# Rapid Publications

# Regulation of Glucose-Transporter Gene Expression by Insulin in Cultured Human Fibroblasts

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**To clarify the effect of insulin on glucose-transporter (GT) biosynthesis, we determined GT mRNA levels in human cultured skin fibroblasts, using HepG2 GT cDNA as a probe. Insulin specifically increased the GT mRNA level in a time- and dose-dependent manner. Time-course study demonstrated that the mRNA level peaked within 3 h of insulin ($1 \times 10^{-7}$ M) addition. After remaining elevated for several hours, mRNA decreased and returned to the basal level after 24 h. In the cell strains from seven normal subjects, the mean (±SE) GT mRNA level determined after 3 h of treatment with $1 \times 10^{-7}$ M insulin was 164.3 ± 8.5% of the level found in untreated control cells. The insulin dose-response curve of GT mRNA levels showed that the maximum stimulation was elicited at $1 \times 10^{-7}$ M, and the half-maximum stimulation occurred at $\sim 5 \times 10^{-10}$ M. Degradation rates of GT mRNA determined in the presence of actinomycin D were not different between insulin-treated and untreated cells. These results suggest that insulin increases GT gene expression in cultured human fibroblasts. *Diabetes* 37:1583–86, 1988**

Insulin stimulates glucose transport by promoting the translocation of glucose transporters (GTs) from the intracellular pool to plasma membrane in isolated adipose cells and muscle cells (1–4). However, the effect of insulin on the biosynthesis of GTs remains to be clarified. In short-term studies with insulin, some investigators failed to find any effect on total cellular GT levels measured by the cytochalasin B binding method (1,3), although others have reported an increase in GTs of adipose cells from rats that had received insulin infusions for several days (5,6). Recently, the complete amino acid sequence of the GT was deduced from the nucleotide sequences of cDNA clones in human HepG2 hepatoma cells (7). To clarify the effect of insulin on GT biosynthesis, we determined GT mRNA levels in human cultured skin fibroblasts in this study, using HepG2 GT cDNA as a probe.

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## MATERIALS AND METHODS

**Cell culture and treatments.** Human diploid fibroblast cultures were established from specimens obtained by forearm incision biopsy from normal volunteers. Cells were grown as monolayers in αMEM (Eagle's minimum essential medium supplemented with amino acids; Flow, Irvine, CA) supplemented with 10% (vol/vol) fetal calf serum (Gibco, Grand Island, NY) and antibiotics in a humidified atmosphere of 95% air/5% $CO_2$ at 37°C. Stock cultures, maintained in 75-$cm^2$ flasks (no. 25110, Corning), were subcultured at 1:6 splits with 0.05% trypsin and 0.02% EDTA in 100-mm dishes (no. 25020, Corning) and grown to confluence. When the cells attained the confluence, the medium was aspirated and replaced with serum-free αMEM (100 mg/dl glucose) containing 0.1% (wt/vol) bovine serum albumin. After 36 h, a small aliquot of phosphate-buffered saline with or without insulin was added, and the cells were incubated at 37°C. After incubations of various duration (0–24 h), the cells were scraped off the dish with a rubber policeman and harvested quickly for determination of GT mRNA levels. In an experiment to determine the effect of actinomycin D (Markor, Jerusalem, Israel), the cells were incubated for 1.5 h with or without $10^{-7}$ M insulin, and then 5 μg/ml actinomycin D was added (time 0). The incubation was continued for 8 h, and changes in GT mRNA levels were determined at 0, 4, and 8 h.

**RNA extraction and hybridization.** Total cellular RNA was prepared with guanidine thiocyanate cesium chloride (8). Northern and dot-blot hybridization studies were performed with high-stringency conditions as previously described (9,10). For Northern blot analysis, 30 μg of total cellular RNA was denatured with 1 M glyoxal and 50% dimethyl sulfoxide, electrophoresed on a 1.0% agarose gel, and transferred to

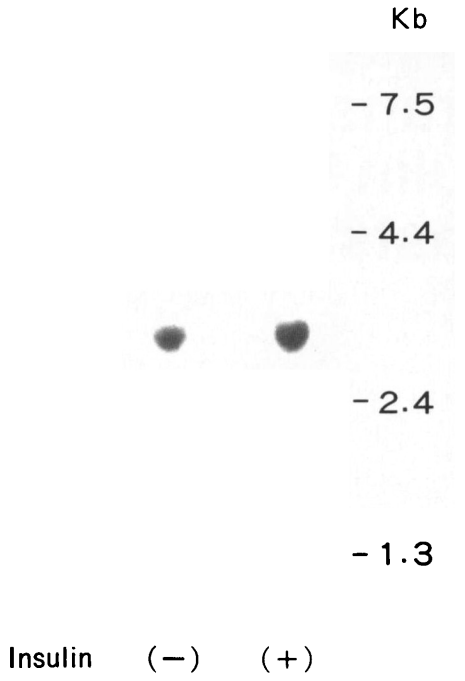


**FIG. 1. Northern hybridization of total cellular RNA from cultured human fibroblasts. Thirty micrograms of total RNA prepared from insulin-treated (3 h) or untreated cells was resolved on 1% agarose gel and hybridized with $^{32}$P-labeled HepG2 glucose-transporter cDNA. Size markers (*right*) indicate location of RNA standards. Kb, kilobase.**

a nylon filter (Gene Screen, New England Nuclear). The transferred RNA was then baked, prehybridized, and hybridized with nick-translated cDNA probes (9). For dot-blot studies, 20 μg of the total cellular RNA denatured with 7% formaldehyde was immobilized on a nitrocellulose filter (BA-85, Schleicher & Schuell), baked, prehybridized, and hybridized (10) as described for Northern blot analysis. The filters were washed and exposed to Konika X-ray films with an intensifying screen at −70°C and developed. The spots were quantitated by densitometric scanning. In preliminary experiments we found that dot-blotting density was linear up to a 25-μg total cellular RNA blot. The intra-assay coefficient of variation determined with 20-μg total cellular RNA blots

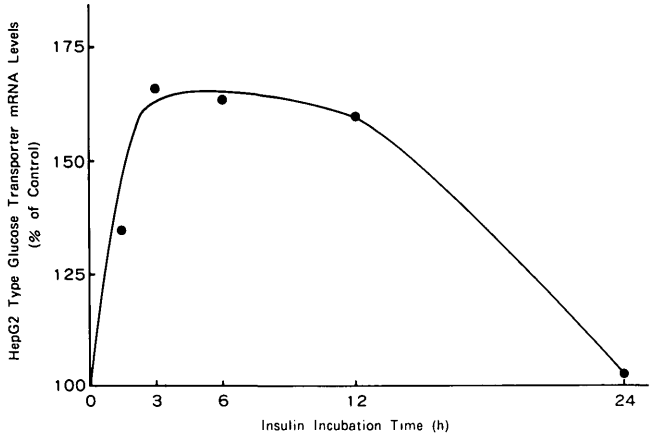


**FIG. 2. Time course of changes in HepG2 glucose-transporter mRNA level in cultured human fibroblasts stimulated with $10^{-7}$ M insulin. Each *point* is mean ± SE of 3 strains and is expressed as percentage of untreated control.**

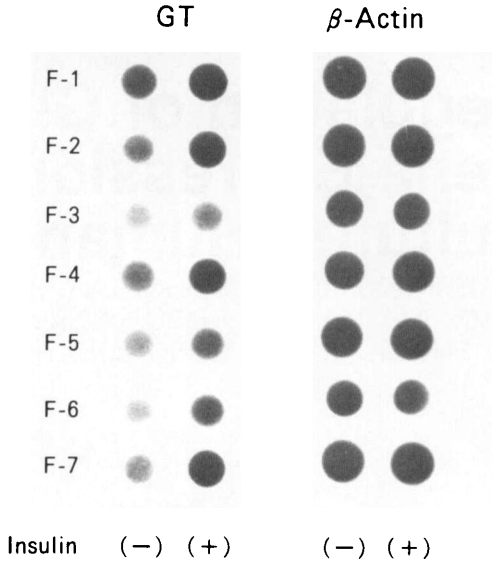


**FIG. 3. Dot-blot hybridization of total cellular RNA from cultured human fibroblasts (F-1 through F-7). Twenty micrograms of total RNA was dotted onto nitrocellulose filter and hybridized with $^{32}$P-labeled HepG2 glucose-transporter (GT) or β-actin probe.**

was 4.0%, as determined with a Schleicher & Schuell microfiltration manifold. The GT probe used was an ~2.0–kilobase pair fragment of HepG2 GT cDNA (phGT2-1) that included the region downstream from the internal *Eco*RI site (7), and the β-actin probe was a 443–base pair *Hin*fI fragment of human β-actin pseudogene (11). These fragments were nick translated with [α-$^{32}$P]dCTP (Amersham, Arlington Heights, IL). Specific activity of the probes was $>10^8$ counts · $min^{-1}$ · $\mu g^{-1}$ DNA.

**Statistical analysis.** All data are expressed as means ± SE. Statistical significance was evaluated by Student's *t* test.

## RESULTS

Northern blot hybridization of HepG2 GT mRNA from cultured human fibroblasts exhibited a single band of ~2.9 kilobases (Fig. 1), as previously reported (12). To study the effect of insulin on cellular GT mRNA levels, fibroblasts obtained from normal subjects ($n$ = 3) were incubated in the presence or absence of $10^{-7}$ M insulin for various lengths of time (0–24 h) and analyzed for GT mRNA content by dot-blot hybridization. Insulin increased the GT mRNA level, which attained a plateau at 3 h. The mRNA level remained elevated for several hours and then decreased to the basal level at 24 h regardless of the continued insulin stimulation (Fig. 2). Insulin was added only at the beginning of the incubation and was not replenished. However, a decrease in insulin concentration in the medium was slight (~15%) during the 24 h. In the cell strains from seven normal subjects, the mean (±SE) GT mRNA level determined after 3 h of treatment with $10^{-7}$ M insulin was 164.3 ± 8.5% of the level of untreated cells (42.9 ± 5.0 without insulin vs. 71.6 ± 10.4 with insulin, arbitrary densitometric units; $P < .01$). On the other hand, the β-actin mRNA level did not differ significantly between insulin-treated and untreated cells (105.6 ± 5.9% of value for untreated cells; Fig. 3). Glucose concentration in the medium was not significantly different during the course of the experiment between insulin-treated and un-

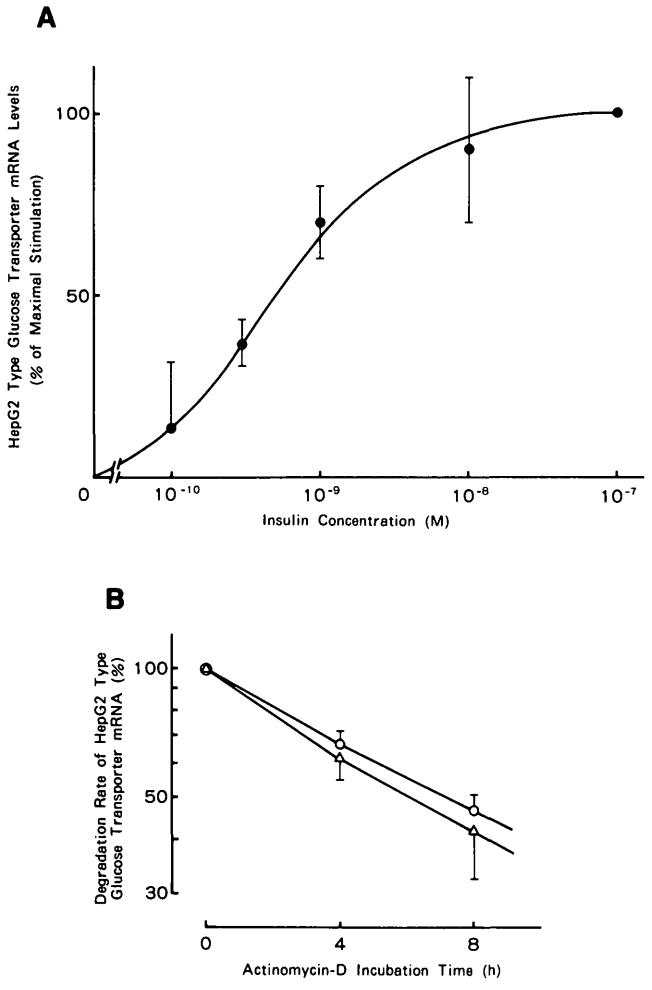


**FIG. 4. *A*: dose-dependent effects of insulin on HepG2 glucose-transporter mRNA in cultured human fibroblasts. Fibroblasts were incubated with insulin for 3 h at 37°C, and total cellular RNA was isolated. HepG2 glucose-transporter mRNA levels were determined by dot-blot hybridization as described in MATERIALS AND METHODS. Each *point* is mean ± SE of 3 strains and is expressed as percentage of maximal stimulation. *B*: effect of insulin (○, without insulin; △, with insulin) on degradation rate of HepG2 glucose-transporter mRNA. Degradation rates were determined with actinomycin D treatment for 0, 4, and 8 h by dot-blot hybridization as described in MATERIALS AND METHODS. Degradation rate was expressed as percentage of basal rate. Results are means ± SE of 3 strains.**

treated cells. The dose-response relationship for the insulin stimulation of GT mRNA level is shown in Fig. 4*A*. The cells were exposed for 3 h to various concentrations of insulin (0–$10^{-7}$ M). A slight increase of GT mRNA level was evident at insulin concentrations as low as $1 \times 10^{-10}$ M, and the maximum stimulation was elicited at $1 \times 10^{-7}$ M. The half-maximum stimulation occurred at $\sim 5 \times 10^{-10}$ M, which is within the physiologic range of insulin concentration.

To determine whether insulin-stimulated accumulation of GT mRNA is due to an increase in the rate of transcription of GT mRNA or a decrease in the degradation rate, we studied the effect of actinomycin D on the cellular accumulation of GT mRNA. After the addition of actinomycin D, GT mRNA levels were decreased in both insulin-treated and untreated cells (Fig. 4*B*). The degradation rates of GT mRNA were similar under both conditions ($t_{1/2}$ of GT mRNA; 7.4 ± 2.8 h with insulin vs. 7.5 ± 0.9 h without insulin).

## DISCUSSION

Insulin is known to specifically regulate the expression of several genes (13–17). In this study, we demonstrated that insulin stimulates the accumulation of HepG2 GT mRNA in cultured human skin fibroblasts in a concentration-dependent manner. However, insulin had no significant effect on mRNA levels of human β-actin, indicating that the effect of insulin on GT mRNA level is specific. Using actinomycin D, an inhibitor of gene transcription, we also demonstrated that the cellular HepG2 GT mRNA levels decrease at similar rates in insulin-treated and untreated cells. These findings suggest that insulin results in the cellular accumulation of HepG2 GT mRNA by enhancing its transcription rather than by decreasing its degradation. Because the effect of insulin was observed even in the low concentration, insulin probably operates through insulin receptors rather than insulin-like growth factor I receptors on the cell surface.

Recently, Santisteban et al. (13) studied thyroglobulin gene expression in FRTL-5 thyroid cells and found that insulin (10 μg/ml) elevated thyroglobulin mRNA levels in a time-dependent manner, attaining the maximal level ~24 h after insulin was added to the medium. In contrast, our results show that insulin-induced increases in HepG2 GT mRNA occurred more rapidly and for a shorter period. After remaining elevated for several hours, the mRNA levels in fibroblasts began to decrease and returned to the basal level regardless of continued exposure of the cells to a high concentration of insulin. A similar time course was also reported by Messina et al. (14) for insulin-induced increases in p33 mRNA levels in rat hepatoma cells. They demonstrated that p33 mRNA level was increased maximally by 1 h after insulin ($10^{-10}$ or $10^{-11}$ M) stimulation and decreased thereafter. Reasons for the decrease in mRNA level with time regardless of continued insulin stimulation remain to be clarified.

Matthaei et al. (18) have reported an increase in GTs in high- and low-density microsomal membrane fractions of rat brain microvessels incubated with insulin for 2 h. With in vivo studies, an increase in GT level also has been demonstrated in adipose cells from rats that had received continuous subcutaneous insulin infusion (5,6). In agreement with these studies, our study suggests that insulin regulates GT biosynthesis, and cultured skin fibroblasts may be helpful in the study of insulin regulation of GT biosynthesis in patients with non-insulin-dependent diabetes mellitus and insulin-resistant syndrome.

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