obese Zucker rats with that of lean controls using relative quantitative RT-PCR. Fat depot specific differences in IGF-1 and IGF-1 receptor expression were detected, with expression generally higher in tissues from the obese as compared with the lean rats. The IGF-1 mRNA levels were higher than that of IGF-1 receptor within the same depot. In vivo the regulation of IGF-1 and IGF-1 receptor in adipose tissue of obese rats is depot-specific and happens at the transcriptional level. The activation of local IGF-1 system in adipose is mainly through the increase of IGF-1 receptor levels in obesity.

INDEX WORDS: Obesity, Preadipocytes, Primary Cell Culture, Immunocytochemistry, Zucker rats, Adipose tissue, Insulin-Like Growth Factor-1, Insulin-Like Growth Factor-1 Receptor, Expression, RT-PCR

ACTIVATION OF IGF-1 SYSTEM DURING ADIPOGENESIS: EXAMINATION IN VITRO AND IN VIVO

by

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CHAPTER 1

INTRODUCTION

Obesity is beginning to replace undernutrition and infectious disease as the most significant contributor to ill health, an epidemic threatening global well-being (1). More than half of adult Americans are overweight or obese (2). There is considerable evidence indicating that higher levels of body weight and body fat are associated with an increased risk for the development of numerous adverse health consequences (2). Understanding of the mechanisms regulating the growth of adipose tissue could lead to the development of effective methods for both the prevention and treatment of obesity.

The growth of adipose tissue depends on stem cell recruitment to become preadipocytes, the proliferation of preadipocytes and the differentiation of preadipocytes to mature adipocytes (3).The factors which stimulate formation of new fat cells and the mechanism adopted in the process have not been clearly identified. Recent studies show that cells within adipose tissue participate actively in the development of fat tissue in a paracrine/autocrine fashion by secreting a large number of factors (4). IGF-1 is highly expressed in adipose tissue (5) and may act as a paracrine factor for adipose tissue growth. Previous studies clearly showed the presence of IGF-1 receptors in preadipocytes cell lines (6, 7, 8). However, the available data of the presence of IGF-1 receptor on preadipocytes in primary cell cultures are controversial (9,10,11,12,13). Because of the complexity of the cell types included in primary cultures of stromal vascular cells derived from adipose tissue, there is still no direct evidence of the presence of IGF-1 receptor specifically on preadipocytes.

In vitro IGF-1 can stimulate both the proliferation and differentiation of preadipocytes based on the cell stage at the time of treatment (14). Long-term, local delivery of IGF-1 by microspheres induced de novo adipose tissue generation in vivo in a

rat model (15). However, little has been done to characterize in vivo IGF-1 and IGF-1 receptor expression levels in white adipose tissue at adulthood stage during periods of rapid adipose tissue expansion in the development of obesity.

We designed two experiments to test the hypothesis that the IGF-1 system is activated to stimulate adipogenesis in obese animals undergoing rapid adipose tissue expansion.

The first experiment is designed to provide direct evidence of the presence of IGF-1 receptors on preadipocytes in primary cultures using fluorescence double-label immunocytochemistry. This experiment employs AD3 monoclonal antibody as a marker of preadipocytes in rat stromal vascular (SV) cell culture. Immunohistology of adipose tissue sections of IGF-1Rα staining provide further in vivo support of the presence of IGF-1 receptors in adipose tissue.

The second study compares IGF-1 and IGF-1 receptor mRNA expression in white adipose tissue of adult genetically obese Zucker rats with that of lean controls during a rapid phase of adipose tissue expansion. Relative quantitative RT-PCR is used in this study to detect the expression of IGF-1 and IGF-1 receptor mRNA in inguinal, mesenteric and epididymal fat depots. The 18S RNA is the internal standard for relative quantitative RT-PCR.

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CHAPTER 2

REVIEW OF THE LITERATURE

Obesity, defined as an increased amount of body fat and estimated by different techniques (1), is a chronic and costly disease that is rarely curable. The clinical problem of obesity is associated with an increase in numerous health risk factors and severity of the associated diseases increases with the degree of expansion of the adipose tissue mass (2). Improvements of our understanding of the molecular mechanisms regulating body fat mass will provide potential opportunities for therapeutic intervention and bring renewed hope and vitality to the development of anti-obesity drugs (3).

Models for Studying Obesity

IN VITRO MODELS:

Much of our current understanding of the molecular and cellular events in adipogenesis comes from in vitro cell culture systems (4, 5). The 3T3 and the C3H10T1/2 cell lines may represent pluripotent stem cells, which can be differentiated into multiple cell types when exposed to 5-azacytidine, a DNA demethylating agent (6). Other frequently employed cell lines are 3T3-F442A, 3T3-L1 and Ob1771 cells. These are already committed to the adipocyte lineage and therefore are considered to be preadipocytes. By using appropriate stimuli, embryonic stem cells can be differentiated into mature adipocytes in vitro and are useful systems for investigating the commitment to the adipose lineage (7).

Another in vitro system used in studying adipocyte development is primary cell culture. Primary cell culture of preadipocytes is derived from the seeded stromal-vascular fraction (SV) of adipose tissue. Primary SV cells contains preadipocytes as well as

multiple other cell types, such as endothelial cells, fibroblasts and mesothelial cells (8). A recent report showed that multipotent stem cells are present within SV fraction of human adipose tissue (9). In general, preadipocytes within the SV fraction from various animals are similar, but their responses to growth factors vary considerably (10). The interpretation of results among studies using different source of SV cells should be done carefully.

IN VIVO MODELS:

Humans, rats, mice and pigs are often used for the in vivo examination of adipose tissue development. The Zucker fatty rat is one of the widely studied genetic animal models of obesity used to characterize the development of adipose tissue. Obesity in the Zucker rat is transmitted through autosomal recessive inheritance of the missense mutation in the leptin receptor gene (11). Previous studies revealed that in Zucker rats adipocyte hypertrophy precedes hyperplasia, resembling the pattern of early onset obesity in humans (12, 13). Also fat cell hypertrophy accompanies the development of the hyperplastic obesity in the Zucker rat (13, 14, 15). Starting from an early age (3-5 wk), the mass of all fat depots studied is greater in obese rats compared with lean rats (16, 13, 14, 15). From 3 to 15 wk the pad weights of inguinal and epididymal depots increase progressively in obese Zucker rats and a similar pattern of development is also observed in the epididymal depot of lean rats (16). In contrast, the inguinal pad weight of lean rats increases progressively only until 12 wk, with no further significant increase observed at week 15 (16). In the epididymal depot of the developing obese rat, thymidine kinase activity is elevated until 273 days of age and total tissue DNA accretion increases until 182 days of age (13). The secretion of paracrine growth factors from adipose tissue of obese Zucker rats was reported to be associated with fat cell size (16). Conditioned medium of adipose tissue of obese Zucker rats demonstrated significantly higher proliferative activities as compared with that from age-matched lean controls when assayed utilizing cultured stromal-vascular cells (including preadipocytes). Furthermore,

12-wk-old age groups had the highest proliferative activity of all ages studied (16). These studies demonstrate that obese Zucker rats at 12-wk-old are undergoing rapid adipose tissue expansion and suggest the possible involvement of paracrine factors in the regulation of adipose tissue expansion.

Adipose Tissue as an Endocrine and Paracine Organ

Adipose tissue is actively involved in the regulation of the development of fat tissue and energy balance, through a network of endocrine, paracrine and autocrine signals (17). The discovery of the adipocyte-derived hormone leptin has greatly changed obesity research and our understanding of energy homeostasis (18). Increased tumor necrosis factor alpha (TNF-alpha) production has been observed in adipose tissue derived from obese rodents or human subjects and TNF-alpha has been implicated as a causative factor in obesity-associated insulin resistance and the pathogenesis of type 2 diabetes (19, 20). The regulated production of interleukin-6 (IL-6) in adipose tissue may modulate regional adipose tissue metabolism and contribute to the reported correlation between serum IL-6 and the level of obesity (21). Elevated expression of plasminogen activator inhibitor 1 (PAI-1) in adipose tissue also have been reported (22). Recently resistin, a new unique signaling molecule, was reported to be secreted by adipocytes and potentially links obesity to diabetes (23). IGF-1 is another paracrine factor that is highly expressed in adipose tissue (24). Various studies have suggested that the IGF system could play an important role in regulating the development of adipose tissue in obesity and our present study is designed to test this hypothesis.

IGF System and Adipose Tissue Development

INTRODUCTION TO IGF-1 SYSTEM:

IGF-1, IGF-1 receptor and IGF binding proteins (IGFBPs) are three major components of IGF system:

IGF-1. The study of insulin-like growth factors (IGFs) began in 1978 with the purification and sequencing of human IGF-1 (25). IGF-1 peptide is a 70-residue single-chain peptide consisting of four linearly contiguous domains, termed B, C, A, and D. IGF-1 is highly conserved among mammals. In rats and in humans, the single-copy IGF-1 gene spans more than 80kb of chromosomal DNA, and consists of six exons and five introns. Exon 3 encodes the distal common 27 amino acids of the signal peptide and most of the B domain of mature IGF-1, whereas exon 4 encodes the remainder of the 70-residue IGF-1 (26). IGF-1 is widely expressed and produced across different tissues in the body (24); liver is the principle source of circulating IGF-1 (27).

IGF-1 Receptor. The mature IGF-1 receptor is a tyrosine kinase receptor with four disulfide-linked subunits as a β-α-α-β heterotetramer. The single-copy IGF-1 receptor gene spans more than 100 kilobases of genomic DNA and contains 21 exons. Exons 1-10 encode from the N-terminal signal peptide to the α-subunit and the β-subunit is encoded by exons 12-21. The proteolytic cleavage site that generates mature α- and β-subunits from polypeptide precursor is encoded by exon 11. In the adult rat, highest levels of expression of IGF-1 receptor are seen in the central nervous system, whereas, intermediate levels of mRNA are produced in other tissues such as kidney, stomach, testes, lung and heart (28).

IGF Binding Proteins. IGFBPs bind IGF-1 with high affinity, but share no homology with the IGF-1 receptor. Due to primary structure differences, these structurally related proteins are characterized by different biochemical properties and biological functions. Basically there are six high-affinity IGF-binding proteins: IGFBP-1, -2, -3, -4, -5 and -6. IGFBPs can modulate IGF-1 actions both positively and negatively, besides their own IGF-independent actions (29, 30).

IGF system. IGF-1 is a potent mitogen for many different cell types (31). Locally produced IGF-1 has an important role in tissue development and this process involves mitogenic stimulation and cell division which needs to be well controlled. First, the strength of the IGF-1 signal is determined by the number of receptors expressed on target cells. Second, the larger soluble IGF-1 binding proteins regulate the biological activities of IGF-1 by interaction with IGF-1. The binding proteins can protect IGF-1 against degradation, facilitate the transportation of IGF-1 across body compartments and interfere with the interaction between IGF-1 and cell surface receptors (32). Last, the amounts of IGF-1 produced and secreted by the cells capable of its synthesis regulate the IGF-1 bioavailability (33)

LOCAL IGF-1 SYSTEM IN WHITE ADIPOSE TISSUE:

IGF-1. It is well established that IGF-1 is produced by white adipose tissue. Studies showed that in pigs fetal hypophysectomy reduced IGF-1 expression in subcutaneous (SQ) adipose tissue and thyroxine treatment increased expression of IGF-1 in the outer SQ adipose tissue layer (34). In adipose tissues of growing Wistar rats the expression of IGF-1 is region-specific, quantitatively in the order: retroperitoneal ≈ epididymal > mesenteric > subcutaneous inguinal (35). The region-specific expression of IGF-1 is highly correlated to the expression of leptin expression in adipose tissues (35). In adult rats, abundant IGF-1 mRNA and IGF-1 peptide levels have been found in white

adipose tissue (WAT), particularly the IGF-1 mRNA level in WAT is comparable to that found in liver, the principle source of circulating IGF-1 (24). Growth hormone (GH) is one of the main regulators of IGF-1 expression in some fat depots (epididymal and perirenal fat) in adult individuals (24,36). IGF-1 mRNA and IGF-1 peptide levels decrease drastically (95%) in WAT after hypophysectomy and are restored to near normal (80%) by GH treatment in vivo (24). In cultured human preadipocytes GH positively regulates IGF-1 secretion, but shows no effect on IGF-1 secretion in cultured human adipocytes (37). Insulin is found to be a positive regulator of and cortisol is to be found to be a negative regulator of IGF-1 secretion in cultured human preadipocytes and adipocytes (37, 38).

IGF-1 receptor. Previous studies have clearly demonstrated the presence of IGF-1 receptors on preadipocyte cell lines by ligand binding (39, 40) and mRNA expression (41). The presence of IGF-1 receptors on primary cultures of stromal vascular (S-V) cells derived from adipose tissue of many species has also been detected by ligand binding (42, 43), mRNA (44), Western blotting (45), and immunocytochemistry (46). Due to the complexity of cell types included in primary cultures of stromal vascular cells derived from adipose tissue, there is still no direct evidence of the presence of IGF-1 receptors specifically on preadipocytes.

There are very few reports about the regulation of IGF-1 receptor expression in white adipose tissue in adult individuals. Androgens and estrogens were reported to have opposite effects on adipogenesis in rat preadipocytes (45). 17 β-estradiol (0.01 uM) increased the proliferative capacities of preadipocytes in superficial (femoral sc) fat depot from adult female rats and IGF-1 receptor levels were enhanced by 17 β-estradiol (0.01 uM) in sc preadipocytes from female ovariectomized rats. Dihydrotestosterone (DHT) decreased both IGF-1 receptor levels (0.01 uM DHT) and glycerol 3-phosphate dehydrogenase activity (0.1 uM DHT) in epididymal preadipocytes, but failed to affect

growth rate of preadipocytes (45). The possible involvement of the IGF-1 receptor in the process is suggested (45).

IGFBPs. There is considerable evidence that IGFBPs are produced by adipocyte/preadipocyte and adipose tissue (47, 48). In adult rats, northern blot analysis demonstrated that white adipose tissue contains mRNAs for IGFBP-2,3,4,5, and 6. IGFBP-5 and 6 are the two most abundant IGFBPs and correlate with GH level (24). IGFBP-3 expression was unchanged by hypophysectomy, but rose after IGF-1 and GH treatment. The IGFBP-4 mRNA level was not regulated by GH. IGFBP-2 mRNA levels were very low in WAT, but were regulated by GH (24).

IGF SYSTEM AND ADIPOGENESIS:

IGF-1 is an adipogenic factor (49). Mediated through its own specific receptors, IGF-1 plays a significant role in regulating the development of adipose tissue and this regulation is modulated by IGFBPs.

In vitro, IGF-1 stimulates the proliferation of preadipocytes from both cell lines like 3T3-L1 cells and primary cell cultures. Exogenous IGF-1 increased replication of porcine preadipocytes in primary cultures of stromal vascular (S-V) cells (50). Once growth arrest occurs at confluence and cells become quiescent, IGF-1 stimulates differentiation (51, 52).

In vivo, long-term, local delivery of IGF-1 by microspheres induced de novo adipose tissue generation in a rat model. At the 4-week harvest period, multiple ectopic islands of adipose tissue were observed on the abdominal wall of the animals treated with IGF-1 containing microspheres which were administered directly to the deep muscular fasica of the adult rats abdominal wall (53). Insulin-like growth factor binding protein-1 transgenic mice have impaired adipogenesis. When fed with a sucrose-enriched diet, IGFBP-1 transgenic mice gained significantly less body weight and the epididymal fat

mass was significantly reduced compared with wild-type mice (54). All these studies support that IGF-1 is a very important adipogenic factor for preadipocytes.

Local IGF-1 System and Obesity

REGULATION:

IGF-1. Our preliminary data demonstrated that significantly more IGF-1 is present in each fat pad of obese Zucker rats as compared with lean controls (55). Total IGF-1 content of fat pads studied was 3-fold higher in obese vs. lean rats. Expressed as ng IGF-1/mg protein, there were no differences in IGF-1 levels within adipose tissues of lean animals. Within the obese adipose tissues, IGF-1 levels in the inguinal fat pads was significantly greater than that of the epididymal or retroperitoneal fat pads.

Hypophysectomy and growth hormone infusion studies in adult normal rats demonstrated that GH is the main regulator of IGF-1 gene expression in epididymal fat depots (24, 36). It is well established that GH secretion is markedly blunted both in obese humans and in animal models of obesity (56, 57). But in contrast to classic GH deficiency, obesity is not associated with a decrease of circulating IGF-1 and reduced somatic growth (57). Whether the decrease in GH secretion is secondary to the obese state remains to be determined. In genetically Obese Zucker rats, the secretion of growth hormone is impaired (58), however the pattern of tissue-specific response of IGF-1 expression to obesity-associated GH decline (59) is different from that of GH deficiency in hypophysectomized rats (60).

We still do not know how the IGF-1 expression in white adipose tissues is regulated in obese individuals compared to lean controls. Further, body fat is not a unitary organ. Considering the regional difference of fat depots, there are more studies needed to address the detailed expression pattern of IGF-1 in different fat depots. When normalized for total protein content, IGF-1 levels were lower in epididymal and retroperoitoneal fat depots of obese compared to lean controls, but similar as lean

controls for the inguinal fat depot (55). 14-day infusion of GH plus IGF-1 induced a decrease in the relative fat weight to the inguinal site in the growing Zucker rats (61). However the effect was limited to the inguinal site, GH infusion has no effect on retroperitoneal fat weight.

IGF-1 receptor. There are few studies on the expression IGF-1 receptor in white adipose tissue of obese subjects. Studies on other cell types showed that IGF-1 down-regulated the expression of IGF-1 receptor (62). Liver, the principle source of circulating IGF-1, expressed high levels of IGF-1 (the same level as adipose tissue) and low levels of IGF-1 receptor (28). Thus, highly expressed IGF-1 in adipose tissue could act on its own receptor expression. The regulation of IGF-1 receptor expression in adipose tissues of obese individuals remains to be determined.

IGFBPs. A decrease in the expression of IGFBPs is another possibility to activate the IGF system in obese animals undergoing rapid adipose tissue expansion. In ob/ob mice, IGFBP-5 mRNA expression decreased compared with lean controls (63). In vivo, the expression of IGFBP-6, one of the major IGFBPs in adipose tissue, decreased under the GH decline by hypophysectomy, while there was no change for IGFBP-5 expression (24).

FUNCTION:

IGF-1 is well demonstrated to stimulate both the proliferation and differentiation of preadipocytes/adipocytes in vitro. Our studies demonstrated that IGF-1 mediated high-fat (HF) diet-induced adipogenesis in Osborne-Mendel rats. Proliferative activity of adipose tissue conditioned medium (ATCM) prepared from HF-fed rats was attenuated after the stripping of IGF-1 from the medium (64). All of these studies as mentioned above suggest IGF-1 may be a paracrine factor physiologically involved in the adipose tissue expansion during obesity. Further studies should be done to explore the exact

functions of IGF system in vivo in the development of obesity, such as how the whole system is regulated in obese animals compared to lean controls. Further, it remains to be determined whether blockage of IGF-1 signaling pathway could stop or attenuate the adipose tissue expansion in obesity.

Summary

Obesity is an epidemic threatening global well-being. However, the mechanisms employed in the adipose tissue expansion in this disease are not clear. Both in vitro studies using preadipocytes cell culture and in vivo studies using obese animal models have improved our understanding the physiological and molecular events associated with adipose tissue growth. These studies have established that adipose tissue is actively involved in the regulation of the development of obesity by the production of numerous paracrine factors. IGF-1 is one of the strong candidates for paracrine factors that stimulate adipogenesis during the progression of obeisty. The functions of these factors in adipose tissue have not been clearly identified. Understanding the mechanisms and effect of paracrine factors in adipose tissue could lead to the development of effective methods for both the prevention and treatment of obesity.

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CHAPTER 3

IMMUNOCYTOCHEMICAL LOCALIZATION OF IGF-1 RECEPTOR ON RAT PREADIPOCYTES[1]

[1]Peng X, Martin RJ, Hausman DB. To be submitted to Obesity Research.

Abstract

Insulin-like growth factor-1 (IGF-1) plays a significant role in regulating the development of adipocytes, but the available data of the presence of IGF-1 receptors on preadipocytes are controversial. We report the co-localization of IGF-1 receptors and AD-3 antigen (marker of preadipocytes) in primary cultures of rat adipose tissue stromal-vascular cells using fluorescence double-label immunocytochemistry. After varying times in serum-free culture, cells were fixed and then incubated with a combination of primary antibodies (AD-3 and the IGF-1 receptor). Cells were incubated sequentially with two fluorescent secondary antibodies (FITC anti-mouse IgG and TRITC anti- rabbit IgG). Preparations were examined using a BioRad confocal laser microscope system. Immunocytochemical results directly show the localization of IGF-1 receptors on rat preadipocytes specifically identified by AD-3. Most AD-3 labeled cells also stained for the IGF-1 receptor. Also other cell types in primary cell culture of rat adipose tissue stromal-vascular cells contain IGF-1 receptors, in agreement with our studies in rat tissue sections. The finding of IGF-1 receptors on preadipocytes is support that IGF-1 can directly act on preadipocytes.

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Introduction

Insulin-like growth factor-1 (IGF-1) plays a significant role in regulating the development of adipocytes, but the available data of the presence of IGF-1 receptors on preadipocytes are controversial. Previous studies have clearly demonstrated the presence of IGF-1 receptors on preadipocyte cell lines by ligand binding (1, 2) and mRNA expression (3). The presence of IGF-1 receptors on primary cultures of stromal vascular

(S-V) cells derived from adipose tissue of many species has also been detected by ligand binding (4 (rabbit), 5 (pig)), mRNA (6 (pig)), Western blotting (7 (rat)), and immunocytochemistry (8 (pig)). However, because of the complexity of cell types included in primary cultures of stromal vascular cells derived from adipose tissue, there is still no direct evidence of the presence of IGF-1 receptors specifically on preadipocytes. We report here the co-localization of IGF-1 receptors and AD-3 antigen (marker of preadipocytes) in primary cultures of rat adipose tissue stromal-vascular cells using fluorescence double-label immunocytochemistry.

Materials and Methods

Primary Cell culture. Stromal-vascular (S-V) cells, including preadipocytes, were obtained from the inguinal adipose tissue of specific pathogen-free male Sprague-Dawley rats (80-100g: Harlan Sprague Dawley, Indianapolis, IN) as described by Kras et al (9). Briefly, animals were anethetized and inguinal adipose tissue was removed aseptically and pooled. Minced adipose tissue was incubated with collagenase type 1 (Worthington Biomedical, Freehold, NJ) in a 37°C shaking water bath. After 90 minutes, digests were filtered and centrifuged. Cells in the S-V pellet were washed with plating medium (DMEM/F12 + 10% fetal bovine serum), diluted and seeded on 35-mm tissue culture plates at 1.0×10^4 cells/cm^2. Cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 24hr, plating medium was removed and replaced with serum-free DMEM/F12 medium containing 100 nM insulin, 7.4 nM transferin, 3.4 nM sodium selenite and 2 nM triiodothyronine. Medium was subsequently changed every 2-3 days. Dishes were removed from culture 24hr, 72hr, 120hr, 168hr, 195hr, 238hr, after plating.

Fluorescent Double Staining of Primary Cell culture. Dual-label immunohistochemisty was conducted as described (8) with some modifications. Briefly,

after varying times in serum-free culture, cells were fixed in 4% paraformaldehyde for 30 min at room temperature or 4°C overnight, rinsed for 15 min in phosphate buffered saline (PBS), and then incubated with a combination of primary antibodies (AD-3; 9) and the IGF-1 receptor (1:50 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA). After rinsing with PBS for 15 min, cells were incubated sequentially with two fluorescent secondary antibodies (1:100 dilution) (FITC anti-mouse IgG (Sigma, St. Louis, MO) and TRITC anti- rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA)), rinsed three times, mounted with elvanol and covered with cover slips. Preparations were examined using a BioRad confocal laser microscope system. Control experiments were performed in which dishes were incubated without one or two primary antibodies.

Immunohistology. Inguinal tissues from young (4-6 wk) male Sprague-Dawley rats were fixed with formalin, sectioned and deparaffinized. Following the manufacturer's protocol, tissue sections were unmasked by using heat treatment in sodium citrate buffer (pH 6, 10mM) at 95 °C for 15 min before incubation with anti-IGF-1Rα primary antibody (1: 100 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibodies were detected using mouse ExtrAvidin® Peroxidase Staining kit (Sigma, St. Louis, MO) and 3-Amino-9-ethylcarbazole (AEC) as peroxidase substrate.

Results

The immuno-reactivity of both AD3 antigen and IGF-1 receptor increases with the increasing culture time (Fig 1, 2). With the differentiation into mature adipocytes, the signal of individual staining, both AD3 and IGF-1 receptor, increased on the single cells. Both AD3 and IGF-1 receptor positive cells were detectable at all the time points. Preadipocytes, fibroblast-like cells marked by AD3 staining, were IGF-1 receptor positive (Fig 1, 2). Clearly, lipid-filled mature adipocytes were IGF-1 receptor positive,

also AD3 positive (Fig 2). Many IGF-1 receptor positive but AD3 negative cells occurred at all the time points. Immunohistology of adipose tissue sections indicated IGF-1 receptor staining of both lipid-filled fat cells as well as other types of cells (Fig 3).

Discussion

Recent studies have suggested that a large number of factors are secreted by adipose tissue and that these factors are actively involved in the regulation of the growth of adipose tissue in a paracrine/autocrine fashion (reviewed in 11). The function of these paracrine factors in adipose tissue has not been clearly defined. In order to meet the criteria for a paracrine role in adipose tissue expansion we propose the following criteria must be met by the potential factors: 1) it has a direct effect on in vitro preadipocyte proliferation, 2) it is synthesized and released from fat cells or other cell types located in fat pads, 3) there are specific receptors for the factor on preadipocytes, 4) it is released at concentrations in vivo that affect proliferation in vitro, and 5) there is a loss of proliferative activity if the factor is not present.

In this study we directly demonstrated the presence of IGF-1 receptors on preadipocytes in primary cultures of rat adipose tissue stromal-vascular cells using fluorescence double-label immunocytochemistry. This result strongly supports the notion that IGF-1 may stimulate the proliferation of preadipocytes in vivo through mediation by the specific IGF-1 receptor. Not all AD3 positive cells stained for IGF-1R, however, suggesting that the population of adipocytes (AD3 positive) is heterogenous. The further characterization of AD3 antigen could help us identify the AD3 cell population more precisely. We also demonstrated that lipid-filled mature adipocytes are IGF-1Rα positive. This is in agreement with some previous studies (6, 3, 12, 13), but not with other studies (14, 8). Further studies are needed to determine whether the differences are related to the species or to the methods used to detect the IGF-1 receptor.

In conclusion, in this study we provide direct evidence for the presence of IGF-1 receptors on preadipocytes. This, along with other studies of the IGF-1 stimulation of preadipocyte proliferation, and higher levels of IGF-1 in fat tissue from obese subjects, lends further support for IGF-1 as a physiologically relevant paracrine factor involved in adipose tissue expansion during obesity.

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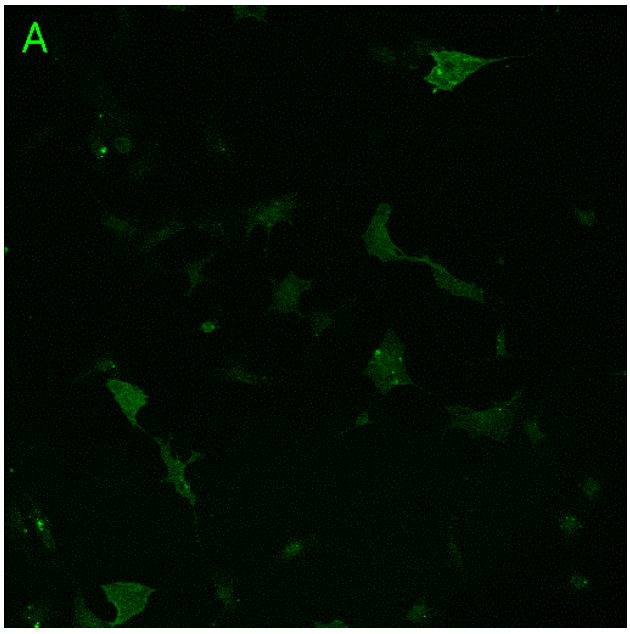
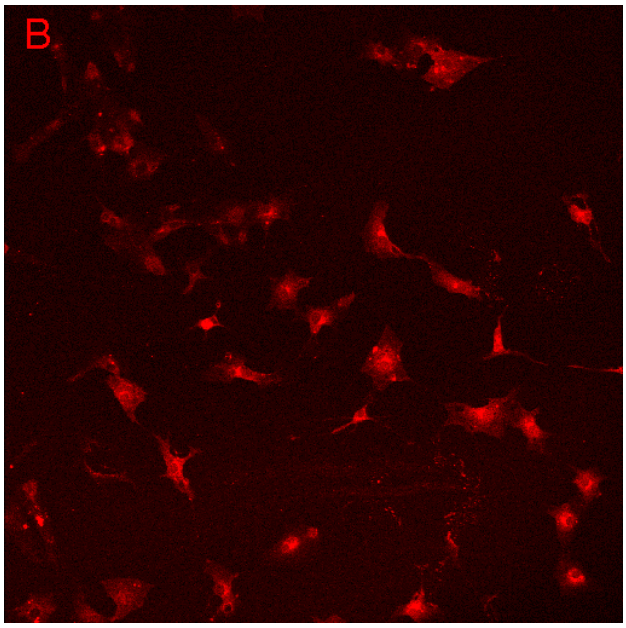
Figure 3.1 Immunofluorescent microscopy of double-labeled preadipocyte primary cell culture (72 hr after plating). A shows reactivity for AD3 and B for IGF-1Rα, C is the overlapping of A and B. White arrow shows a cell both IGF-1Rα and AD3 positive, red arrow shows a cell IGF-1Rα positive but AD3 negative. ×200.


A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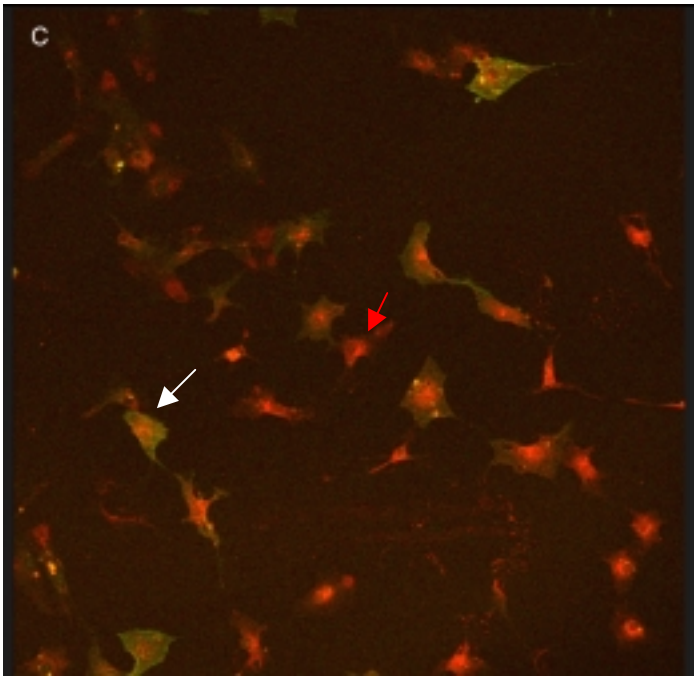
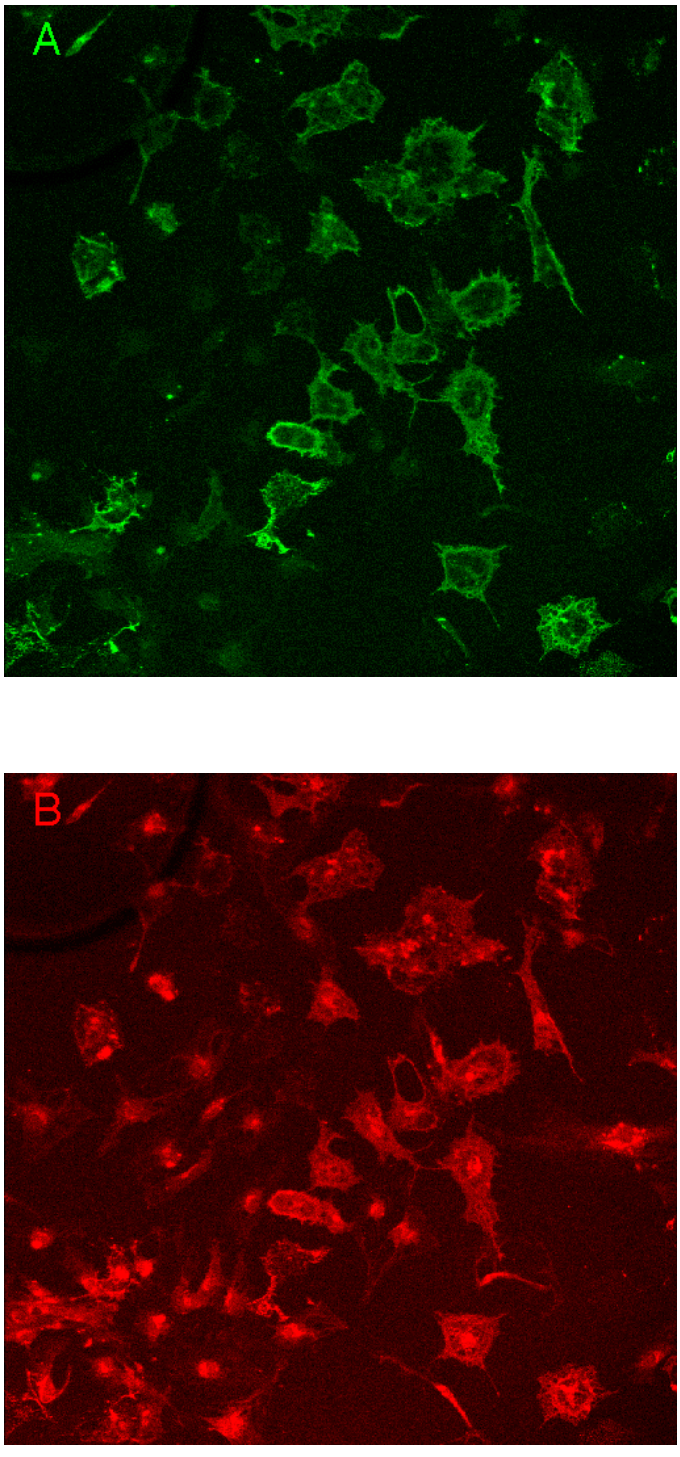

C

Figure 3.2 Immunofluoresent microscopy of the double-labeled preadipocyte primary cell culture (168 hr after plating). A shows reactivity for AD3 and B for IGF-1Rα, C is the overlapping of A and B. White arrow shows a cell both IGF-1Rα and AD3 positive, red arrow shows a cell IGF-1Rα positive but AD3 negative. ×200.


A
B

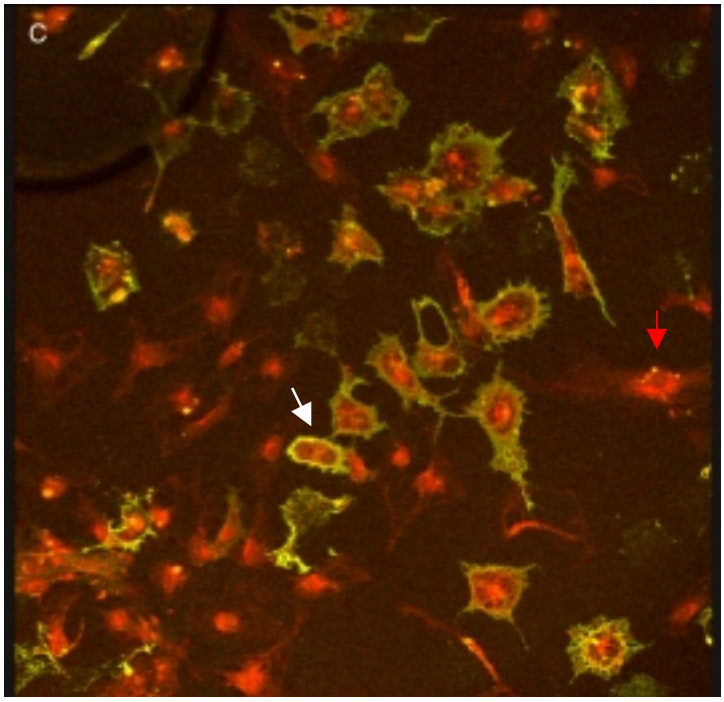
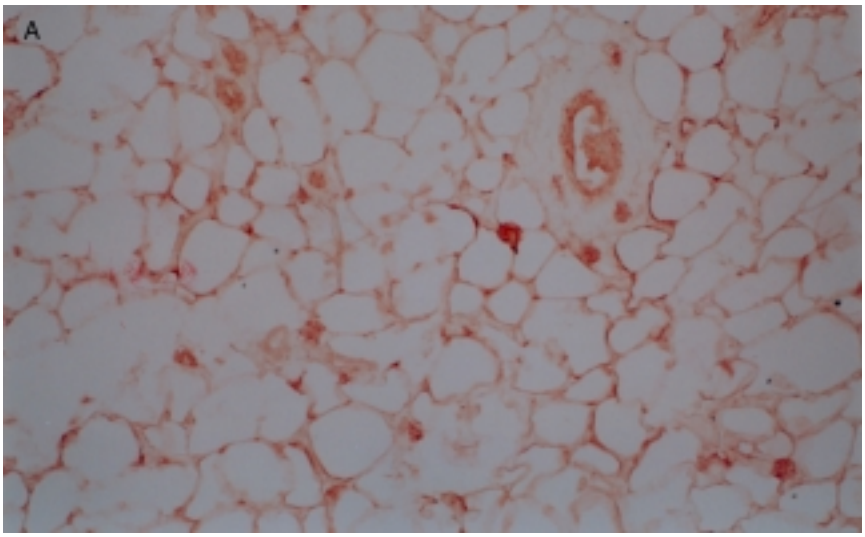

C

Figure 3.3 Light microscopy of immunohistological-stained section of inguinal adipose tissue sections from young (4-6 wk) rat showing A the reactivity of adipocytes and other types of cells for IGF-1Rα. B negative control without primary antibody. ×200.


A


B

CHAPTER 4

CHARACTERIZATION OF IGF-1 SYSTEM IN WHITE ADIPOSE TISSUE OF 12 WEEK OLD MALE LEAN AND OBESE ZUCKER RATS[1]

[1]Peng X, Dean RG, Martin RJ, Hausman DB. To be submitted to Obesity Research.

Abstract

The expressions of insulin-like growth factor-1 (IGF-1) and IGF-1 receptor were studied in inguinal, epididymal and mesenteric fat depots of both obese and lean 12-wk-old male Zucker rats. Using 18S RNA as the internal standard, relative quantitative RT-PCR was employed to characterize IGF-1 and IGF-1 receptor mRNA levels. IGF-1 mRNA levels were found higher than IGF-1 receptor mRNA levels within the same depot in all the depots studied, based on the optimal ratio of 18S primers:competimers used for the amplification of IGF-1 and IGF-1 receptor cDNA. In the inguinal fat depot of obese rats, both IGF-1 and IGF-1 receptor mRNA levels were increased compared to that of lean controls (IGF-1: $p < 0.01$; IGF-1 receptor: $p < 0.05$). IGF-1 receptor mRNA levels were similar between obese and lean rats in epididymal fat depots, whereas IGF-1 mRNA levels were decreased in obese rats as compared with lean rats ($P < 0.05$). In the mesenteric depot the expression of IGF-1 receptor was greater for the obese rats as compared with the lean controls ($P < 0.05$), however, the increase was smaller than that observed in the inguinal fat depot. There was no change of IGF-1 mRNA levels between obese and lean rats in this depot. Results in this study demonstrated that both IGF-1 and IGF-1 receptor are regulated at transcriptional level in adipose tissues of obese rats as compared with lean controls during obesity. The region-specific pattern suggests different pathways are involved in the regulation of IGF-1 and IGF-1 receptor in different depots. The higher IGF-1 mRNA levels than that of IGF-1 receptor within the same depot implies that IGF-1 receptor numbers on cell surfaces plays an important role in controlling the passage of IGF-1 signals. The tendency to increase IGF-1 receptor mRNA levels suggests that the stimulation of adipogenesis by the activation of the IGF system succeeds through the increase of IGF-1 receptor numbers as a result of increased expression of IGF-1 receptor.

Introduction

Obesity is beginning to replace undernutrition and infectious disease as the most significant contributor to ill health, an epidemic threatening global well being (1). More than half of adult Americans are overweight or obese (2). There is considerable evidence indicating that higher levels of body weight and body fat are associated with an increased risk for the development of numerous adverse health consequences (2).

Recent studies show that cells within adipose tissue participate actively in the development of fat tissue in a paracrine/autocrine fashion by secreting a large number of factors (reviewed in 3). The discovery of the adipocyte-derived hormone leptin has greatly changed obesity research and our understanding of energy homeostasis (4). Increased tumor necrosis factor alpha (TNF-alpha) production has been observed in adipose tissue derived from obese rodents or human subjects and TNF-alpha has been implicated as a causative factor in obesity-associated insulin resistance and the pathogenesis of type 2 diabetes (5, 6). The regulated production of interleukin-6 (IL-6) in adipose tissue may modulate regional adipose tissue metabolism and contribute to the reported correlation between serum IL-6 and the level of obesity (7). Elevated expression of plasminogen activator inhibitor 1 (PAI-1) in adipose tissue also have been reported (8). Recently resistin, a new unique signaling molecule, was reported to be secreted by adipocytes and potentially links obesity to diabetes (9).

IGF-I is another regulatory factor highly expressed in adipose tissue (10). In vitro IGF-I can stimulate both the proliferation and differentiation of preadipocytes based on the cell stage at the time of treatment (11). Long-term, local delivery of IGF-I by microspheres induced de novo adipose tissue generation in vivo in a rat model (12). However, little has been done to characterize in vivo IGF-1 and IGF-1 receptor expression levels in white adipose tissue at the adulthood stage during the development of obesity.

This study compares IGF-I and IGF-I receptor mRNA expression in white adipose tissue of adult genetically obese Zucker rats with that of lean controls during a rapid phase of adipose tissue expansion.

Materials and Methods

Animals. Sixteen 12 week old male Zucker rats (the University of Georgia colony) were grouped into two groups, obese and lean control. The number of animals per group (n = 8) was determined using power analysis (95%) and the standard deviation was estimated based on a published study (13). Animals were housed in hanging wire cages in a room environmentally regulated for temperature (23 ± 3 °C) and humidity (40-50%), with a 12:12-h light-dark cycle. Rats had free access to pelleted diet (Ralston Purina, St. Louis, MO) and tap water throughout the duration of the study. All procedures for the care of the animals used in this study were approved by the University of Georgia Institutional Animal Care and Use Committee.

Tissue Collection. Animals were anesthetized by CO_2 and killed by decapitation. Body weights were recorded and animals sacrificed via decapitation in the fed state. Inguinal, mesenteric and epididymal fat pads were rapidly excised, weighed and ~ 1 g samples were frozen in liquid nitrogen and stored at -70 °C until analysis.

Total RNA preparation. Total RNA from adipose tissue was extracted using RNeasy total RNA kit (Qiagen, Valencia, CA) following the instructions of the manufacturer. The ratio of absorption (260/280 nm) of all preparations was between 1.9 and 2.1 and the yield of total RNA was measured by the absorbance reading at 260 nm. The integrity of total RNA purified was checked by denaturing-agarose gel electrophoresis and ethidium bromide staining.

Relative Quantitative RT-PCR. Target-of-interest mRNA in adipose tissue total RNA preparations was quantified by reverse-transcription (RT) followed by co-amplification of target cDNA and 18S cRNA using polymerase chain reaction (PCR). In this system quantitation is based on 18S mRNA levels which were titrated to match levels of the mRNA of interest using QuantumRNA 18S Internal Standards (Ambion, Austin, TX). The rat IGF-1 (Genbank access: M15481) primers used were: 5'-GTCTTCACACCTCTTCTACCTG-3', 5'-CGAGCTGACTTTGTAGGCTTCA-3', the PCR product is 272 bases long. The rat IGF-1 receptor (Genbank access: L29232) primers used were: 5'-ATCGATGTGGAGGAGGTGAC-3', 5'-GAACTCTTCCGGGTCTGTGA-3', and the PCR product is 282 bases long. The annealing temperature of both primer pairs was 60 °C. Primers were picked up using Primer 3 (14). The multiple sequence analysis using CLUSTAL X 1.81 (15) was performed first with highly homologous sequences acquired by BLAST (16) search to avoid non-specific amplification.

Total RNA was reverse transcribed into single-strand cDNA using a Reverse Transcription System (Promega, Madison, WI) according to the product protocol (5mM MgCl2, 10mM Tris-HCl [pH 9.0 at 25 °C], 50mM KCl, 0.1% Triton®X-100, 1mM deoxynucleotide triphosphates, 1u/μl Recombinant RNasin® Ribonuclease inhibitor, 15u/μg AMV Reverse Transcriptase, 0.5μg Random Primers, 1μg total RNA in a 20μl reaction). The Hot-start PCR reaction was amplified using HotStarTaq Polymerase (Qiagen, Valencia, CA) according to the product protocol. For each RT reaction, 2μl RT products were added to each PCR reaction (160 pmol of each primer, 1.5mM MgCl2, 0.2mM deoxynucleotide triphosphates, 18S primers and Competimers, and 1.25 U HotStar Taq polymerase in 50 μl total). For each fat depot RNA preparation, the number of cycles (95 °C, 1 min; 60 °C, 1 min; 72 °C, 1 min; Biometra® TGradient Thermocycler, Biometra, Göttingen, Germany) was determined to be in the exponential range and the optimal ratio of 18S primers:competimers was titrated to have both target-

of interest and the 18S control amplify to give similar yields of products (Table 1). Equal volumes of the PCR products for each sample were run on 2% agarose gels (SeaKem® LE Agarose, FMC Bioproducts, Rockland, ME) and stained using SYBR® Gold nucleic acid gel stain (Molecular Probes, Eugene, OR). The image were captured electronically using FluorChem™ (Alpha Innotech, San Leandro, CA), and the bands were quantified using AlphaEaseFC™ image analysis software (Alpha Innotech, San Leandro, CA).

Data analysis. Results are expressed as means±SE. Data were analyzed using SAS® software (SAS release 8.00, SAS Institute Inc. NC). Simple linear regression and sample correlation coefficients were calculated to determine the linear range of PCR cycles. Student's t-test was used for comparisons of means between like fat pads of obese vs. lean rats. Differences were accepted at significance level of 0.05 ($P < 0.05$).

Results

Body weight and fat depots weight. As shown in Table 2, at 12 weeks obese Zucker rats were significantly heavier than lean controls ($P < 0.001$). The mass of the inguinal, mesenteric and epididymal fat depots was significantly greater in obese rats compared with lean controls ($P < 0.001$; Table 2).

Region-Specific Expression of IGF-1 and IGF-1 Receptor in White Adipose Tissue. Both IGF-1 and IGF-1 receptor mRNAs were easily detected in all three fat depots studied using RT-PCR (data not shown). For the quantitative analysis of gene expression, 18S RNA was chosen as the endogenous standard and the optimal ratios of 18S primers:competimers were determined first to have both 18S and the RNA under study amplified in the same linear range. A decrease of ratios of 18S primers:competimers decreases the signals from 18S PCR products. In general, 3:7 is

appropriate for most genes and 2:8 or less will be applied for genes of rare abundance. As shown in Table 1, IGF-1 mRNA levels were much higher in all three adipose tissue than those of IGF-1 receptor, evaluated by both the optimal ratios of 18S primers:competimers and the PCR cycles used within the linear range. In general, IGF-1 is normally expressed (optimal ratios of 18S primers:competimers was 3:7) in inguinal and epididymal fat depots and more highly expressed in mesenteric depots (1:2) (Table 1). IGF-1 receptor expression is relatively lower in general (2:8) in inguinal and epididymal fat depots, again similarly as IGF-1, and higher in mesenteric depots (2:5) (Table 1).

To quantitate IGF-1 and IGF-1 receptor gene expression, the signals of PCR products were normalized against that obtained from the 18S amplicon (Figure 1). Compared with lean controls, the expression of IGF-1 and IGF-1 receptor in white adipose tissues of obese Zucker rats was depot-specific. In the inguinal fat depot of obese rats, both IGF-1 and IGF-1 receptor mRNA levels were increased as compared with that of lean controls (IGF-1: $p < 0.01$; IGF-1 receptor: $p < 0.05$; Figure 2). IGF-1 receptor mRNA levels were similar between obese and lean rats in epididymal fat depots, whereas IGF-1 mRNA levels were decreased in obese rats as compared with lean rats ($P < 0.05$; Figure 3). In the mesenteric depot the expression of IGF-1 receptor was greater for the obese rats as compared with the lean controls ($P < 0.05$), however, the increase was smaller than that observed in the inguinal fat depot. There was no difference in IGF-1 mRNA levels between obese and lean rats in this depot (Figure 4). Table 3 summarizes the results of all three fat depots.

Discussion

At the tissue level, expressed on a per cell basis (mRNA levels normalized against 18S rRNA, protein levels normalized against total protein), the expression data of IGF-1 mRNA levels in various adipose tissue depots in this study agrees with the data of IGF-1

protein measurement in our preliminary studies (17). Specifically there is a similar tissue-specific pattern of change of IGF-1 status in obese rats compared to lean controls. The decrease of IGF-1 mRNA levels reported here in epididymal fat depot of obese rats as compared with lean controls agrees with a similar decrease of IGF-1 protein levels in 15 weeks old male Zucker rats (17). A decrease of IGF-1 expression in adipose tissue was also reported in studies of male obese SHFF/Mcc-*fa*^{cp} rats as compared with lean controls (18). However, in the inguinal fat pads, we see a 60% increase of IGF-1 expression level of obese rats vs. lean controls. In our preliminary data, expressed as ng IGF-1/ mg total protein, IGF-1 levels in the inguinal fat pads were significantly greater than that of the epididymal or retroperitoneal fat pads. There were no fat-depot specific differences in IGF-1 levels in the lean rats (17). However, the IGF-1 protein levels in inguinal fat pads of obese rats were the same as in that of lean controls, even though IGF-1 mRNA levels were much higher in obese than that in lean controls. This implies that the regulation of IGF-1 protein levels happens at both the transcriptional and translational levels.

A possible reason of the decrease of IGF-1 in adipose tissues, like in epididymal depot, is the obesity associated growth hormone (GH) deficiency. It is well established that GH secretion is markedly blunted both in obese humans and in animal models of obesity (19, 20). GH is apparently one of the main regulators of IGF-1 mRNA levels in adipose tissue. Hypophysectomy of normal rats remarkably decreases IGF-1 mRNA and IGF-1 protein levels in epididymal fat depots. GH treatment restores IGF-1 mRNA and IGF-1 protein levels to near normal in vivo (10, 21). However, the increase of IGF-1 mRNA levels in the inguinal depot of our obese rats implies that some factors other than GH are involved in the regulation of IGF-1 levels in adipose tissue. In the development of hyperplastic obesity, the secretion of paracrine growth factors is associated with fat cell size (22, 23). The increase of enlarged adipocytes in adipose tissues of obese rats vs. lean rats could lead to the reported increases of IGF-1 expression. Direct innervation of white adipose tissue depots by the sympathetic nervous system may play a role in the region-specific IGF-1 expression. In vivo denervation stimulates adipose tissue proliferation (24,

25), and inguinal depot has lowest innervation and highest proliferative capacity (26, 27).The expression of IGF-1 is also regulated by many other factors: hormones, oncogenes and other growth factors. One of possible candidates is insulin, as obesity is associated with chronic hyperinsulinemia. Insulin is reported to stimulate the secretion of IGF-1 in cultured human preadipocytes and adipocytes (28). Insulin receptor affinity is significantly higher in subcutaneous than in omental fat cells (29, 30), and visceral fat shows a decreased response to insulin as compared with peripheral fat (31). Additional candidates could be epidermal growth factor and platelet-derived growth factor (32). Further, adipose tissue is not a unitary organ, depot specific expression patterns could arise, in part, from distinct intrinsic characteristics of adipose cells (33).

In this study, the 18S mRNA levels, the internal standard for relative quantitative RT-PCR, were titrated to match levels of the mRNA of interest by adjusting the ratio of 18S primers:competiters. In all the depots studied, IGF-1 required a much higher ratio than IGF-1 receptor did, demonstrating much higher abundance of IGF-1 transcripts than that of IGF-1 receptor. It was reported that IGF-1 mRNA and protein levels in adipose tissue are comparable to those in liver, the major source of circulating IGF-1 (10). The data reported here, together with previous studies, lead to the hypothesis that the IGF-1 peptide content could saturate the binding sites on the cell surface, assuming they both have similar translation efficiency. Thus the IGF-1 receptor would be the bottleneck of the signaling pathway. The amount of IGF-1 receptors on the cell surface controls the net influx of IGF-1 signals. The number of IGF-1 receptors plays a critical role in the switch of the cell from a "non-mitogenic" to a "mitogenic" mode. Fibroblasts with less than 15,000 receptors grow only in serum-containing media, whereas cells with 22,000 binding sites grow in serum-free media supplemented with IGF-1 (34). Cells expressing more than 30,000 receptors will proliferate in soft agar (34; reviewed in 35). An associated increase of IGF-1 receptor levels was observed with the increased proliferation of preadipocytes by 17β-estradiol treatment, while IGF-1 receptor levels were decreased

along with the antiadipogenic effect of androgen (36). In two of the three depots studied herein, the significant increases of IGF-1 receptor mRNA levels in adipose tissue in obese compared to lean rats suggests an increase of IGF-1 signal influx into the cells, leading to the activation of the IGF system.

This study did not examine IGF binding proteins (IGFBPs), an important player in the IGF system, because of limitations of resources and time. IGFBP-1 transgenic mice have impaired adipogenesis (37). The decrease of the expression of IGFBPs is another possibility to activate the IGF system in obese animals undergoing rapid adipose tissue expansion. In ob/ob mice, IGFBP-5 mRNA expression decreased compared with lean controls (38). The expression of IGFBP-6 in vivo decreased under the GH decline by hypophysectomy (10). As with all studies at the mRNA level, the changes of mRNA levels reported here can not guarantee corresponding changes in protein levels, even though we found high agreement of expression data reported with our preliminary studies on protein levels. The direct measurement of proteins is needed to elucidate the exact mechanisms of regulation of the IGF system. In addition, the question of which cell types in adipose tissue undergo what kind of changes and examination of IGFBPs, the component proteins of IGF system, should be included in the future more comprehensive studies to address the whole picture of this complicated IGF system.

In this study we demonstrated the region-specific IGF-1 and IGF-1 receptor expression pattern in adipose tissues of obese rats compared to lean controls. The region-specific pattern of IGF-1 and IGF-1 receptor expression suggests that both IGF-1 and IGF-1 receptor are regulated at the transcriptional level in adipose tissues of obese rats as compared with lean controls and that different pathways are involved in the regulation of IGF-1 and IGF-1 receptor in different depots. The higher IGF-1 mRNA levels than that of IGF-1 receptor within the same depot implies that IGF-1 receptor numbers on cell surfaces may play an important role in controlling the passage of IGF-1 signals. Our results suggest the activation of the IGF-1 system mediates the rapid adipose expansion during the development of obesity. The activation of the IGF-1 system is mainly through

the increase of IGF-1 receptor levels. Further more complete studies including IGFBPs should be done to address the whole picture of the IGF system in adipose tissue. The studies of the cell types contributing to the specific expression changes in adipose tissue is another important issue to address to elucidate functions of the IGF system.

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TABLE 4.1
Parameters for relative quantitative RT-PCR

Inguinal depot		
	IGF-1	IGF-1 receptor
PCR cycles	26	31
Ratio of 18S primers:competimers	3:7	2:8
Epididymal depot		
	IGF-1	IGF-1 receptor
PCR cycles	24	28
Ratio of 18S primers:competimers	3:7	2:8
Mesenteric depot		
	IGF-1	IGF-1 receptor
PCR cycles	26	30
Ratio of 18S primers:competimers	1:2	2:5

TABLE 4.2
Body weight and fat depot weights
of obese and lean Zucker rats at 12 weeks of age

	Obese	Lean
body weight (g)	516.25±20.91[b]	361±8.86[a]
inguinal weight (g)	29.17±1.89[b]	4.06±0.32[a]
epididymal weight (g)	12.27±0.94[b]	2.57±0.26[a]
mesenteric weight (g)	9.48±0.63[b]	1.99±0.01[a]

TABLE 4.3
IGF-1 and IGF-1 receptor mRNA levels in three fat depots of obese and lean Zucker rats

Inguinal depot		
	Obese	Lean
IGF-1	1.01±0.06[b]	0.63±0.11[a]
IGF-1 receptor	0.82±0.05[b]	0.62±0.06[a]
Epididymal depot		
	Obese	Lean
IGF-1	0.76±0.02[b]	1.13±0.04[a]
IGF-1 receptor	0.73±0.03	0.66±0.04
Mesenteric depot		
	Obese	Lean
IGF-1	1.04±0.05	0.88±0.09
IGF-1 receptor	0.73±0.01[b]	0.69±0.01[a]

Figure 4.1 Comparison of IGF-1 (a) and IGF-1 receptor (b) mRNA levels in adipose tissue of obese and lean Zucker rats using RT-PCR. (a) A representative gel of SYBR Gold-agarose analysis of the rat IGF-1 and 18S rRNA amplification at 26 cycles in inguinal tissue. The amplicon of rat 18S rRNA is 488-bp and the amplicon of rat IGF-1 is 272-bp. The image shows samples from four obese (ob) and four lean (lean) controls. (b) A representative gel of SYBR Gold-agarose analysis of the rat IGF-1 receptor and 18S rRNA amplification at 31 cycles in inguinal tissue. The amplicon of rat 18S rRNA is 488-bp and the amplicon of rat IGF-1 is 282-bp. The image shows samples from four obese (ob) and four lean (lean) controls.

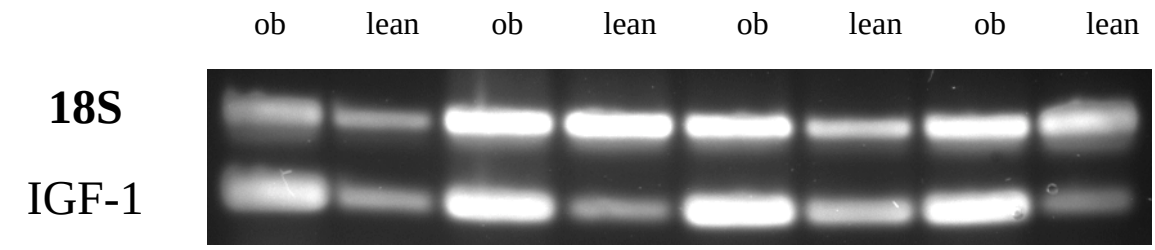


Figure 4.1a Relative quantitative RT-PCR of IGF-1mRNA in adipose tissues

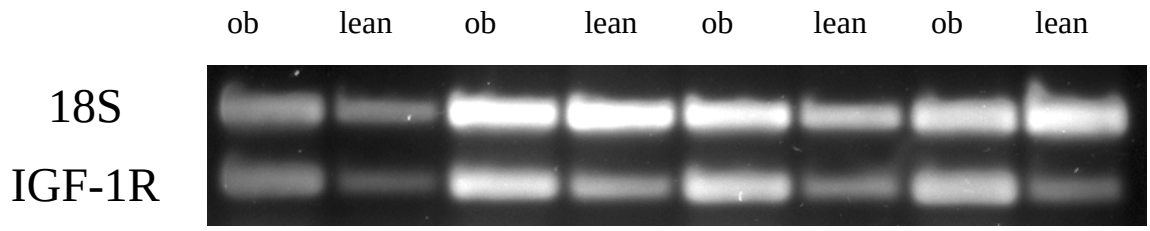


Figure 4.1b Relative quantitative RT-PCR of IGF-1 receptor mRNA in adipose tissues

Figure 4.2 IGF-1 and IGF-1 receptor mRNA levels in inguinal fat depot. Values represent mean SE (n = 8) of the ratio of signals from gene of interest (IGF-1 or IGF-1 receptor) to 18S rRNA signals. Single (*) or double (**) asterisks indicate a significant difference between obese and lean rats within mRNA levels of the same gene ($P < 0.05$).

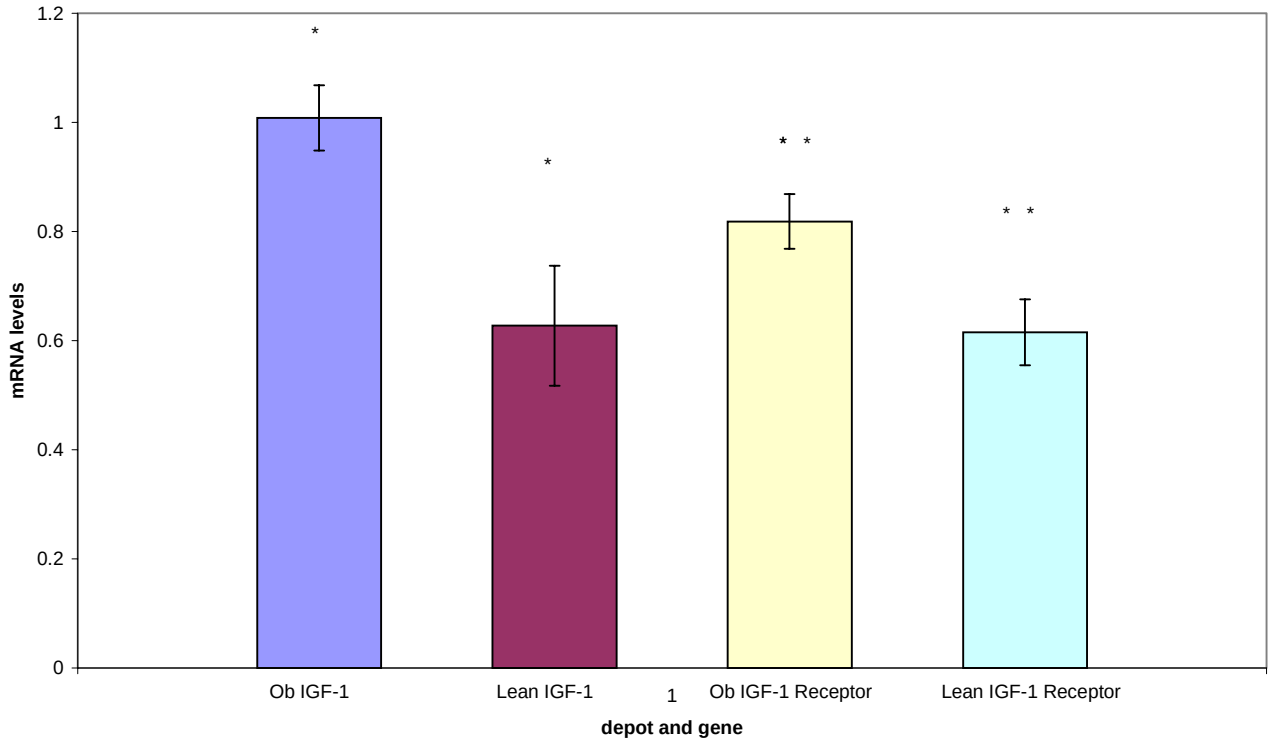
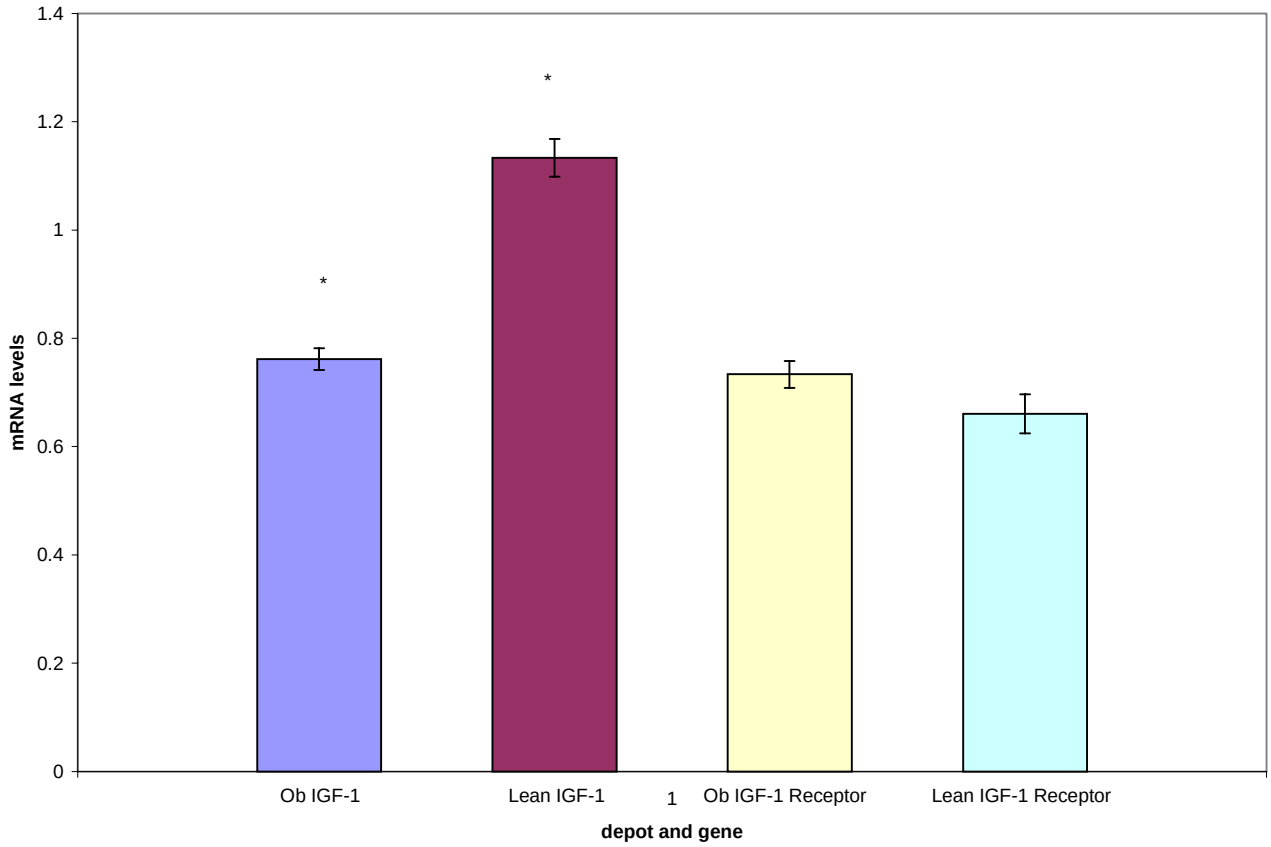
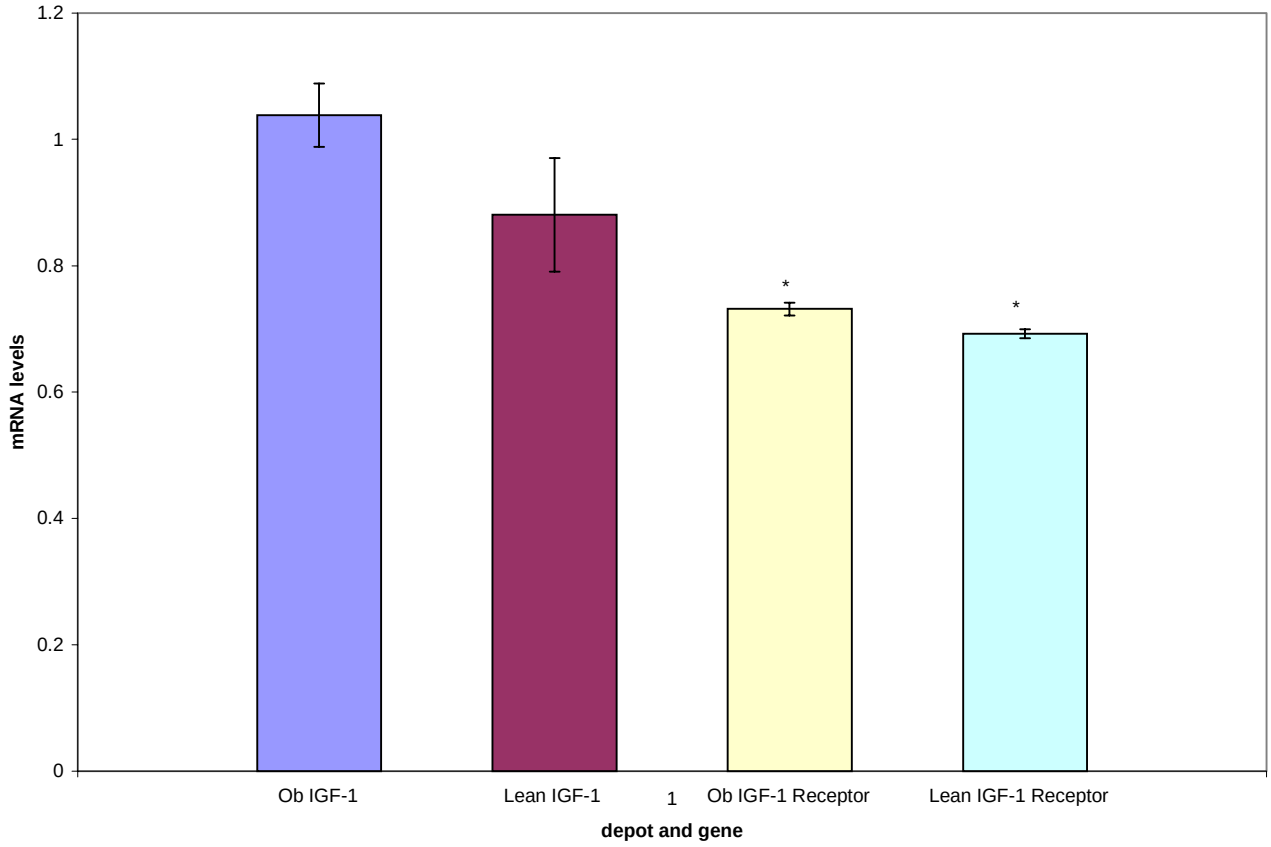

1.2
1
0.8
0.6
0.4
0.2
0
mRNA levels
*
*
* *
* *
Ob IGF-1
Lean IGF-1
Ob IGF-1 Receptor
Lean IGF-1 Receptor
depot and gene

Figure 4.3 IGF-1 and IGF-1 receptor mRNA levels in epididymal fat depot. Values represent mean SE (n = 8) of the ratio of signals from gene of interest (IGF-1 or IGF-1 receptor) to 18S rRNA signals. Single (*) asterisks indicate a significant difference between obese and lean rats within mRNA levels of the same gene ($P < 0.05$).


1.4
1.2
1
0.8
0.6
0.4
0.2
0
mRNA levels
*
*
Ob IGF-1
Lean IGF-1
Ob IGF-1 Receptor
Lean IGF-1 Receptor
depot and gene

1

Figure 4.4 IGF-1 and IGF-1 receptor mRNA levels in mesenteric fat depot. Values represent mean SE (n = 8) of the ratio of signals from gene of interest (IGF-1 or IGF-1 receptor) to 18S rRNA signals. Single (*) asterisks indicate a significant difference between obese and lean rats within mRNA levels of the same gene ($P < 0.05$).


1.2
1
0.8
0.6
0.4
0.2
0
mRNA levels
*
*
Ob IGF-1
Lean IGF-1
Ob IGF-1 Receptor
Lean IGF-1 Receptor
depot and gene

1

CHAPTER 5

SUMMARY AND CONCLUSIONS

Two experiments were designed to test the hypothesis that the IGF-1 system is activated to stimulate adipogenesis in obese animals undergoing rapid adipose tissue expansion.

The purpose of the first experiment is to give direct evidence of the presence of IGF-1 receptors on preadipocytes. Primary cultures of stromal vascular cells derived from rat adipose tissue and fluorescence double-label immunocytochemistry was utilized. Use of AD3 monoclonal antibody allowed specific identification of preadipocytes in rat stromal vascular (SV) cell culture. Immunocytochemical results directly show the localization of IGF-1 receptors on rat preadipocytes specifically identified by AD-3. Most AD-3 labeled cells also stained for the IGF-1 receptor. Also other cell types in primary cell culture of rat adipose tissue stromal-vascular cells contain IGF-1 receptors, in agreement with our studies in rat tissue sections.

The second study compared IGF-1 and IGF-1 receptor mRNA expression in white adipose tissue of 12-wk-old genetically obese Zucker rats with that of lean controls. Animals at this age are undergoing rapid adipose tissue expansion. Relative quantitative RT-PCR is used in this study to detect the expression of IGF-1 and IGF-1 receptor mRNA expression in inguinal, mesenteric and epididymal fat depots. The 18S RNA is the internal standard for relative quantitative RT-PCR. Results demonstrated region-specific IGF-1 and IGF-1 receptor expression pattern in adipose tissues of obese rats compared to lean controls. The region-specific pattern of IGF-1 and IGF-1 receptor expression suggests that both IGF-1 and IGF-1 receptor are regulated at the transcriptional level in adipose tissues of obese rats as compared with lean controls and that different pathways are involved in the regulation of IGF-1 and IGF-1 receptor in

different depots. The higher IGF-1 mRNA levels than that of IGF-1 receptor within the same depot implies that IGF-1 receptor numbers on cell surfaces play an important role in controlling the passage of IGF-1 signals.

The data from these studies strongly support that IGF-1 could directly act on preadipocytes to stimulate the proliferation of preadipocytes and their differentiation into mature adipocytes. In vivo the regulation of IGF-1 and IGF-1 receptor in adipose tissue of obese rats happens at the transcriptional level. Depot-specific regulation produces the region-specific patterns of regulation of IGF-1 and IGF-1 receptor expression. The activation of local IGF-1 system in adipose tissue may mediate the rapid adipose expansion during the development of obesity. The activation of IGF-1 system could be mainly through the increase of IGF-1 receptor levels.