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# Analysis of insulin-stimulated insulin receptor activation and glucose transport in cultured skeletal muscle cells from obese subjects☆

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**Abstract**

Obesity is associated with impaired insulin-stimulated glucose disposal in the skeletal muscle, but whether this is an intrinsic or acquired factor is unknown. In many patients with type 2 diabetes mellitus (T2D) and their nondiabetic relatives, who have a genetic predisposition for diabetes, insulin resistance is maintained in cultured muscle cells. To study the association of obesity with defects in insulin action, we investigated insulin stimulation of both insulin receptor (IR) autophosphorylation and subsequent glucose transport in primary skeletal muscle cell cultures obtained from both nonobese and obese nondiabetic subjects. In these 2 groups, there was no difference in the ability of insulin to induce autophosphorylation of the IR, phosphorylation of the downstream serine kinase Akt/PKB, or stimulation of glucose transport. Moreover, there were no major differences in cultured muscle cell content of either the IR, the IR antagonist PC-1, or GLUT 1 and GLUT 4. These data therefore indicate that the insulin resistance associated with obesity is not maintained in cultured muscle cells and suggest that this insulin resistance is an acquired feature of obesity.


## 1. Introduction

Insulin resistance in the skeletal muscle precedes and contributes to the development of type 2 diabetes mellitus (T2D) and the metabolic syndrome [1-3]. Impaired responses to insulin in the skeletal muscle may result from both intrinsic genetic factors as well as acquired components related to diet, activity, and other lifestyle factors [1,4]. Obesity, for example, is associated with a reduced capacity for insulin-stimulated glucose disposal in muscle both in vivo and in vitro [5,6]. However, in obesity, whether skeletal muscle insulin resistance is an acquired or intrinsic factor has not been determined.

Primary skeletal muscle cultures have been used to directly study insulin action in human muscle [7,8]. This model system has been used both to study intrinsic defects in muscle insulin action and to characterize the effects of potential mediators of insulin resistance. Insulin resistance has consistently been reported in muscle cells from patients with T2D [7,9,10]. Whether insulin resistance also occurs in cultured muscle cells from obese individuals is unknown.

In our previous studies, we compared insulin action in skeletal muscle strips prepared from rectus abdominis muscles of both nonobese and obese nondiabetic individuals [6,11]. Insulin stimulation of glucose transport and downstream insulin receptor (IR) signaling events including IRS-1-PI3K–associated activity and Akt activation were impaired in muscle strips from obese individuals [6,11]. We have now investigated insulin action in cultured muscle cells from rectus abdominis muscles that were obtained from a similar population of individuals. When cultured muscle cells from nonobese individuals are compared with cells from obese individuals, the cells from obese subjects respond to insulin with normal activation of IR signaling and insulin stimulated glucose transport. These data suggest therefore that skeletal muscle cells from obese individuals do not retain their insulin-resistant phenotype.

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## 2. Materials and methods

### 2.1. Materials

Microtiter plates (Maxisorp Immunoplates) were from Nunc (Copenhagen, Denmark). Bovine serum albumin (BSA) was from Intergen (Purchase, NY). Crystalline porcine insulin was obtained from Eli Lilly (Indianapolis, Ind). Biotin-conjugated antiphosphotyrosine antibody was from UBI (Lake Placid, NY). Horseradish peroxidase (HRP)–conjugated streptavidin was from Pierce (Rockford, Ill). The ELAST amplification kit used was from DuPont NEN (Boston, Mass). The biotinyl tyramide (TMB) reagent kit was from Kirkegaard and Perry Laboratories (Gaithersburg, Md). Defined media components were from Clonetics (Walkersville, Md). All other reagents were from Sigma (St Louis, Mo).

### 2.2. Subjects

Nonobese and obese nondiabetic subjects were individuals undergoing elective abdominal surgery. Body mass, height, ethnicity, sex, age, and diabetic status were recorded as part of preoperative procedures. A fasting blood sample was obtained for subsequent analysis of insulin and glucose. Subjects were categorized into groups based on body mass index (BMI) (nonobese, $<30$; obese, $\geq 30\ kg/m^2$), and the presence of diabetes was exclusionary. All the nonobese subjects were women and 2 of the obese subjects were men. During surgery, a biopsy specimen of the rectus abdominis was obtained for subsequent cell culture. All procedures were approved by the East Carolina University Human Studies Committee.

### 2.3. Plasma analysis

Plasma was analyzed for glucose (YSI 2300 STAT Plus Glucose and Lactate Analyzer, YSI Inc, Yellow Springs, Ohio) and for insulin with a microparticle enzyme immunoassay (IMx, Abbott Laboratories, Abbott Park, Ill). Glucose and insulin concentrations were used to determine homeostasis model assessment (HOMA) values {(fasting glucose [mg/dL] $\times$ 0.05551) $\times$ fasting insulin [$\mu$U/mL]/22.1} as an index of in vivo insulin action [12].

### 2.4. Cell culture

The harvesting and subsequent culturing of satellite cells from rectus abdominis skeletal muscle tissue were adapted with modification from Henry et al [8]. Muscle biopsy specimen was immediately placed in ice-cold Dulbecco's Modified Eagle's Medium (DMEM) and transported to the cell culture facility. The tissue was then dissected free of adipose and connective tissue in ~3 mL DMEM + ~5 mL $Ca^{2+}Mg^{2+}$ free Hanks buffered saline (HBBS) at room temperature. The tissue was minced (2-mm pieces) and washed twice by centrifugation for 10 minutes with 20 mL of Hanks at 550 g (Beckman TJ-6). Satellite cells were isolated by subjecting the tissue to a trypsin digestion cocktail containing 0.25% (wt/vol) trypsin, 0.1% (wt/vol) type IV collagenase, and 0.1% (wt/vol) BSA for 30 minutes on a low shaker setting at room temperature. The cellular suspension was treated with 5% fetal bovine serum (FBS) (wt/vol) to terminate digestion and preplated in uncoated 25-$cm^2$ flasks for 1 to 3 hours at 37°C to remove fibroblasts. The residual cellular suspension was carefully transferred to collagen I–coated 25-$cm^2$ flasks in 3 mL SkGM (skeletal growth medium [skeletal basal medium supplemented with 10% FBS, 0.5 mg/mL BSA, 0.5 mg/mL fetuin, 20 ng/mL human epidermal growth factor, 0.39 $\mu$g/mL dexamethasone, and 50 $\mu$g/mL gentimicin/amphotericin B]) and incubated in a 5% $CO_2$, 37°C humidified atmosphere. The volume of SkGM was increased to 5 mL after 24 hours of incubation, speeding myoblast attachment. The SkGM was changed every 5 days. After reaching 70% confluence, myoblasts were subcultured onto 6- or 24-well type I collagen–coated plates at densities of $6.4 \times 10^4$ cells or $1.6 \times 10^4$ cells per well, respectively. Cultures used for experimental procedures were between the fourth and eighth population doubling. When cells reached 80% confluence, they were switched to low-serum media (2% FBS) to induce differentiation. At 8 to 9 days postdifferentiation and before analysis of insulin action, myoblasts were serum starved for 18 hours. For studies of insulin signaling, cells growing in 6-well plates were exposed to various concentrations of insulin for 5 minutes. Cells were then washed with phosphate-buffered saline at 4°C and then solubilized in 50 mM HEPES, pH 7.6, 150 mM NaCl, 1% Triton X-100, 2 mM $Na_3VO_4$, 1 mM PMSF, 2 $\mu$M leupeptin, 2 $\mu$M pepstatin A. Lysates were allowed to solubilize for 1 hour at 4°C and were then centrifuged for 20 minutes at 14 000 rpm. Supernatants were collected and stored at −70°C for subsequent study. Protein content of cellular extracts was determined by the Bradford method [13]. Differentiation states of myoblasts were determined by assaying extracts for creatine phosphokinase (CPK) activity (Sigma) [8,14]. Assessment of myoblast CPK activity confirmed that each cell line was of a muscle-specific lineage. Undifferentiated myoblast morphology was confirmed visually by the absence of fused myotubes and a relatively low CPK activity [8,14].

### 2.5. Glucose transport

To assess 2-deoxyglucose uptake, muscle cells were cultured in 24-well, collagen-coated plates. Myotubes were allowed to differentiate for 10 days and serum starved 16 hours before assay. Cells were then washed and incubated with transport buffer (20 mM HEPES, pH 7.4, 140 mM NaCl, 5 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% BSA), containing varying concentrations of insulin, for 1 hour at 37°C. Cytochalasin B (final concentration, 10 $\mu$M) was added to wells to determine nonspecific incorporation of label. 2-Deoxy-[$^3$H]glucose was added to all wells (final