Role of fatty acid synthesis in the control of insulin-stimulated glucose utilization by rat adipocytes

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Abstract A decreased capacity for fatty acid synthesis is associated with a decreased insulin effect on glucose metabolism in large fat cells and fat cells from rats fed a high-fat diet. We have investigated the relationship between these processes by specifically inhibiting fatty acid synthesis with (−)-hydroxycitrate (2.5 mM), an inhibitor of citrate cleavage enzyme, and cerulenin (0.05 mM), an inhibitor of fatty acid synthetase. (−)-Hydroxycitrate and cerulenin decreased maximally insulin-stimulated fatty acid synthesis from [6-^{14}C]glucose to 10% and 25% of controls, respectively, while only (−)-hydroxycitrate decreased basal values. Oxidation of [1-^{14}C]glucose in the presence of insulin was markedly depressed by each inhibitor. Thus, the percent increase over basal value was decreased from 540% in controls to 151% and 154% by (−)-hydroxycitrate and cerulenin, respectively. In contrast, oxidation of [6-^{14}C]glucose was slightly enhanced by both inhibitors. Thus, oxidation of glucose via the pentose shunt was reduced, while Krebs cycle oxidation was unaffected. Basal and insulin-stimulated incorporation of [1-^{14}C]glucose and [6-^{14}C]glucose into glyceride-glycerol and basal lactate production was unchanged by the inhibition of fatty acid synthesis. Insulin-stimulated lactate production was halved by the inhibition of fatty acid synthesis. Total glucose utilization, as assessed by measuring the disappearance of glucose from the medium, was not detectably changed by inhibiting fatty acid synthesis under basal conditions, but insulin-stimulated values were decreased to 52% and 64% of control by (−)-hydroxycitrate and cerulenin, respectively. This occurred despite the fact that neither agent affected the initial rate of 2-deoxyglucose uptake, or glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase activities. These data therefore provide direct evidence that a limitation of the fatty acid synthetic pathway decreases the ability of insulin to stimulate both pentose shunt glucose oxidation and overall glucose utilization, but not Krebs cycle oxidation or glyceride-glycerol synthesis. The enzymatic capacity of the fat cell for fatty acid synthesis is therefore an important determinant of insulin-stimulated glucose utilization.—**Fried, S. K., M. Lavau, and F. X. Pi-Sunyer.** Role of fatty acid synthesis in the control of insulin-stimulated glucose utilization by rat adipocytes. *J. Lipid Res.* 1981. **22:** 753–762.

Supplementary key words insulin resistance · pentose shunt · (−)-hydroxycitrate · cerulenin

The ability of insulin to stimulate glucose metabolism is markedly diminished in adipocytes from rats fed high-fat diets (1–3) and in large adipocytes from older rats (4–7) compared to small adipocytes from younger animals. In both cases, the decreased insulin effect cannot be attributed to major alterations of insulin binding or to the ability of insulin to stimulate glucose transport, and therefore must be due to a limited capacity to metabolize glucose (1, 2, 4–7). It is unknown, however, which step(s) of glucose metabolism restricts glucose utilization by these fat cells.

The decreases in both basal and insulin-stimulated glucose metabolism in large adipocytes, as well as in adipocytes from rats fed a high-fat diet, involve reductions of fatty acid synthesis and pentose shunt activity, but not Krebs cycle oxidation or glyceride-glycerol synthesis (1, 4, 6, 8–10). Marked reductions in the activities of the key fatty acid synthesis enzymes seem to be responsible for the decreased fatty acid synthesis by these cells (1, 4). Since reductions in the activities of the glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase cannot account for the depressed rates of glucose oxidation by large fat cells, Richardson and Czech (4) have proposed that the primary defect in insulin-resistant large adipocytes is a decreased capacity for fatty acid synthesis which secondarily inhibits the flux of glucose through the pentose shunt. We have found a similar situation in adipocytes from rats fed a high-fat diet (1). Although the glucose dehydrogenases are reduced by high-fat feeding (1), the activities are still sufficient to support control rates of [1-^{14}C]glucose oxidation. In addition, the indirect inhibition of

Abbreviations: KRBB, Krebs Ringer bicarbonate buffer; HC, (−)-hydroxycitrate; C, cerulenin; I, insulin.

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fatty acid synthesis by the electron transport inhibitor rotenone diminishes pentose shunt glucose oxidation (4, 11), supporting the hypothesis that changes in fatty acid synthesis are primary. However, the relationship of changes in these two pathways to total glucose utilization and the response to insulin of the other major pathways of glucose metabolism have not been established.

Agents that specifically inhibit fatty acid synthesis are required to test the hypothesis that a limited capacity for fatty acid synthesis alone could lead to an altered pattern of glucose metabolism and a blunted response to insulin. We have therefore used (−)-hydroxycitrate, an inhibitor of citrate cleavage enzyme (12), and cerulenin (13), an inhibitor of fatty acid synthetase, to probe these relationships. (−)-Hydroxycitrate is an analog of citrate that competes with citrate for citrate cleavage enzyme (12), while cerulenin is thought to act by binding to the β-keto-acyl-ACP-synthetase of fatty acid synthetase (13). Both agents have been shown to inhibit fatty acid synthesis in rat adipocytes (14, 15).

MATERIALS AND METHODS

Animals

Four-week-old male Wistar rats (Charles River Laboratories) were fed Purina laboratory chow and water ad libitum and kept in a temperature-controlled room with a 12-hr light/dark cycle for at least 1 week before study.

Preparation of isolated adipocytes

In each experiment, five to six fed rats, weighing 145–160 g (6–8 weeks of age) were decapitated at 10 AM and the entire epididymal fat pads were removed and pooled. Fat cells were isolated by digestion with collagenase by modification (1) of the method of Rodbell (16). Collagenase (Sigma, type I, lot no. 126C0435) was present at a final concentration of 1.5 mg/ml of Krebs-Ringer bicarbonate buffer (KRBB), pH 7.4, with 5 mM glucose and 2% bovine serum albumin, fraction V (Reheis Chemical Co., Armour Pharmaceutical Co., lot R58706). After 1 hr of digestion at 37°C, fat cells were filtered through a 250 μm nylon screen and were washed three times with KRBB, without collagenase, by allowing the cells to float, aspirating away the infranatant buffer, and then resuspending the cells in fresh buffer.

Glucose metabolism studies

Glucose metabolism studies were performed as previously described (1). In brief, aliquots of the isolated fat cell suspension (approximately 10^6 cells) were incubated with either [1-^{14}C]glucose or [6-^{14}C]-glucose (New England Nuclear) (0.2 μCi/flask, final concentration of glucose 5 mM) plus: *a*) no additions; *b*) 2.5 mM (−)-hydroxycitrate (Na salt);[2] *c*) 0.05 mM cerulenin (Calbiochem-Behring Corp.), and with or without 0.67 nM insulin (rat insulin, Novo). The final volume was 1 ml. This concentration of insulin produced a maximal stimulation of glucose metabolism. In some experiments, fat cells were incubated at the described conditions, and also with 0.2 mCi of ^3H_2O (New England Nuclear) to assess overall rates of fatty acid synthesis (17). The specific activities of substrates were determined in each experiment by assaying the radioactivity of the incubation medium. The concentration of water in the medium was assumed to be 55.5 M. All conditions were run in each experiment.

After 2 hr of incubation, the reaction was stopped by the addition of 0.5 ml of 6N H_2SO_4 to the medium. Hyamine hydroxide (0.2 ml) was added to hanging center wells to collect $^{14}CO_2$ liberated during an additional 1-hr incubation. The incorporation of radioactivity into total lipids, fatty acids, and glyceride-glycerol was determined as previously described (1).

Determination of fat cell number

Fat cell number was estimated by measuring the DNA content of 1 ml of fat cell suspension (18, 19) and assuming that each cell has 6.7 pg DNA (20).

Measurement of nonradioactive metabolites

Total glucose uptake and lactate production were assessed by measuring the concentration of these substances in the medium both before and after the 2-hr incubation. For this purpose, in each experiment, aliquots of fat cells were incubated in parallel to the radiolabeled glucose metabolism studies. The cells plus medium were added to 1 ml of ice-cold 1.2 M $HClO_4$ and mixed thoroughly. This mixture was neutralized with 0.6 ml of imidazole buffer (0.4 M imidazole, 2 N KOH, 0.4 M KCl) (21). After centrifugation in the cold, the supernatant was removed and the precipitate was washed with 0.5 ml of distilled water. This wash was combined with the original extract, which was frozen at −20°C and later used for the fluorometric determination of glucose (22) and the measurement of lactate by a spectrophotometric, enzymatic technique (23) as modified by Engel and Jones (i.e., pH 9 and 12 mM EDTA) (24). Stand-

[2] (−)-Hydroxycitrate was a gift of Dr. A. C. Sullivan of Hoffman-LaRoche, Inc.

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TABLE 1. Effect of inhibitors of fatty acid synthesis on the uptake of 2-deoxyglucose

	Basal		Insulin	
	Experiment	Mean	Experiment	Mean
	pmol/mg lipid/2 min			
Control	a. 17.6 b. 33.1	25.4	a. 51.9 b. 68.6	60.2
(−)-Hydroxycitrate	a. 19.7 b. 26.2	23.0	a. 62.5 b. 71.9	67.2
Cerulenin	a. 18.2 b. 25.8	22.4	a. 60.5 b. 66.9	63.7

Effect of (−)-hydroxycitrate (2.5 mM) and cerulenin (0.05 mM) on the initial rate of uptake of 0.3 mM 2-deoxy[1-^{14}C]glucose, in the presence or absence of insulin (0.67 nM). Adipocytes were isolated and preincubated with each inhibitor, with or without insulin for 60 min at 24°C in Krebs-Ringer phosphate buffer, pH 7.4, 2% BSA, before assay with deoxyglucose, for 2 min. Data represent the mean of triplicate values of two separate experiments (a and b) and their mean.

ards for glucose and lactate were prepared in KRBB and treated identically to the samples.

Enzyme activity assays

One ml of isolated fat cell suspension was homogenized in 0.25 M sucrose with 1 mM EDTA and 1 mM dithiothreitol (1) and centrifuged at 100,000 *g* for 1 hr. The clear internatant was used to assay the combined activities of glucose-6-phosphate dehydrogenase (E.C.1.1.1.4.9) and 6-phosphogluconate dehydrogenase (E.C.1.1.1.4.4) at substrate concentrations of 0.005–1 mM glucose-6-phosphate and 6-phosphogluconate by a spectrophotometric technique (25), in the presence or absence of up to 5 mM (−)-hydroxycitrate or up to 0.1 mM cerulenin.

Glucose transport measurement

The uptake of 2-deoxy[^{14}C]glucose (final concentration 0.3 mM) was assessed by the method of Livingston and Lockwood (26) as previously described (1). Adipocytes for this assay were isolated in Krebs Ringer phosphate buffer, pH 7.4, without added glucose and were then preincubated for 60 min at 24°C in the presence or absence of 2.5 mM (−)-hydroxycitrate or 0.05 mM cerulenin, with or without insulin (0.67 nM) prior to the assay with 2-deoxyglucose. The low substrate concentration and temperature conditions of this assay were employed to ensure the linearity of the assay with time and to make it less likely that the rates of phosphorylation limited the uptake of hexose.

Statistical analysis

All data were analyzed by an analysis of variance (ANOVA) for replicated experiments (27) on log-transformed values. The log transformation was necessary to provide homogeneity of variance between the basal and insulin-stimulated values. The method of least significant difference was used to compare individual means if the ANOVA was significant ($P < 0.05$).

RESULTS

Since the purpose of this study was to determine the effect of the specific inhibition of fatty acid synthesis on glucose utilization, it was important to determine first whether the inhibitors had any direct effect on the glucose transport process itself, or on the activities of the enzymes of the pentose pathway, since alterations in glucose metabolism via this pathway were expected. Neither inhibitor of fatty acid synthesis significantly affected basal or maximally insulin-stimulated 2-deoxyglucose uptake (see **Table 1**), even after a 1-hr pre-incubation. Each inhibitor was also without effect on the combined activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase when added to assays of these enzymes performed on the 100,000 *g* supernatant of homogenates of isolated fat cells at substrate concentrations of 0.005–1 mM (data not shown). Thus, the inhibitors did not alter either the

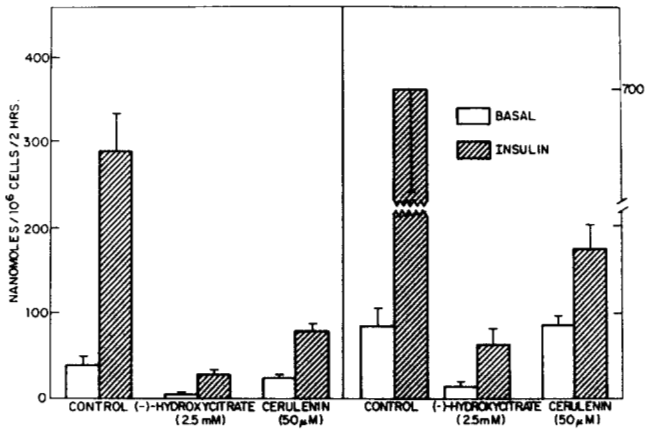

Fig. 1. Effect of inhibitors of fatty acid synthesis on the conversion of [1-^{14}C]glucose (left panel) and [6-^{14}C]glucose (right panel) to fatty acids by isolated adipocytes. Values are expressed in nmol of glucose converted to product/10^6 cells/2 hr on the vertical scales of both panels. Epididymal adipocytes from 150 g male Wistar rats were incubated for 2 hr at 37°C in Krebs-Ringer bicarbonate buffer, 2% BSA, pH 7.4, 5 mM glucose, with a), no additions; b), insulin (I) (0.67 nM); c), 2.5 mM (−)-hydroxycitrate (HC); d), HC + insulin; e), 50 μM cerulenin (C); f), C + insulin (I). Data are the mean ± SEM of four separate experiments. All incubation conditions were run on the same cell suspension in each experiment. b) versus d): $P < 0.005$ for both labels; b) versus f): $P < 0.05$ for both labels; a) versus c): $P < 0.025$ for [6-^{14}C]glucose, $P < 0.05$ for [1-^{14}C]glucose.

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TABLE 2. Incorporation of ^3H_2O and [^{14}C]glucose into fatty acids by isolated adipocytes in the presence of insulin: effect of (-)-hydroxycitrate and cerulenin

	^{3}H	% of Control	^{14}C	% of Control
	μg-atoms incorporated into fatty acids/10^6 cells/2 hr			
Control	2.3	100%	2.4	100%
(-)-Hydroxycitrate	0.29	13%	0.37	15%
Cerulenin	0.69	30%	0.72	30%

Adipocytes were incubated for 2 hr at 37°C with ^3H_2O and either 5 mM [6-^{14}C]glucose or [1-^{14}C]glucose, in the presence of insulin (0.67 nM) and with or without 2.5 mM (-)-hydroxycitrate or 0.05 mM cerulenin. The number of μg atoms of ^{14}C was calculated from the specific yields of each labeled glucose and the specific activity of the triose phosphates as described by Katz et al. (28). Values obtained with [1-^{14}C]- and [6-^{14}C]glucose were averaged. Data represent the means of triplicate values in one experiment.

maximal velocity of these enzymes or their affinity for their substrates.

Fatty acid synthesis

As shown in **Fig. 1**, basal fatty acid synthesis from [1-^{14}C]glucose was significantly reduced to 12% of control by (−)-hydroxycitrate (2.5 mM) and was not significantly affected by cerulenin (0.05 mM). Insulin-stimulated fatty acid synthesis was reduced to 10% of control values by (−)-hydroxycitrate and to 28% of controls by cerulenin (Fig. 1). The inhibition of the incorporation of [6-^{14}C]glucose into fatty acids was similar to that from [1-^{14}C]glucose. Preliminary experiments revealed that these concentrations of inhibitors produced the maximum achievable inhibition of fatty acid synthesis.[3] Furthermore, the incorporation of ^3H_2O into fatty acid was reduced to a similar extent, indicating that overall rates of fatty acid synthesis, regardless of carbon precursor, were also suppressed by these inhibitors (**Table 2**).

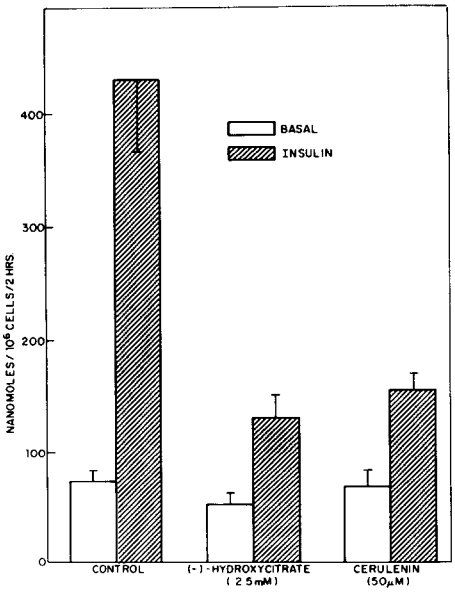

Fig. 2. Effect of inhibition of fatty acid synthesis on [1-^{14}C]glucose oxidation. Data are means ± SEM from the same experiments described in the legend to Fig. 1. HC Basal versus control, $P < 0.05$; HC + I versus I control+, $P < 0.025$; C + I versus I control+, $P < 0.025$.

Oxidation of glucose by isolated fat cells in the presence and absence of inhibitors of fatty acid synthesis

Basal [1-^{14}C]glucose oxidation was depressed to 73% of control values by 2.5 mM (−)-hydroxycitrate, but was not affected by 0.05 mM cerulenin (**Fig. 2**). The latter result was expected since this agent did not alter basal fatty acid synthesis. In the presence of a maximally stimulating concentration of insulin (0.67 nM), [1-^{14}C]glucose oxidation was reduced to 30% of insulin-stimulated control values. Thus, the insulin effect was markedly reduced from 520% in the controls, to 151% and 154% when fatty acid synthesis was inhibited by (−)-hydroxycitrate and cerulenin, respectively. Although interpretation of percent changes is difficult due to the changing baseline, this type of analysis is useful as a reflection of relative changes in insulin effects.

In contrast to the results for [1-^{14}C]glucose oxidation, [6-^{14}C]glucose oxidation was not affected by either inhibitor in the basal state, and was actually enhanced in the presence of insulin and each inhibitor (**Fig. 3**).

Incorporation of [1-^{14}C] and [6-^{14}C]glucose into glyceride-glycerol

As shown in **Fig. 4,** neither (−)-hydroxycitrate nor cerulenin significantly affected basal or insulin-stimulated [1-^{14}C]glucose or [6-^{14}C]glucose incorporation into the glycerol moiety of triglyceride. However, there was a trend toward a lower incorporation of both labels into this product. The insulin effect on glyceride-glycerol production was not altered by the inhibition of fatty acid synthesis by either (−)-hydroxycitrate or cerulenin.[4]

[3] It is possible that the concentration of (−)-hydroxycitrate that gave maximal inhibition of fatty acid synthesis might have been lower if the bovine serum albumin used in the buffer had been dialyzed to remove citrate.

[4] This conclusion is based on the fact that the interaction terms in the ANOVA (IxC; IXHC) were not significant for glyceride-glycerol, whereas they were highly significant for CO_2 production and fatty acid synthesis. This indicates that the insulin effect was

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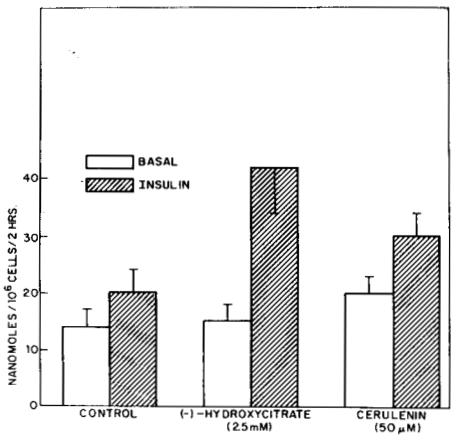

Fig. 3. Effect of inhibition of fatty acid synthesis on [6-^{14}C]-glucose oxidation. Data are from the same experiments described in Fig. 2. Insulin-stimulated control is less than HC + I ($P < 0.005$) and C + I ($P \leq 0.05$).

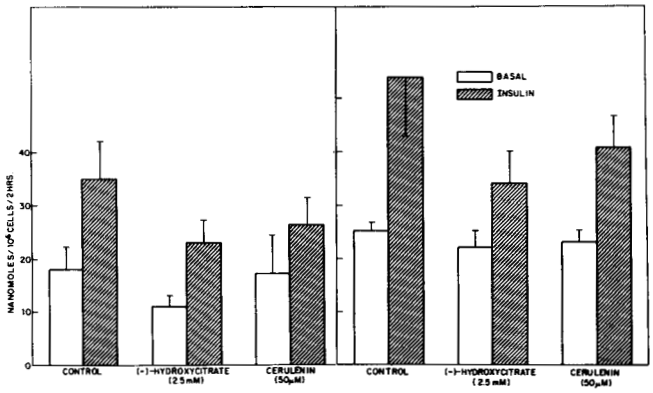

Fig. 4. Effect of inhibition of fatty acid synthesis on [1-^{14}C]-glucose (left panel) and [6-^{14}C]glucose (right panel) incorporation into glyceride-glycerol. Results are from the same experiments described in Fig. 3.

Lactate production in the presence of inhibitors of fatty acid synthesis

Basal lactate production was unaffected when fatty acid synthesis was inhibited by (−)-hydroxycitrate or cerulenin (**Fig. 5**). Lactate release was decreased to 48% of control by (−)-hydroxycitrate and to 53% of insulin-stimulated control by cerulenin.

Total glucose utilization in the presence of inhibitors of fatty acid synthesis

Glucose utilization was estimated by summing the incorporation of labeled glucose into all end-products (CO_2, fatty acids, glyceride-glycerol) and assuming that one mole of glucose forms two moles of lactate (i.e., lactate values are divided by 2, and added to the total incorporation of each label (**Table 3**)); and also, more directly, by measuring the disappearance of glucose from the medium (**Table 4**). Basal glucose utilization (as calculated by either method) was not significantly affected by either inhibitor of fatty acid synthesis. Under insulin-stimulation, both the sum of the products and the measurement of glucose disappearance indicate that glucose utilization is depressed by each inhibitor. However, the extent of the inhibition is greater when estimated by the summing method (60–70%) than by the direct measurement (30–50%).

influenced by the inhibitors for these latter pathways, but was unaffected in glyceride-glycerol. However, since the insulin effect in the presence of cerulenin did not reach statistical significance ($P < 0.15$) for [1-^{14}C]glucose, some caution must be observed before conclusions are drawn with regard to the effect of insulin on glyceride-glycerol synthesis in the presence of this inhibitor.

DISCUSSION

These studies clearly demonstrate that a direct inhibition of fatty acid synthesis in adipocytes decreases the ability of insulin to stimulate both pentose shunt glucose oxidation and overall glucose utilization. We have shown that inhibition of fatty acid synthesis by either (−)-hydroxycitrate or cerulenin, specific inhibitors of citrate cleavage enzyme and fatty acid synthetase, respectively, produced similar alterations of glucose metabolism. The similarity in the results with these two agents makes it unlikely that these data are artifactual. Furthermore, since neither agent directly affected the insulin-stimulation of

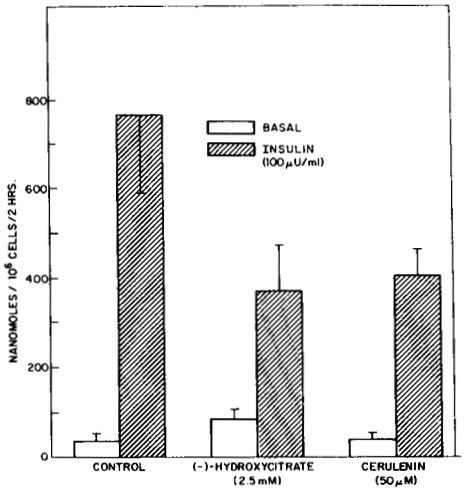

Fig. 5. Effect of inhibition of fatty acid synthesis on total lactate production. Aliquots of the same cell suspension used for radioactive incorporation studies (Figs. 1–4) were incubated in parallel, but without addition of radioactivity. Lactate production was measured enzymatically. $P < 0.05$ HC + I versus I control; $P < 0.05$ C + I versus I control.

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TABLE 3. Calculation of total glucose utilization from the sum of the incorporation of [1-^{14}C]glucose or [6-^{14}C]glucose into CO_2, fatty acids, glyceride-glycerol, plus lactate

	Basal	Insulin
	nmol/10^6 cells/2 hr	
Sum of [1-^{14}C]glucose + lactate		
Control	150 ± 17	1133 ± 177
(-)-Hydroxycitrate (2.5 mM)	112 ± 18	366 ± 42
Cerulenin (50 μM)	133 ± 33	464 ± 31
Sum of [6-^{14}C]glucose + lactate		
Control	144 ± 28	1159 ± 214
(-)-Hydroxycitrate	93 ± 12	323 ± 53
Cerulenin	152 ± 17	451 ± 48

Data were obtained from Figs. 1–5. Values for total lactate production in Fig. 5 were divided by 2 for the calculation (see footnote 5).

glucose transport or the activities of the glucose dehydrogenases of the pentose shunt, conclusions about the role of fatty acid synthesis seem justified.

Each inhibitor of fatty acid synthesis caused a blunted response to insulin of [1-^{14}C]glucose oxidation, but not [6-^{14}C]glucose oxidation. This indicates a decrease in the activity of the pentose shunt, but normal Krebs cycle oxidation. Conversion of the [1-^{14}C]glucose label to $^{14}CO_2$ takes place specifically by the action of 6-phosphogluconate dehydrogenase in the pentose cycle, and also through the Krebs cycle. Oxidation of [6-^{14}C]glucose, in contrast, occurs predominantly in the Krebs cycle, and not through the pentose pathway. When fatty acid synthesis was inhibited, insulin failed to stimulate [1-^{14}C]glucose oxidation to the same extent as in controls, suggesting that the activity of the pentose shunt was diminished. Furthermore, the fact that [6-^{14}C]glucose oxidation was actually enhanced by these inhibitors of fatty acid synthesis supports the hypothesis that there is a specific reduction in glucose oxidation via the pentose pathway.

More exact calculations of the contribution of the pentose shunt to glucose metabolism by the equations of Katz, Landau, and Bartsch (28) confirm the conclusion that the pentose pathway is reduced by the inhibition of fatty acid synthesis (mean values of 17% (control) versus 11% (hydroxycitrate) and 12% (cerulenin)). These calculations are based on specific yields (percent of the total glucose utilized converted to product) and represent an estimation of flux through the pentose pathway. The values obtained are similar whether or not the methods that assume triose-phosphate isomerization are used. The other assumptions involved in these calculations have been extensively examined and were determined to be valid for adipose tissue (28).

The decrease in the flux of glucose through the pentose shunt under insulin-stimulated conditions, when fatty acid synthesis was inhibited as calculated by the equations of Katz et al. (28) (approximately 40%), a more quantitative estimate of flux, was somewhat less than expected from the marked decline (70%) in insulin-stimulated [1-^{14}C]glucose oxidation in the face of a slightly enhanced oxidation of [6-^{14}C]-glucose. The decreased oxidation of [1-^{14}C]glucose oxidation produced by the inhibitors may therefore be due in part to the decreased total glucose uptake.

These data also provide further evidence for a regulatory link between the rate of fatty acid synthesis and the activity of the pentose shunt. A correlation between pentose shunt activity and the rate of fatty acid synthesis has been observed by other investigators (4, 28, 30, 31) in a variety of physiological situations and when fatty acid synthesis is inhibited indirectly with the electron transport inhibitor rotenone (4, 11). Moreover, the data confirm more indirect observations (4, 11) that the decrease in pentose shunt activity can be secondary to a decrease in fatty acid synthesis.

Kather, Rivera, and Brand (30) have shown that the reduction in [1-^{14}C]glucose oxidation following the inhibition of fatty acid synthesis by rotenone can be overcome by phenazine methosulfate which reoxidizes NADPH non-enzymatically. This strongly suggests a role for NADPH levels or the availability of $NADP^+$ in the control of the pentose pathway under these conditions. Thus, the present data are consistent with the hypothesis of Richardson and Czech (4) that

TABLE 4. Effect of inhibition of fatty acid synthesis on total glucose utilization by isolated adipocytes

	Glucose Utilization	
	Basal	Insulin
	% of control	
Control	100	100
(-)-Hydroxycitrate (2.5 mM)	106 ± 8	52 ± 14
Cerulenin (50 μM)	110 ± 27[a]	64 ± 10

[a] Mean of two experiments.

Aliquots of the fat cell suspension (see Fig. 2) were incubated in parallel. The concentration of glucose in neutralized perchloric acid extracts of the incubation medium before and after the incubation was measured by a fluorometric assay. Data are expressed as a percentage of the glucose utilization of the controls in each experiment and are presented as the mean percentage ± SEM of three separate experiments. The values for glucose utilization in the controls were 628 ± 250 and 1578 ± 351 nmol/10^6 cells/2 hr in the basal and insulin-stimulation conditions, respectively.

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the NADPH/$NADP^+$ ratio is elevated under conditions of low fatty acid synthesis and that this secondarily inhibits the activity of the pentose shunt. This is based on the observation of Eggleston and Krebs (32) that an increased ratio of NADPH/$NADP^+$ can markedly inhibit the activity of glucose-6-phosphate dehydrogenase, the probable rate-limiting enzyme of the pentose shunt. It is also conceivable that the concentration of some other metabolite or co-factor that is affected by a change in the rate of fatty acid synthesis may play a role in decreasing the activity of the pentose shunt and/or total glucose utilization.

The contribution of the pentose shunt to the NADPH required for lipogenesis can also be calculated (28). In agreement with others (28, 30), the pentose shunt contributed an average of 49 ± 4% (standard error) of the NADPH required in controls. When fatty acid synthesis was inhibited with cerulenin, the values were similar (59 ± 13%), while the contribution of the pentose shunt was extremely variable and often exceeded the requirement when fatty acid synthesis was more severely restricted with (−)-hydroxycitrate (145 ± 56%). Similar variable results were obtained by Katz et al. (28) when fatty acid synthesis was inhibited by epinephrine. A production of NADPH by the pentose shunt equal or greater than that utilized was also seen in fat cells from fasted rats in which fatty acid synthesis is also very low (30).

One can only speculate on the cause of the increased insulin-stimulated [6-^{14}C]oxidation induced by (−)-hydroxycitrate and cerulenin. It is possible that as the extramitochondrial concentration of citrate rises when fatty acid synthesis is inhibited, the efflux of citrate from the mitochondria is decreased. This could lead to increased oxidation within the mitochondria. However, a direct effect of these agents on [6-^{14}C]glucose oxidation cannot be excluded.

It is not possible to draw conclusions about the effect of the inhibitors on basal glucose utilization (even if this increase were as high as 50%) since these small changes are within the error of the direct assay (33). The assumptions required for the summing method (see footnote 5) coupled with the fact that the two methods do not agree makes it impossible to rule out an effect of the inhibitors on basal glucose utilization with these data. In contrast, the assay of glucose disappearance is reliable at the higher rates of glucose utilization in the presence of insulin (33). The sum of metabolites accounts for an average of 83% of the insulin-stimulated glucose utilization as assessed by the direct assay of glucose in the medium. A systematic underestimation of total glucose utilization by the sum of ^{14}C-labeled metabolites has been noted by others (33). This is probably due largely to metabolites that were not measured, such as pyruvate, glycogen, glycerol, and amino acids, as well as to the inherent inaccuracy of subtracting two large numbers to determine the disappearance of glucose. Thus, although it is uncertain whether the reduction in insulin-stimulated glucose utilization is as much as 60–70%, as estimated by the summing method, or only 30–50%, as assessed by the disappearance of glucose from the medium, it is clear that each inhibitor of fatty acid synthesis causes an underutilization of glucose in the presence of insulin.

Neither inhibitor of fatty acid synthesis altered the incorporation of glucose into glyceride-glycerol in both the presence and absence of insulin, but an increased percentage of the glucose metabolized was diverted into this product. Absolute rates of insulin-stimulated lactate production were decreased, but relative to the amount of glucose utilized, this pathway was also increased. Thus the inhibition of fatty acid synthesis produces an altered pattern of glucose metabolism with a shunting of glucose carbons into three carbon metabolites and oxidation via the Krebs cycle.

The pattern of glucose metabolism produced by the pharmacological inhibition of fatty acid synthesis in this work is strikingly similar to that seen in insulin-resistant fat cells from rats fed high-fat diets (1–3, 8, 9) and in enlarged fat cells (4–7, 29). In both, fatty acid synthesis is less than 10% of controls (young animals fed low-fat diets), even in the presence of high concentrations of glucose and insulin. In both cases this is attributable to decreases in the activities of the key enzymes of fatty acid synthesis, acetyl CoA carboxylase and fatty acid synthetase (1, 4). In the large fat cell, the oxidation of glucose via the pentose cycle is also severely depressed while the pathways leading to the incorporation of glucose into glyceride-glycerol, lactate, and oxidation via the Krebs cycle are intact (4–6). The situation with regard to the contribution of the pentose cycle to glucose metabolism in adipocytes from rats fed a high-fat diet is less clear (8, 9), but

[5] This calculation assumes that the two halves of the glucose molecule are converted equally to lactate. In fact, this is highly unlikely as evidenced by the unequal incorporation of [1-^{14}C] and [6-^{14}C]glucose into fatty acids. The error could be greater with [1-^{14}C]glucose since unlabeled pentose formed in the pentose shunt can re-enter glycolysis via the transketolase and transaldolase reactions, subsequently form lactate, and therefore enter the calculation twice. However, we think the calculations are adequate for the purposes of this work. Lactate production was measured primarily to rule out the possibility that lactate was increased in the presence of inhibitors. The opposite was in fact observed.

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[1-^{14}C]glucose oxidation is impaired while the pathways leading to glyceride-glycerol, lactate production, and Krebs cycle oxidation function normally in these fat cells (3, 10). Thus, like the effect of inhibition of fatty acid synthesis, the defects in intracellular glucose metabolism in these examples of decreased insulin responsiveness are pathway-specific, involving only fatty acid synthesis and [1-^{14}C]glucose oxidation.

In addition to altered pattern of glucose utilization in large fat cells and fat cells from rats fed high-fat diets, the response of total glucose utilization to insulin is also blunted (6, 8). This occurs without major alterations of insulin binding or in the insulin stimulation of glucose transport, implying that glucose utilization is being regulated at the level of one or more enzymes of glucose metabolism. The results of this work clearly demonstrate that a decreased enzymatic capacity for fatty acid synthesis alone could lead to the decreased response of both [1-^{14}C]glucose oxidation and total glucose utilization to insulin that are observed in these models of insulin resistance.

The mechanism by which a diminished rate of fatty acid synthesis would lead to a decrease in total glucose utilization rather than merely diverting glucose carbons into other pathways can only be speculated upon. Czech, Richardson, and Smith (34) have hypothesized that, since fatty acid synthesis and the pentose shunt constitute the two major pathways of glucose metabolism in fat cells, the simultaneous limitation of their activities decreases the capacity of the cells for glucose metabolism. Net glucose transport would be depressed as the concentration of intracellular free glucose increased, decreasing the transmembrane concentration of glucose. Indirect evidence that an elevation of intracellular glucose occurs in large fat cells, even under basal conditions, has recently been obtained (35). This mechanism is consistent with our observation of decreased glucose utilization in the presence of inhibitors of fatty acid synthesis despite unaltered initial rates of 2-deoxyglucose uptake. Alternatively, an elevated concentration of intracellular glucose or some other metabolite such as ATP or glucose-6-phosphate could directly depress the activity of the glucose transport system (36, 37).

Another mechanism which could contribute to the decreased glucose utilization is an inhibition of glycolysis at the level of phosphofructokinase by elevated citrate levels (38). An elevation of intracellular citrate by (−)-hydroxycitrate has been demonstrated in adipose tissue (15). During the course of this work, Brunengraber, Boutry, and Lowenstein (39) reported that (−)-hydroxycitrate causes a metabolic crossover point at the level of phosphofructokinase in the perfused liver. Based on the fact that an accumulation of glycolytic intermediates could not be demonstrated, these authors hypothesized that (−)-hydroxycitrate caused a coordinated decrease in glucose utilization. However, the high medium glucose concentration and glycogen content of the livers prevented a change in glucose utilization to be measured.

Conclusions about the role of fatty acid synthesis in the control of glucose utilization by adipocytes are limited to conditions of constant re-esterification. That is, if basal lipolysis were increased or there were a greater influx of free fatty acids into the cell, the requirement for re-esterification would increase and therefore so would the rate of glyceride-glycerol synthesis. This would lead to higher glucose-6-phosphate utilization and would upset the relationship between fatty acid synthesis and total glucose utilization. This occurs when glucose metabolism is stimulated by epinephrine, which inhibits fatty acid synthesis but stimulates glucose incorporation into glyceride-glycerol, lactate, and oxidation via the Krebs cycle (10, 40, 41). However, if the glyceride-glycerol pathway became saturated, glucose utilization would then depend on the capacity of the cell for fatty acid synthesis.

In conclusion, these studies provide direct evidence that an inhibition of fatty acid synthesis decreases the ability of insulin to stimulate both pentose shunt oxidation and total glucose utilization in isolated fat cells. The enzymatic capacity for fatty acid synthesis is therefore an important factor in the control of insulin-stimulated glucose utilization by adipose cells.

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