Recovery of Maximal Insulin Responsiveness and Insulin Sensitivity after Induction of Insulin Resistance in Primary Cultured Adipocytes*

(Received for publication, September 9, 1988)

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Treatment of primary cultured adipocytes with 50 ng/ml insulin and 20 mM glucose for 0–6 h resulted in a loss of maximal insulin responsiveness (MIR) which was immediate (no lag period), rapid ($t_{1/2}$ of 3 h), linear, and extensive (80% of that seen at 24 h), whereas loss of insulin sensitivity from 0–24 h was slow ($t_{1/2}$ = 8 h), extensive (insulin $E_{50}$ of 0.3 and 1.45 ng/ml at 2 and 24 h, respectively), and was preceded by an initial 2-h lag. Recovery of MIR and insulin sensitivity was assessed by inducing desensitization for various times from 2–24 h, removing insulin and glucose, and then measuring MIR and insulin sensitivity over a subsequent 1–6-h period. After 2 h, recovery of MIR in desensitized cells was rapid (251 pmol of glucose/3 min/h), whereas after 24 h, recovery was much slower (35 pmol/min/h). In contrast, the opposite trend was seen for recovery of insulin sensitivity: at early times recovery of insulin sensitivity was slow (0.05 ng/ml/h) but was rapid after 24 h (0.12 ng/ml/h). Thus, it appears that MIR and insulin sensitivity can be independently regulated since recovery rates for MIR and insulin sensitivity diverged with the progression of insulin resistance. When the effects of insulin and glucose on recovery were examined, we found that insulin alone was unable to block recovery of MIR or insulin sensitivity. Glucose alone, however, was effective in preventing recovery of insulin sensitivity but not recovery of MIR. In the presence of 20 mM glucose, low doses of insulin (treatment $E_{50}$ = 0.22–0.46 ng/ml) effectively prevented recovery of both MIR and insulin sensitivity. De novo protein synthesis apparently is not involved in the development of insulin resistance or the reversal of desensitization since inhibition of protein synthesis by cycloheximide had no effect on the insulin resistance. When the effects of insulin and glucose on recovery were examined, we found that insulin alone was unable to block recovery of MIR or insulin sensitivity. Glucose alone, however, was effective in preventing recovery of insulin sensitivity but not recovery of MIR. In the presence of 20 mM glucose, low doses of insulin (treatment $E_{50}$ = 0.22–0.46 ng/ml) effectively prevented recovery of both MIR and insulin sensitivity. De novo protein synthesis apparently is not involved in the development of insulin resistance or the reversal of desensitization since inhibition of protein synthesis by cycloheximide had no effect on the loss of MIR and insulin sensitivity or recovery. From these studies we conclude that: 1) the insulin-responsive glucose transport system in adipocytes is regulated through two mechanistically distinct but integrated control systems—regulation of insulin sensitivity and MIR; 2) desensitization of the glucose transport system is characterized by rapid and progressive loss of MIR followed by a much slower decline in insulin sensitivity; 3) recovery of MIR and insulin sensitivity is observed after desensitization, but recovery rates are differentially regulated and determined by both the extent of desensitization and by the ambient concentrations of glucose and insulin; and 4) development of insulin resistance and subsequent recovery are both independent of de novo protein synthesis. Based on the rapidity of desensitization and recovery, it appears likely that daily fluctuations in circulating levels of insulin and glucose acutely regulate the adipocyte glucose transport system by modulating MIR and insulin sensitivity.

Desensitization of peripheral target tissues to the biological actions of insulin is the hallmark of certain pathophysiological states such as Type II diabetes and obesity and is characterized by impaired insulin action that is manifested by decreased insulin responsiveness and/or insulin sensitivity (1–6). Although the cellular and molecular mechanisms underlying insulin resistance have not been fully elucidated, recent advances have provided important new insights into this process. Of particular importance are the independent studies of Cushman and Wardzala (7) and Suzuki and Kono (8) which clearly demonstrated that insulin enhances the rate of glucose uptake into isolated rat adipocytes primarily through a rapid, reversible, and energy-dependent translocation of glucose transporters from a large intracellular pool to the plasma membrane. With the formulation of this translocation hypothesis, subsequent adipocyte studies revealed that a marked decrease in the number of intracellular glucose transporters was associated with several insulin-resistant states including streptozotocin-induced diabetes (9), aging (10), and high fat feeding (11). Thus, at least one cause of insulin resistance can be attributed to a depletion in the actual number of intracellular glucose transporters.

As exemplified by the above studies, isolated rat adipocytes are ideally suited for investigating the cellular mechanisms underlying insulin resistance of the glucose transport system. However, development of insulin resistance in adipocytes could not be studied under defined in vitro conditions because cell viability restricted studies of insulin action to experimental times under 6 h. To overcome this obstacle, in 1983 we developed a method for maintaining isolated adipocytes in primary culture for times ranging from 6 h to 2 weeks (12, 13). We used this new culture system to address two questions pertaining to the development of insulin resistance: 1) can prolonged insulin treatment induce desensitization of the glucose transport system, and if so, 2) does the extracellular concentration of glucose play a role in the induction of insulin resistance? In 1986, we reported that prolonged insulin treatment for 2–24 h can indeed lead to progressive desensitization of the insulin-responsive glucose transport system. Moreover, loss of insulin sensitivity, maximal insulin responsiveness (MIR), and insulin binding were found to occur at different times.

* This work was supported in part by Research Grant DK38754 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: MIR, maximal insulin responsiveness; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMEM, Dulbecco's modified Eagle's medium containing 25 mM Hepes and 1% bovine serum albumin; HBSS, Hepes-buffered balanced salt solution; TmR, treatment.
rates, indicating sequential regulatory effects at both receptor and multiple postreceptor sites along the insulin action pathway (14, 15).

When the role of glucose in the induction of insulin resistance was examined, several important observations were made which advanced our understanding of insulin-induced desensitization (16). We found that neither insulin alone nor glucose alone could induce desensitization of the glucose transport system; however, in the presence of insulin, physiological concentrations of glucose decreased MIR in a dose-dependent manner. Our interpretation of this observation was that the intracellular metabolism of glucose is the primary regulator of the insulin-responsive glucose transport system and that glucose (or a glucose metabolite) forms part of a negative feedback loop that decreases both insulin responsiveness and sensitivity. In this scheme, the role of insulin is simply to enhance glucose uptake into the cell. A particularly important aspect of this study related to a novel mechanism involved in desensitization. Specifically, we found that treatment with glucose and insulin diminishes MIR not by depleting the number of intracellular glucose transporters but rather by impairing the ability of glucose transporters to translocate from the cell interior to the cell surface in response to insulin. Thus, diminished translocation of insulin-responsive glucose transporters represents an additional mechanism mediating desensitization of the insulin-responsive glucose transport system.

In the current study, we have examined the other arm of insulin resistance, namely, recovery of insulin action after in vitro induction of insulin resistance. Overall, these studies demonstrate that recovery of MIR and insulin sensitivity can be measured in vitro and that rates of recovery are related to the extent of desensitization.

**EXPERIMENTAL PROCEDURES**

**Materials—Porcine monoclonal insulin was generously supplied by Dr. Ronald Chance of Lilly. Collagenase was obtained from Worthington; Dulbecco's modified Eagle's medium (DMEM) and DMEM specially formulated without D-glucose were obtained from GIBCO; Hepes, phloretin, and cycloheximide were from Sigma; bovine serum albumin (CRG-7) was from Armour; silicone oil was from Arthur H. Thomas; and 2-deoxy-D-[3H]glucose, D-3-O-methyl[U-14C]glucose, and [125I]A14 moniodoinsulin were from Du Pont-New England Nuclear.

**Preparation of Sterile Isolated Adipocytes—Male Sprague-Dawley rats weighing 160-225 g were killed by cervical dislocation, and the epididymal fat pads were removed under sterile conditions. Isolated adipocytes were then obtained as described previously (17) using a method modified from that of Rodbell (18). Briefly, minced tissue (1-2 g) was shaken in 4-ounce sterile polycarbonate containers at 37°C for 50 min in 4 ml of DMEM containing 25 mM Hepes, collagenase (1.5 mg/ml), and albumin (40 mg/ml). Cells were then filtered through nylon mesh (1000 μm), centrifuged at 100 rpm for 25 s, washed three times in sterile glucose-free DMEM containing 20 mM Hepes, 1% bovine serum albumin, 2% fetal calf serum, penicillin (20 units/ml), and streptomycin (20 mg/ml). Cells were then diluted to a final volume equal to 10 ml of buffer/g of fat (final concentration about 5 × 10^7 cells/ml) in a glucose-free and insulin-free HBSS and insulin sensitivity (ED_50) of the glucose transport system was determined from complete insulin dose-response curves.
tivity can be attributed to either a decrease in insulin receptor number or postreceptor regulation at sites along the insulin action pathway (or both). Quantitative differences in insulin sensitivity among treatment groups can be assessed by calculating and comparing half-maximally effective insulin concentrations (insulin ED$_{50}$).

A decrease in the ability of insulin to stimulate maximally the biological action under study is termed decreased "insulin responsiveness." Usually, decreased MIR reflects a regulatory change distal to insulin binding since most cells possess spare insulin receptors (e.g., in adipocytes only 10% of available insulin receptors need be occupied to elicit a maximal biological response). Although in most cases we refer to changes in insulin action by the specific terms MIR or insulin sensitivity, occasionally we use the more general terms "insulin resistance" or "desensitization." These terms are used interchangeably and imply impaired insulin action as manifested by a decrease in insulin responsiveness, insulin sensitivity, or both.

RESULTS

Loss of Maximal Insulin Responsiveness: Time Course and Kinetic Studies on the Effects of Insulin and Glucose—Fig. 1 depicts an experiment in which adipocytes were cultured for various times in the presence of 20 mM glucose alone (controls) or glucose plus insulin (50 ng/ml insulin). After thoroughly washing the cells in cold (10 °C) insulin-free and glucose-free buffer, we determined the MIR of the glucose transport system by acutely restimulating cells with 25 ng/ml insulin and then measuring the 2-deoxyglucose uptake rates. Based on the progressive loss of MIR in insulin-treated cells, it is apparent that induction of desensitization occurs in two phases: a fast phase (0–6 h) in which the rate of loss is immediate (no lag period), rapid (t$_{50}$ of 3 h), extensive (80% of that seen at 24 h), and linear; and a much slower phase (6–24 h). It should also be mentioned that the loss of MIR occurred more rapidly (t$_{50}$ of 3 h) than previously reported (t$_{50}$ of 8 h, Ref. 16). In control cells, MIR remained constant from 0 to 6 h and decreased by only 15% by 24 h. Nearly identical results were obtained when glucose transport was assessed using radiolabeled 3-O-methylglucose (a 40% decrease in MIR after 4 h and a 70% decrease after 24 h). These findings indicate that loss of MIR occurs at the level of glucose transport and that 2-deoxyglucose uptake rates accurately reflect this change.

Normally, under in vivo conditions, adipocytes would be continuously exposed to circulating insulin; therefore, it was of interest to determine if deactivation of the glucose transport system was a prerequisite for the expression of desensitization. Our in vitro approach to address this question was to expose cells to both insulin and glucose for 4 h to induce desensitization and then divide the cells into two groups (Fig. 2). The first group of cells (deactivated and restimulated) was washed extensively in cold insulin-free glucose-free buffer. After the stimulated glucose transport system deactivated to basal values, maximally insulin-stimulated rates of glucose transport were determined by reexposing cells to insulin (25 ng/ml for 30 min at 37 °C) and measuring 2-deoxyglucose uptake rates. In the second group (no deactivation), cells were washed in glucose-free buffer containing 25 ng/ml insulin (to prevent deactivation of stimulated glucose transport), and after a 10-min preincubation period at 37 °C, the rate of 2-deoxyglucose uptake was measured. As can be seen, loss of MIR in the continuous presence of insulin was equivalent to that observed in deactivated and insulin-restimulated cells. Thus, deactivation of the glucose transport system is not required for desensitization of MIR to be manifested.

Loss of Insulin Sensitivity: Time Course and Effects of Insulin and Glucose—Fig. 3 depicts an experiment in which adipocytes were cultured for various times with 20 mM glucose alone (controls) or with glucose plus 50 ng/ml insulin. After thoroughly washing the cells (control and desensitized), we determined the insulin sensitivity of the glucose transport system at each of the indicated times. This was accomplished by acutely restimulating cells with various concentrations of insulin, measuring the rate of 2-deoxyglucose uptake, and calculating the half-maximally effective insulin concentration (insulin ED$_{50}$) from complete insulin dose-response curves.

As can be seen in Fig. 3, insulin sensitivity remained constant for up to 24 h in control cells (ED$_{50}$ of 0.25 ± 0.01 ng/ml), whereas sensitivity progressively diminished in insulin-treated cells (insulin ED$_{50}$ of 0.24–1.45 ng/ml at 0 and 24 h, respectively). It is important to note that loss of insulin sensitivity was characterized by an initial 2-h lag period in which little or no change in sensitivity was observed (ED$_{50}$ of 0.26 versus 0.30 ng/ml at 0 and 2 h, respectively). From 2 to 24 h, sensitivity decreased slowly and continually with a t$_{50}$ of 8 h. Based on our current results revealing that insulin and glucose treatment of adipocytes causes an almost immediate loss in MIR which was rapid (t$_{50}$ of 8 h) and 80% completed by 6 h, we conclude that the time-dependent loss of insulin

![Graph](image-url)
sensitivity differs markedly from the loss of MIR.

Because loss of insulin sensitivity may be attributed to down-regulation of cell surface receptors, we performed the insulin-binding studies summarized in Table I. These studies revealed that cell surface insulin receptors remain constant during the first 6 h of desensitization and were decreased by only about 30% after 24 h. Since only about 10% of the insulin receptors need be occupied to elicit a maximal response in glucose transport activity (20) and since receptor loss was negligible at 6 h and modest (30%) at 24 h, the observed loss of MIR and insulin sensitivity must be the result of postreceptor regulatory events.

**Figure 3.** Progressive loss of insulin sensitivity in adipocytes exposed to high insulin and glucose. Cells were cultured at 37 °C from 2 to 24 h with 20 mM glucose in the absence (control) or presence of 50 ng/ml insulin. At the indicated times, the cells were washed (at 10 °C) in glucose-free and insulin-free HBSS. Cells were then pre-treated for 30 min at 37 °C with a full range of insulin concentrations (0–25 ng/ml), and the insulin-stimulated rates of 2-deoxyglucose uptake were measured. Insulin sensitivity (insulin ED50) of the glucose transport system was determined at the indicated times from full insulin dose-response curves. Each point represents the mean ± S.E. of six experiments.

**Table 1**

| Treatment time (h) | Specific 125I-insulin binding (B/F \*100) | Loss of receptors |
|--------------------|------------------------------------------|------------------|
|                   | Control                                  | Insulin-treated  |
| 0                  | 5.5 ± 0.1                                | 5.6 ± 0.2        | 0                |
| 6                  | 5.4 ± 0.3                                | 5.2 ± 0.4        | 4                |
| 24                 | 5.2 ± 0.2                                | 3.5 ± 0.4        | 33               |

Recovery of Maximum Insulin Responsiveness: Direct Relationship to the Extent of Desensitization—To determine whether adipocytes can regain their ability to respond maximally to insulin after desensitization of the glucose transport system, we performed the experiments depicted in Fig. 4. After treating cells at 37 °C for 2–24 h with high glucose (20 mM) and insulin (50 ng/ml), cells were washed and further incubated in glucose-free and insulin-free buffer. At various times from 0.5 to 6 h, glucose uptake rates were measured. Results of these experiments revealed that adipocytes can recover their capacity to respond maximally to insulin. More importantly, however, it should be noted that recovery slopes (as determined by linear regression analysis) diminished as a function of treatment time, indicating a progressive slowing in the rate at which MIR recovers. To obtain more quantitative information on the relationship between recovery of MIR and insulin incubation time, we calculated recovery rates based on the linear regression data in Fig. 4A. As shown in Fig. 4B, recovery rate was maximal after 2 h (250 pmol/3 min/h), exhibited the greatest change during the first 6 h of treatment (250–100 pmol/3 min/h), and slowed dramatically between 6 and 24 h (100–40 pmol/3 min/h). It should be noted that the t1/2 for recovery is 3.3 h, which is remarkably similar to the t1/2 for induction of desensitization (3 h), suggesting a possible link between these two processes. Fig. 4C illustrates that recovery of MIR is highly correlated (R = 0.98) with maximal rates of 2-deoxyglucose uptake measured immediately after desensitization. Thus, it
appears that recovery of the insulin-responsive glucose transport system is related to the extent of desensitization rather than actual insulin treatment time.

**Recovery of Insulin Sensitivity**—To determine whether recovery of insulin sensitivity also slows as a function of desensitization, we performed the experiments depicted in Fig. 5. Adipocytes were cultured for 2–24 h in the presence of high glucose and insulin, extensively washed, and then allowed to recover at 37 °C in insulin-free and glucose-free buffer. At the indicated times, complete insulin dose-response curves were obtained and sensitivity determined by calculating the insulin ED_{50}. As can be seen in Fig. 5A, recovery of insulin sensitivity can be quantitated in vitro, and the initial rates of recovery are linear over 6 h.

To quantify better the relationship between insulin treatment time and subsequent recovery of sensitivity, we calculated recovery rates based on linear regression analysis of the data in Fig. 5A. These rates are plotted in Fig. 5B and reveal that recovery of sensitivity actually increases as desensitization progresses. Thus, after 2 h of insulin treatment recovery of sensitivity was 0.654 ng/ml/h, whereas after 18 h it was 0.115 ng/ml/h. This trend is in marked contrast to changes in MIR where recovery slows as desensitization proceeds. It should also be noted that recovery of sensitivity is much slower than that of MIR (t_{1/2} of 7.7 h versus 3.3 h for sensitivity and MIR, respectively).

**Effects of Insulin and Glucose on the Recovery of MIR and Insulin Sensitivity**—Shown in Fig. 6 are the dose-dependent effects of insulin (in the presence of 20 mM glucose) on recovery of MIR and insulin sensitivity. From these results several salient points emerged. First, it is apparent that neither insulin nor glucose alone can prevent recovery of maximal insulin responsiveness over 3 h (Fig. 6A). However, low concentrations of insulin, in the presence of high glucose, can effectively blunt recovery in a dose-dependent manner (MIR EC_{50} = 0.22 ng/ml). It should also be noted that insulin concentrations of 0.5–1 ng/ml decreased MIR below the value observed immediately after the 4-h treatment period. This indicates that recovery was completely inhibited and that further desensitization occurred during the 3-h recovery period. Finally, it is important to highlight the fact that MIR did not increase in control cells during the first 4 h of the experiment. This suggests that after collagenase digestion, freshly isolated cells are maximally responsive to insulin and are not partially desensitized from their in vivo environment. However, we cannot exclude the possibility that rapid recovery occurred during the 1 h required to isolate cells from the epididymal fat pads.

From the data in Fig. 6B, it can be seen that glucose alone prevented recovery of insulin sensitivity (insulin ED_{50} of 0.46 ng/ml immediately after desensitization versus 0.51 ng/ml after a 3-h recovery period), whereas insulin alone had no effect on recovery. However, insulin markedly diminished sensitivity (ED_{50}) in a dose-dependent manner in the presence of high glucose (MIR EC_{50} = 0.46 ng/ml). Moreover, combinations of insulin and 20 mM glucose effectively lowered
sensitivity to values far below those seen immediately after 4 h of desensitization.

To assess the dose-dependent effects of glucose on recovery of MIR and insulin sensitivity, we used a protocol similar to that of Fig. 6, and these results are shown in Fig. 7. Again, neither glucose nor insulin alone affected recovery of MIR, whereas in the presence of a fixed insulin dose, a clear dose-dependent inhibition of recovery was observed as a function of glucose concentration (Fig. 7A). It is important to highlight the fact that the concentration of glucose required to block recovery of MIR (Tmt EC50 = 1.2 mM) is much lower than that found to induce desensitization after a 4-h insulin treatment period (Tmt EC50 = 3.3 mM, data not shown).

Shown in Fig. 7B is the dose-dependent ability of glucose (in the presence of insulin) to block recovery of insulin sensitivity. As was observed in Fig. 6, glucose alone blocked recovery of sensitivity, whereas insulin alone was ineffective. Furthermore, desensitization beyond that seen immediately after the 4-h insulin treatment period occurred when cells were allowed to recover in the presence of insulin and concentrations of glucose greater than 1 mM (Tmt EC50 = 2.5 mM).

Inability of Protein Synthesis Inhibitors to Prevent Desensitization or Block Recovery of Maximal Insulin Responsiveness and Sensitivity—In addition to augmenting the rate of glucose uptake into adipocytes, insulin exerts numerous other pleiotropic actions which include rapid enhancement in the rate of de novo protein synthesis. Therefore, we next examined the role of protein synthesis in mediating both desensitization of the glucose transport system and the subsequent recovery of MIR (Fig. 8, A and B). Shown in A is the effect of 5 μg/ml cycloheximide on MIR in control cells (20 mM glucose) and cells treated with glucose and insulin (50 ng/ml). Although cycloheximide inhibited protein synthesis by >90% within 2 min (data not shown), it is apparent from these results that cycloheximide was ineffective in preventing desensitization. These findings add credence to the idea that glucose or a metabolite is the primary regulator of the insulin-responsive glucose transport system and acts independently of insulin's effect on protein synthesis.

In Fig. 8B, it can be seen that when cells were desensitized for 18 h and allowed to recover for 6 h in the absence (control) or presence of cycloheximide, no differences in the extent of recovery were observed between control and the cycloheximide-treated cells. This indicates that recovery of MIR is also independent of protein synthesis.

The role of protein synthesis in the loss and recovery of insulin sensitivity was next examined by performing the experiments depicted in Fig. 8, C and D. When adipocytes were incubated for 4 h in the absence (controls) or presence of maximally effective concentrations of glucose and insulin,
DISCUSSION

Primary cultured adipocytes were used in the current study to examine both the development and recovery of insulin resistance under defined in vitro conditions. When adipocytes were treated with maximally effective concentrations of insulin and glucose for 2–24 h, we found that the MIR of the glucose transport system decreased in a biphasic manner. At early times (1–6 h), induction of desensitization was immediate (no lag period), rapid (t½ of 5 h), linear, and extensive (80% of that seen at 24 h); whereas at later times (6–24 h), desensitization proceeded more slowly. In cells treated with glucose alone, MIR remained constant from 0 to 6 h and decreased less than 15% by 24 h.

Loss of insulin sensitivity, like loss of MIR, was induced by treating isolated adipocytes with glucose and insulin. At various times from 2 to 24 h, sensitivity was determined by washing cells in insulin-free and glucose-free buffer, allowing the stimulated glucose transport system to deactivate, and then constructing complete insulin dose-response curves. From these curves, the half-maximally effective insulin concentration (insulin ED₅₀) was determined and used as an index of insulin sensitivity.

Results from these studies revealed that an initial lag of about 2 h preceded loss of insulin sensitivity and that from 2 to 24 h there was a slow and progressive loss of sensitivity (t½ = 8 h) resulting in a 6-fold decrease in sensitivity (ED₅₀ of 0.25 ng/ml at 0 h versus 1.45 ng/ml after 24 h). When the slower loss of insulin sensitivity is compared with the rapid loss of MIR, it is apparent that there are marked kinetic differences in both the onset and rate, suggesting that MIR and insulin sensitivity may be differentially regulated. Particularly striking was the 2-h time point, where loss of MIR was nearly 50% complete without a concomitant decrease in insulin sensitivity.

We reported previously that incubation of adipocytes with glucose and insulin first leads to a decline in the insulin sensitivity of the glucose transport system followed by a delayed loss in MIR (16). In the current studies, however, we found that MIR declines almost immediately and is 80% complete by 6 h; loss of insulin sensitivity, however, exhibited a 2-h lag and proceeded at a slower rate. Although the temporal loss of insulin sensitivity was in good agreement between the two studies, the rate at which MIR declined was markedly slower in the earlier study. This apparent discrepancy between our past and current studies can be readily explained by differences in the washing procedures, the time allotted for deactivation of the glucose transport system, and differences in the recovery rates of MIR. In previous studies, the washing and deactivation procedures were lengthy, requiring about 3 h, whereas the current studies involved washings with cold buffer (to slow recovery) and immediate restimulation with insulin to assess MIR. Thus, in our previous studies we underestimated the rapidity of desensitization of MIR because of ongoing recovery, particularly at the earlier times when recovery is very rapid.

Relevant to our studies are recent reports indicating that glucose directly regulates the rate of glucose utilization in skeletal muscle. Sasson and Cerasi (22) reported that low concentrations of glucose (3–4 mM) were capable of decreasing glucose transport activity in isolated soleus and skeletal muscle in a time-dependent manner over a period of 2–3 h. Similarly, Richter et al. (23) noted that exposure to moderately high levels of glucose (12 mM) leads to the rapid development of insulin resistance in perfused rat hindlimbs. They found this effect to be enhanced in the presence of insulin which indicates that both insulin and glucose are involved, although glucose seemed to be the predominant modulator. Additionally, the induction of insulin resistance was both rapid and extensive, with an 80% decrease in insulin responsiveness at 5 h. Both the present decline in MIR and the time frame observed by Richter et al. (23) closely parallel our findings with desensitized rat adipocytes, which suggests that the glucose and insulin modulation of insulin-sensitive glucose transport activity in adipose and muscle may occur by a common mechanism.

Recovery of MIR and insulin sensitivity after induction of insulin resistance constitutes the other arm of desensitization and is important since at any point in time the biological responsiveness of cells to insulin would reflect a balance between desensitization and recovery. Using primary cultured adipocytes, we examined recovery of MIR and insulin sensitivity after inducing varying degrees of insulin resistance. This was done by incubating cells for 2–24 h, removing insulin and glucose, and measuring MIR and sensitivity over a subsequent 30-min–6-h period. Results of these studies clearly revealed that adipocytes can recover both their insulin sensitivity and capacity to respond maximally to insulin and that recovery is linear over time.

Most interesting, however, was the finding that recovery of MIR varied inversely with insulin and glucose treatment time. Thus, the fastest recovery rates were seen after 2 h of desensitization (251 pmol of glucose/3 min/h) and the slowest recovery rates after 24 h of treatment (35 pmol/3 min/h). The rapidity with which recovery rates changed (t½ of 3.3 h) and the biphasic nature of these changes are remarkably similar to those observed for the induction of insulin resistance, which suggests a link between the two processes. When recovery rates were plotted as a function of MIR (measured immediately after desensitization), a high correlation was found (R = 0.98). Thus, it appears that recovery of MIR is related to the extent of desensitization rather than the actual treatment times.

An opposite trend was seen for recovery of insulin sensitivity. At early times after desensitization, recovery of sensitivity was slow (0.055 ng/ml/h at 2 h), whereas recovery proceeded more rapidly after 24 h (0.115 ng/ml/h). Thus, recovery rates of maximal insulin responsiveness were decreasing during the period when insulin sensitivity recovery rates were increasing, clearly establishing the operation of distinct regulatory mechanisms.

When we assessed the individual roles of insulin and glucose on recovery of MIR and sensitivity in 4-h-desensitized cells, neither insulin nor glucose alone was capable of blocking recovery of MIR. Low concentrations of insulin, however, did effectively inhibit MIR recovery in a dose-dependent manner in the presence of 20 mM glucose (insulin EC₅₀ = 0.22 ng/ml), and low concentrations of glucose prevented recovery of MIR in the presence of 50 ng/ml insulin (glucose EC₅₀ = 1.2 mM). In studies examining recovery of insulin sensitivity, we found that insulin alone was incapable of blocking recovery, whereas glucose alone could effectively prevent recovery, adding credence to the idea that distinct molecular mechanisms regulate MIR and insulin sensitivity. When both glucose and insulin were present, we found that higher concentrations of each were required to blunt or prevent recovery of insulin sensitivity. Thus, in the presence of a fixed insulin dose (50 ng/ml) the glucose EC₅₀ was 2.5 mM, while in the presence of a fixed dose of glucose (20 mM) the insulin EC₅₀ was 0.46 ng/ml.

Three aspects of the current study are particularly relevant to the cellular mechanisms underlying loss of insulin responsiveness and sensitivity. First, we observed no decrease in insulin binding during the first 6 h of treatment and only
about a 30% decrease after 24 h (consistent with our previous findings, Refs. 13 and 16) which indicates that desensitization of both MIR and insulin sensitivity occur at a postreceptor site. Second, we found that cycloheximide inhibition of protein synthesis did not affect the loss of either MIR or sensitivity (after a 4-h exposure to insulin and glucose). This finding adds support to the idea that glucose or a glucose metabolite is the primary regulator of the insulin-responsive glucose transport system and that desensitization is not mediated by de novo synthesis of regulatory proteins. Finally, we found that deactivation of the glucose transport system was not a prerequisite for the expression of desensitization. Thus, the loss of MIR in the continuous presence of insulin was nearly identical to that observed in cells from which insulin was removed (to allow for deactivation of the glucose transport system) and then restimulated. The finding that deactivation is unnecessary (as is the removal of glucose) for the expression of desensitization is important since under normal in vivo conditions adipocytes would be continuously exposed to circulating insulin and glucose.

For several reasons we believe that our current results support the concept that desensitization and recovery of MIR and insulin sensitivity constitute a physiological control system by which glucose and insulin acutely regulate glucose transport activity under normal in vivo conditions. First, desensitization of the glucose transport system precedes loss in cell surface insulin receptors, indicating that glucose and insulin modulation of glucose transport activity occurs at a postreceptor site. Second, induction of insulin resistance is rapid (i.e., of 3 h) and temporally in accord with postprandial oscillations of plasma glucose and insulin concentrations (24, 25). Further, the finding that loss of insulin sensitivity, unlike MIR, is delayed and then proceeds slowly suggests that preservation of insulin sensitivity may be of physiological importance. Fourth, deactivation of the glucose transport system is not a prerequisite for desensitization, nor is the removal of glucose, which indicates that induction of insulin resistance can take place in the continuous presence of insulin and glucose, as would be encountered in the in vivo situation. Finally, recovery of insulin responsiveness is extremely rapid especially after shorter periods of desensitization.

Based on the data encompassed in this paper, we propose that the insulin-sensitive glucose transport system is regulated through two mechanistically distinct but integrated mechanisms: regulation of insulin sensitivity and regulation of MIR. In this model, insulin target tissues can continuously monitor circulating levels of glucose and insulin and regulate net glucose influx in accordance with prevailing physiological conditions. The overall response of the glucose transport system is likely based on both the extent and duration of hyperinsulinemic and hyperglycemic conditions. In the first phase of desensitization (less than 2 h), MIR would be more rapidly reduced with no change in insulin sensitivity, and insulin responsiveness would rapidly return to normal when elevated levels of insulin and glucose were reduced. Such events would be expected to occur during the postprandial oscillations in circulating levels of insulin and glucose during dietary intake (24, 25).

Should glucose and insulin remain elevated for longer periods of time (2–6 h), further cellular adaptations would reduce glucose intake. This would be mediated by an additional loss of MIR, a decrease in insulin sensitivity, and a more protracted period of recovery. In the third phase (6–18 h), severe insulin resistance would be manifested by a marked loss of both MIR and insulin sensitivity. Finally, as a long term regulatory response to prolonged hyperinsulinemia and hyperglycemia, the number of cell surface insulin receptors would be reduced thereby causing further loss of insulin sensitivity.

In the context of this scheme, the underlying cellular mechanisms that regulate insulin sensitivity and responsiveness would play important roles in the acute regulation of the glucose transport system in response to physiological fluctuations in circulating levels of insulin and glucose associated with dietary intake. Abnormalities in this putative regulatory system may underlie certain insulin-resistant states such as Type II diabetes and obesity.

**Acknowledgment—**We gratefully acknowledge the editorial assistance of Henri Knuse.

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