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MAINTENANCE OF 3T3-L1 CELLS IN CULTURE MEDIA CONTAINING SATURATED FATTY ACIDS DECREASES INSULIN BINDING AND INSULIN ACTION[1]

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Summary

To modify cellular and membrane lipid composition, differentiated 3T3-L1 adipose cells were maintained in media containing dialyzed fetal calf serum supplemented with a variety of fatty acids. Cells exposed to saturated fatty acids exhibited up to an 80% decrease in insulin binding and insulin stimulated 2-deoxyglucose uptake, whereas cells exposed to monounsaturated fatty acids of the same chain length showed less change. The decrease in insulin binding appeared to be due to changes in both receptor number and affinity. Maximal insulin stimulation of deoxyglucose was decreased with little change in sensitivity to insulin. There was an excellent correlation between the inhibition of both insulin binding and insulin action by the fatty acids tested. These results suggest that modification of cellular lipids can produce profound effects on both insulin binding and insulin action.

Introduction

The first step in insulin action is the binding of insulin to its receptor on the plasma membrane (1). Subsequent steps in insulin action are thought to include the transmission of a signal across the plasma membrane, generation of a second messenger, regulation of membrane and cytoplasmic processes, as well as internalization of both insulin and its plasma membrane receptor (2-4). The best characterized of these processes is stimulation of glucose transport in the adipocyte (4) The role of the plasma membrane or cellular lipids in these

1. This work has been presented in preliminary form at the meeting of the American Diabetes Association, Diabetes 30:17A, 1981 (abstract). Address correspondence to Dr. Grunfeld, University of California Service, Veterans Administration Medical Center (111 F), 4150 Clement Street, San Francisco, CA 94121. Present address for Dr. Kahn is Elliott P. Joslin Research Laboratory, 1 Joslin Place, Boston, MA 02215.

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events is largely unknown, although treatment with phospholipase has been shown to increase insulin binding (5,6).

Spector has shown that it is possible to extensively modify the fatty acyl groups of phospholipids of the plasma membrane as well as other cellular neutral and phospholipids by maintaining cultured cells in media containing dialyzed fetal calf serum supplemented with specific fatty acids (7). We have applied this technique to 3T3-L1 cells, a cultured fat cell line which we have used extensively for the study of insulin responsiveness and insulin resistance (8-11)[2]. In this study we demonstrate that specific fatty acids can profoundly affect insulin binding and insulin action.

MATERIALS AND METHODS

Dexamethasone and all fatty acids were purchased from Sigma Chemical Co. Fetal calf serum was purchased from Microbiological Associates (Lot #94062). Other materials were obtained as described previously (8-11). Fatty acid supplemented media were prepared by minor modification of the procedure of Spector (7). Fatty acids (0.2 M in ethanol) were diluted 1:25 into Dulbecco's phosphate buffer saline pH 7.4 containing 20% bovine serum albumin at 60°. The mixture was gently agitated until the fatty acids dissolved. The fatty acid-albumin solution was then readjusted to pH 7.4 with sodium hydroxide. The fatty acid-albumin molar ratio was kept less than 3, which assures that fatty acid is bound to the albumin. The fatty acid-albumin solution was then diluted 1:10 into Dulbecco-Vogt modified Eagle's medium containing 10% dialyzed fetal calf serum to a final concentration of 3.3×10^{-4} M fatty acid and 0.4% ethanol. Media were then filter sterilized. Control cells were treated with medium prepared as above except that only the carrier ethanol was added to the albumin. The albumin and ethanol had little effect on insulin binding or action (data not shown).

3T3-L1 cells were grown in Linbro multiwell plates as previously described (8-11). Differentiation was enhanced by modification of the method of Reed *et al* (13). Confluent cells were treated with 0.25 μM dexamethasone, 0.5 mM 1-methyl-3-isobutyl-xanthine and insulin (1 μg/ml) in Dulbecco-Vogt modified medium containing 10% fetal calf serum for one change of medium (2 days). Cells were then maintained in Dulbecco-Vogt medium supplemented with the fatty acid-albumin mixture as described above for 4-5 feedings (10-12 days). Cells were washed with Krebs Ringer phosphate buffer pH 7.4 containing 2% bovine serum albumin and insulin binding and insulin stimulated 2-deoxyglucose uptake were determined in that buffer by methods previously described in detail (8-11). Insulin binding and biological action were assayed while cells were still attached to plates in order to avoid artifacts that might result during the isolation of cells with fatty acid modification.

RESULTS

Differentiated 3T3-L1 cells were fed with media supplemented with palmitic acid (16:0) or palmitoleic acid (16:1) and insulin binding was measured (Fig.

2. Dialysis of fetal calf serum reduces its biotin content; glucose incorporation into fatty acids is decreased in 3T3-L1 cells maintained in dialyzed serum (12).

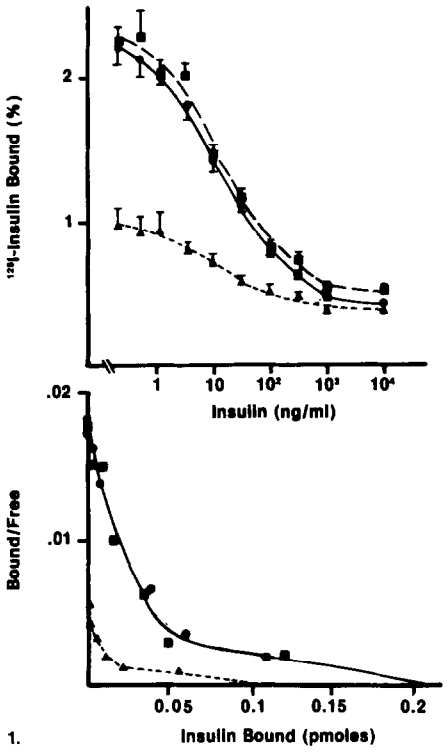
1). Cells maintained in palmitic acid exhibited a 65% decrease in tracer insulin binding (upper panel). This was due to a decrease in specific binding with little change in non-specific binding (^{125}I-insulin bound in the presence of 10 μg/ml of unlabeled insulin). By contrast, insulin binding to cells treated with palmitoleic acid was not significantly different from control cells.

Scatchard analysis shown in the lower panel suggests that the decreased insulin binding seen after treatment with palmitic acid was due to a decrease in receptor concentration (Ro) as well as a decrease in receptor affinity. Assuming the negative cooperativity model (14), the high affinity site ($\bar{K}_e$) was decreased from 8.7 to 5.2 X10^7 M^{-1} while the low affinity site ($\bar{K}_f$) was decreased from 2.0 to 1.2 X10^7 M^{-1}.

In addition to the changes in insulin binding, these cells showed changes in the insulin stimulated component of deoxyglucose uptake (Fig. 2). Maximal insulin stimulation was decreased 75% in cells fed palmitic acid. A smaller change was seen with cells fed palmitoleic acid. In both cases the dose response curve for insulin showed little change in the sensitivity of the cells to insulin.

Insulin binding and deoxyglucose uptake were then compared in cells maintained in media containing a variety of saturated and unsaturated fatty acids (Fig. 3). The upper panel shows tracer insulin binding and nonspecific binding. Cells exposed to the saturated fatty acids, stearic (18:0), palmitic (16:0), myristic (14:0) and capric (10:0) acids exhibited a 50-70% decrease in specific insulin binding whereas cells maintained in monounsaturated fatty acids, oleic (18:1), palmitoleic (16:1) and myristoleic (14:1) acids exhibited only a 5-35% decrease. The lower panel shows the effect of fatty acid treatment on deoxyglucose uptake. Again, saturated fatty acids produced a profound decrease in the maximal insulin response while unsaturated fatty acids had little effect on insulin action. Basal levels of deoxyglucose uptake were only slightly affected.

For both insulin binding and insulin action the saturated fatty acid analogue had a more profound effect than the monounsaturated analogue of the same chain length. The ability of saturated fatty acids to inhibit insulin binding and



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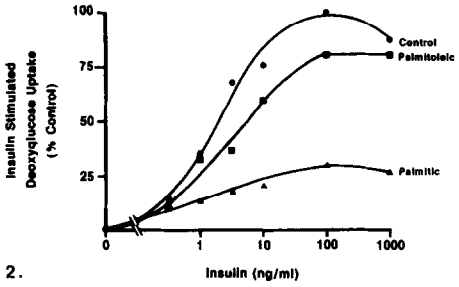

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FIGURE 1. The effect of fatty acid supplementation on insulin binding. Cells were maintained in media containing palmitic acid (▲), palmitoleic acid (■) or carrier ethanol (●) for five feedings (12 days) and insulin binding was measured. The binding assay was performed in Krebs Ringer phosphate buffer containing 2% bovine serum albumin at 24°C for 75 min while cells were still adherent to the plate. The values represent the mean of two experiments, each involving triplicate culture plates for each point. The upper panel represents competition by varying concentrations of unlabeled insulin (x-axis) against ^{125}I-insulin tracer binding (0.2 ng/ml). The lower panel shows analysis of this data by the method of Scatchard.

FIGURE 2. The effects of fatty acids on insulin action. Cells were prepared in an identical manner to those in Figure 1. The insulin stimulated component of deoxyglucose uptake is shown as a function of insulin concentration for control cells and cells fed with palmitic or palmitoleic acid. Deoxyglucose uptake was measured in cells adherent to plates after a 25 min pre-incubation with insulin under the same conditions as insulin binding was studied in Figure 1 by adding a 20 min pulse of 0.2 mM ^{14}C-2-deoxyglucose (0.8 μCi/ml) at 24°C (8,11). Control (●), palmitoleic acid (■), palmitic acid (▲). Basal deoxyglucose uptake was 1.30 ± .11 nmoles/well/20 min. Insulin stimulated deoxyglucose uptake in control cells averaged 5.40 nmoles/well/20 min.

insulin action was not directly proportional to their chain length. Palmitic (16:0) and capric (10:0) acids were more effective than stearic (18:0) and myristic (14:0) acids. An excellent correlation was found between the effects

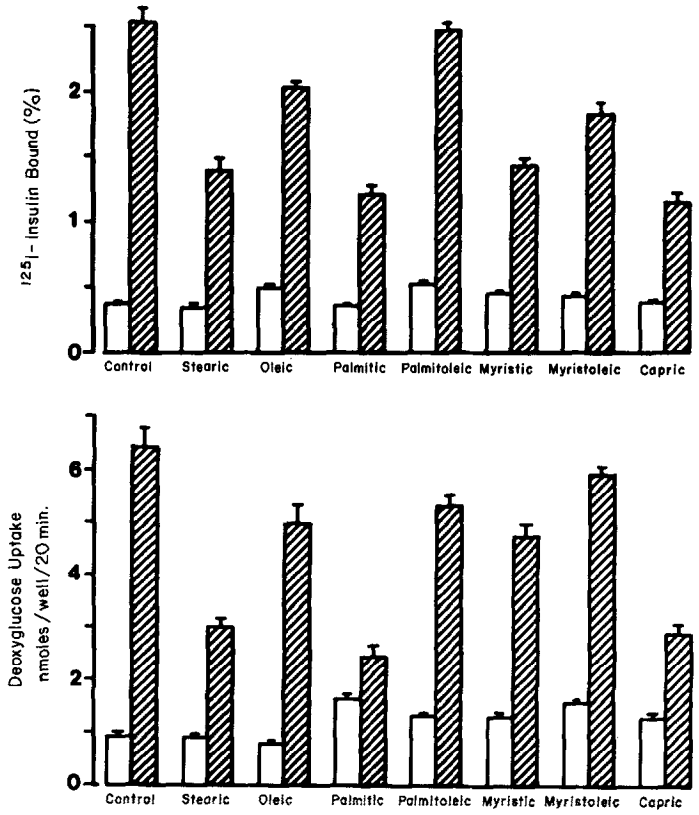

FIGURE 3. The effect of various fatty acids on insulin binding and insulin action. Cells were fed a variety of fatty acids as previously described. Tracer ^{125}I-insulin binding (hatched bar) and nonspecific binding (open bar), measured as described in Fig. 1, are shown in the upper panel. Insulin-stimulated (1 μg/ml) deoxyglucose uptake (hatched bar) and basal deoxyglucose uptake (open bar), measured as described in Fig. 2, are shown in the lower panel. This figure represents the results of a typical experiment although each fatty acid was studied several times.

of a given fatty acid on insulin stimulated deoxyglucose uptake and insulin binding (Fig. 4). Linear regression analysis gave an R value of .881 with a p value of less than 0.01.

DISCUSSION

We have shown that insulin binding and insulin action can be influenced by the feeding of specific fatty acids to cultured cells in a manner which is known to influence the fatty acyl substitution of both cell membrane phospholipids and intracellular triglycerides (7). Cells maintained in media supplemented with

unsaturated fatty acids show a profound decrease in both insulin binding and insulin-stimulated deoxyglucose uptake, whereas supplementation with mono-unsaturated fatty acids produce less effect.

The effects shown here are not due to a general toxicity of fatty acids as they are specific for certain fatty acids. While the saturated fatty acids have a more profound effect on insulin binding and insulin action than unsaturated fatty acids, not all saturated fatty acids effectively reduce insulin binding or insulin action (cf. myristic acid). Likewise, a detergent effect is unlikely since the effects were not directly related to the chain length of the fatty acids.

The locus for the effect of the fatty acids has not yet been determined. The fatty acids may influence intracellular substrates and energy sources. For example, unsaturated fatty acids have been shown to deplete certain cells of ATP (15). However, a more likely locus for the action of saturated fatty acids in these experiments is modification of the phospholipid composition of the plasma membrane (7). Studies of the temperature sensitivity of insulin-stimulated glucose transport have led some authors to propose that changes in membrane fluidity may modulate insulin action (16-18), although the same temperature dependence has not been found by all investigators (19,20). In addition, when solubilized insulin receptors are reconstituted into liposomes of different composition, the affinity of the receptor for insulin is influenced by the fatty acyl substitution of the added phospholipids (21); it is not possible to detect variations in the exposure, i.e., number of insulin receptors, in such reconstitution experiments. The addition of lipids directly to plasma membranes has been shown to influence the exposure of adrenergic receptors and the coupling of receptors to adenylate cyclase (22-24). It is thought that these changes are mediated by differences in membrane fluidity of the lipid modified plasma membrane however, the results seem to imply that the method of modification is as important as the absolute membrane fluidity in determining the nature of the changes.

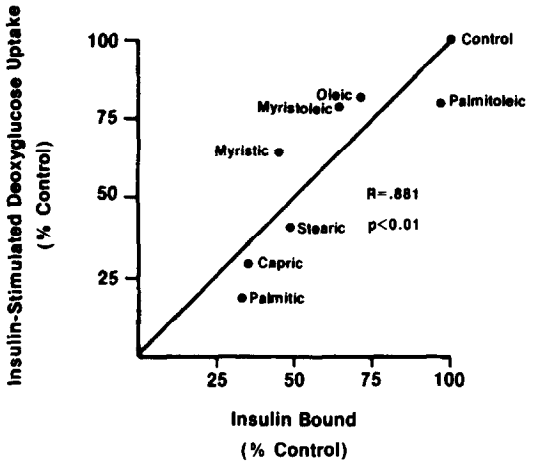

FIGURE 4. The correlation of insulin binding and insulin action in cells fed fatty acids. The insulin stimulated component of deoxyglucose uptake (uptake in the presence of 1 μg/ml insulin - basal uptake) and is plotted on the y-axis; specific insulin binding (tracer insulin binding - nonspecific binding) is plotted on the x-axis. This represents the averaged results of several experiments similar to the one shown in Figure 3. The line is drawn based on least squares linear regression analysis.

One surprising finding of our study was that fatty acid treatment resulted in a decrease in the ability of insulin to maximally stimulate deoxyglucose uptake rather than a change in insulin sensitivity. 3T3-L1 cells contain approximately 60% "spare receptors" for insulin (8); therefore, a small reduction in insulin binding should not necessarily affect maximal insulin response action. The presence of a good correlation between the effects of fatty acids on insulin binding and insulin action as shown in Figure 4 probably implies that a single event (the incorporation of fatty acid) affects both insulin binding and insulin action independently. However, a more complex analysis of the data may yield other more plausible interpretations.

In summary, these data suggest that cellular and membrane lipid composition may have profound effects on insulin binding and insulin action. To better understand these events at a molecular level, studies determining the nature of the changes in the fatty acyl composition of 3T3-L1 cells fed fatty acids and the influence of such modifications on membrane fluidity are in progress.

ACKNOWLEDGEMENTS

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Noted added in proof: Henson, Ginsberg and Spector have recently demonstrated that fatty acid supplementation of 3T3-L1 cells by this technique results in extensive modifications of the fatty acyl groups of the plasma membrane, with corresponding changes in membrane microviscosity (personal communication)

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