INSULIN ALTERS THE PROLIFERATION OF SUBCUTANEOUS AND VISCERAL ADIPOSE CELLS

F. Saraç, S. Yıldız, G. Özgen, C. Yılmaz, T. Kabalak, M. Tüzün
Ege University Endocrinology and Metabolism Department, Izmir, Turkey

ABSTRACT
Objective: Adipose tissue is the most abundant source of accessible stroma. The stromal cells proliferate and differentiate to mature adipocytes by different hormonal stimulus. Insulin was known to be associated with fat cell proliferation. The aim of this study was to investigate the varying concentrations of insulin effect on the proliferation of human subcutaneous and visceral adipose tissue cultures. Subcutaneous and omental adipose tissue was obtained from a 34 years old female donor aged with a body mass index (BMI) of 34.1 kg/m². Stromal vascular cells were isolated and cultured using modified procedures described by Entenmann and Hauner. For the proliferation assay, stromal-vascular cells from subcutaneous and omental adipose tissue cultures were fed with proliferation media containing 100 nM, 200 nM, 400 nM insulin, for 3 days. Cell numbers and sizes of proliferating cultures of human omental adipose tissue cultures increased more than human subcutaneous adipose tissue culture at the level of 100 nM and 200 nM insulin concentrations. All cells of adipose cultures died at the 400 nM insulin concentration. High insulin levels (400 nM) have a toxic effect on adipose cells. Insulin effects the number and size of omental adipose cells more than the subcutaneous adipose cells.

Introduction
Adipose tissue is the most abundant source of accessible stroma. The stromal cells proliferate and differentiate to mature adipocytes by different hormonal stimulus (1, 3-5). These precursor cells found in the stromal vascular fraction of adipose tissue, and can be induced to proliferate and differentiate in vitro chemically defined media. This has allowed investigation of the factors which regulate the production of adipocytes in a number of species including humans, rodents and pigs revealing key roles for insulin or insulin like growth factor-I (IGF-I), and also glucocorticoids and triiodothyroine (T3) in some species (2, 6). Insulin known to be associated with fat cell proliferation (1, 6, 7-10).

The development and regulation of the insulin receptors in preadipocytes of various origin are not clear, as differences seem to exist between the various cell lines studied. Several studies with 3T3-L1cell lines, which develop into adipocytes, have shown an increased insulin binding during differentiation. In the 3T3-C2 cell lines, which do not develop into adipocytes, insulin mediated down regulation and dexamethasone induced upregulation of insulin binding have been demonstrated (10, 12).

The aim of this study was to investigate the varying concentrations of insulin effect on the proliferation of human subcutaneous and visceral adipose tissue cultures.

Materials and Methods
Isolation of stromal vascular (SV) cells from subcutaneous and omental adipose tissue
Subcutaneous and omental adipose tissue was obtained from a female donor aged 34 years with a body mass index (BMI) of 34.1 kg/m². Stromal vascular cells were isolated and cultured using modified pro-
 procedures described by Entenmann and Hauner (3). Adipose tissue was enzymatically digested for 45 min in a Krebs Ringer buffer containing 1 mg/ml collagenase, 15 mg/ml BSA, 5 mM glucose and 100 mM HEPES. The ratio of digestion solution to adipose tissue was 5m/1g. The digesta was then filtered through 200 and 60 micron mesh and pelleted at 600 g for 5 min. The SV cells were resuspended in a RBC lysis buffer (0.154 M ammonium chloride, 10 mM potassium bicarbonate, 0.1 mM EDTA) for 10 min. Then it filtered and centrifugated to remove contaminating endothelial cells.

Cultures of SV cells were grown in proliferation medium containing 90% DMEM/Hams F-12 (1:1), 10% FBS and penicillin/streptomycin/gentamicin/fungisone (50 U/ml; 50 μg/ml; 25 μg/ml; 0.5 μg/ml). Cultures were incubated at 37 °C in O2:CO2 (95.5%) atmosphere. Initially, freshly isolated stromal vascular cells were grown to 80% confluency.

For the proliferation assay, stromal-vascular cells from subcutaneous and omental adipose tissue cultures were fed with proliferation media containing 100 nM, 200 nM, 400 nM insulin, for 3 days. The degree of proliferation of cultures of omental and subcutaneous adipose cell was determined on day 25 by light microscopy

**Induction of cell proliferation**

Freshly split stromal vascular cells were seeded (5x10³/cm²) in 12 well Falcon dishes nd cultured in basal proliferation medium containing 90% DMEM/Hams F-12 (1:1), 10% FBS and penicillin/streptomycin/gentamicin/fungisone (50 U/ml; 50 μg/ml; 25 μg/ml; 0.5 μg/ml). Cultures were incubated at 37 °C in O2:CO2 (95.5%) atmosphere.

Following attachment, subcutaneous and omental adipose tissue cultures were grown in differentiation medium containing 90% DMEM/Hams F-12 (1:1), 10% FBS and penicillin/streptomycin/gentamicin/fungisone (50 U/ml; 50 μg/ml; 25 μg/ml; 0.5 μg/ml), 33 μM biotin, 17 μM pantothenate, 100 nM human insulin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine (IBMX) for the next 3 days. Then cultures were exposed to adipocyte media containing 97% DMEM/Hams F-12 (1:1), 10% FBS and antibiotics/antimycotics, 33 μM biotin, 17 μM pantothenate, 100 nM human insulin, 1 μM dexamethasone, 1 μM hydrocortisone for the next 10 days. Under these growth conditions, oil droplets began to appear by the 10th days. The presence of lipid within adipocytes was visualized by staining with Oil Red O. Nuclei were stained with hematoxylin to determine cell number. To stain adipocytes, cells in 35-mm dishes were fixed for 30 min at 4°C with Baker's Formalin (10 ml of 37% formaldehyde, 10 ml calcium chloride, and 80 ml water). Cells were then washed three times with HBSS and stained for 10 min with 0.3% Oil Red O. This procedure was showed that mature adipocytes differentia-
ted in 20 days.

**Results and Discussion**

In the present study, subcutaneous and omental stromal vascular cells were used to develop culture conditions supporting adipocyte differentiation, because both of tissues can be easily obtained by surgery. We evaluated the effects of insulin at the vari-
ous concentrations in the subcutaneous and omental tissue. Cell numbers and sizes of proliferating cultures of human omental adipose tissue cultures increased more than human subcutaneous adipose tissue culture at the level of 100 nM and 200 nM insulin concentrations. Insulin showed a stimula-
tory effect on adipocyte proliferation at the level of 100 nM and 200 nM concentra-
tions. Insulin effected omental tissue more than subcutaneous tissue. All cells of adi-
pose cultures died at the 400 nM insulin concentration. It has been degenerative at high concentration.

In mammals, adipose tissue occurs as a number of depots, some located in the ab-
**Fig 1.** Photomicrographs of human omental adipose tissue.  
A. Cells in the 100 nM insulin concentration; 
B. Cells in the 200 nM insulin concentration; 
C. Cells in the 400 nM insulin concentration.

**Fig. 2.** Photomicrographs of human subcutaneous adipose tissue cells.  
A. Cells in the 100 nM insulin concentration  
B. Cells in the 200 nM insulin concentration  
C. Cells in the 400 nM insulin concentration.
dominal cavity (e.g. omental), some under the skin (subcutaneous depots), some within the musculature. Accretion of adipose tissue occurs through both hypertrophy of adipocytes and adipocytes hyperplasia (11, 12). Adipocytes do not divide, but rather are formed from precursor cells which proliferate and differentiate into mature adipocytes (13, 14-16, 26). Adipose tissue cellularity is linked to the dynamic role layed by preadipocytes. Preadipocytes, which reside in the adipose tissue stromal vascular compartment are specialized fibroblast like progenitor cells which proceed through a complex program of gene expression to differentiate into terminal mature adipocytes. The cell population can be controlled via proliferation and differentiation of preadipocyte into adipocytes (17).

Adipocyte differentiation therefore requires the cell to process a variety of combinatorial inputs during the decision to undergo differentiation. Hormones and growth factors with a role in adipocyte differentiation act via specific receptors to transduce external growth and differentiation signals through a cascade of intracellular events (19-21). Identification of agents or molecules that modulate the process in either a positive or negative manner provides insight into the signal transduction pathways involved. Extracellular matrix proteins may play an important role in modulating adipocyte differentiation by permitting the morphological changes and adipocyte-specific gene expression that accompany differentiation. The combination of hormones and growth/differentiation factors that trigger or potentiate adipocyte differentiation (22, 23). Depending on the species, the age of the donor, and/or the adipose depot source, agents such as glucocorticoids and MIX are either necessary to trigger the differentiation program or act only to accelerate it. The full complement of inducing agents required for differentiation varies with each cell culture model, insulin, IGF-I, cAMP, and glucocorticoids are generally considered necessary for the induction of differentiation either in serum-containing or in serum-free media (24-28).

In many studies, primary preadipocytes derived from young animals require a high concentration of insulin or IGF to be essential for differentiation and dexamethasone to enhance differentiation (26-28). The presence of a very high insulin concentration in the lipid containing medium did not prevent the increase in the insulin binding during the development of the cells (29, 30). This is in contrast to the findings that a high ambient insulin concentration reduces the number of insulin receptors in cultured mature adipocytes (31). However, it has been shown that physiological insulin concentrations can also exert a regulatory effect on the number of insulin receptors in some cells. Thus, the present design cannot completely exclude the observation that the insulin in the 20% human serum added to the medium, producing an average insulin concentration of ~56 pM, exerted some slight regulatory effect (33).

Our results demonstrated that high insulin levels (400 nM) have a toxic effect on adipose cells. Degenerated cells were visualized with microscoph. In summary, high insulin levels (400 nM) have a toxic effect on adipose cells. Insulin effects the number and size of omental adipose cells more than the subcutaneous adipose cells.

REFERENCES