Effects of Insulin and Procaine Hydrochloride on Adipose Tissue Hexose Metabolism in *ob/ob* Mice

Evidence of a Postreceptor Defect

HERBERT F. HOPE-GILL, VINOD NANDA, AND TING-PO KAM

SUMMARY

To determine if insulin resistance of obese hyperglycemic *ob/ob* mice might be due to an initial postreceptor defect, the effects of insulin and procaine HCl (0.1 mM) on glucose and fructose metabolism in their adipose tissue were studied. Procaine, unlike insulin, has been shown to exert its insulin-like effects on trypsinized adipocytes, suggesting an action independent of the insulin receptor. In the present study this agent, like insulin, stimulated glucose uptake, glycogenesis, pentose shunt activity, and lipid synthesis in tissue of lean littermates but not of *ob/ob* siblings. Prewashing has been shown to restore the sensitivity of their adipose tissue to insulin regarding antilipolysis and hexose metabolism, presumably by restoring available receptor number. In the present study, in contrast to the complete restoration of antilipolysis, prewashing only partially restored the effects of insulin (but not of procaine) on glycogenesis and lipid synthesis in tissue of *ob/ob* mice; no return of effects of either agent on glucose uptake or the pentose shunt occurred. Since hexose metabolism remained unresponsive to procaine and since prewashing restored only antilipolysis and a portion of the lipogenic effect of insulin (but not effects on glucose uptake or pentose shunt activity), these studies suggest an underlying irreversible defect in responsiveness of glucose uptake and the pentose shunt in adipose tissue of *ob/ob* mice independent of a deficiency of available insulin receptors. That portion of insulin resistance due to hyperinsulinemia and resulting receptor deficiency might be secondary to these underlying inherent defects. DIABETES *28*:537–543, June 1979.

Obese hyperglycemic *ob/ob* mice are characterized by hyperinsulinemia and by insulin resistance of muscle, liver, and adipose tissue.[1–7] The cause of the resistance to the actions of insulin is unknown. Hypersecretion of insulin (because of hyperphagia[1,8] or a defect in the beta cell[9]) has been implicated as the initiating factor: the resulting hyperinsulinemia is thought to cause the insulin resistance through induction of a decrease in insulin binding to its receptors. Several factors have been implicated in this decrease in binding. Roth, Kahn, and co-workers have demonstrated that the principal cause is a resulting decrease in number of insulin receptors because of hyperinsulinemia.[10–14] Other factors implicated are saturation of insulin receptor sites[3,7] and negative cooperativity of insulin-binding sites.[15] It has been suggested that these effects result in unresponsiveness of tissues to further increases in insulin concentration and hence insulin resistance.

While the evidence that hyperinsulinemia is responsible for much of the insulin resistance seen in *ob/ob* mice is considerable, there is some conflicting evidence concerning whether or not there is an underlying peripheral tissue defect independent of a deficiency of available insulin receptors. In support of the theory that a decrease in available receptor number is the primary cause, several authors have shown that chronic reduction of insulin levels, by food restriction or streptozotocin, ameliorated insulin resistance in *ob/ob* mice,[1,16,17] suggesting that no primary tissue defect exists. However, in support of the presence of an underlying tissue defect, Le Marchand et al.[18] presented evidence that correction of insulin-binding abnormalities by these measures was not accompanied by any detectable metabolic effect of insulin on hepatic or adipose tissue metabolism even though unresponsiveness of muscle was corrected. Moreover, Boozer and Mayer[19] demonstrated that hyperphagia, hypergly-

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From the Departments of Medicine, Sunnybrook Medical Centre, University of Toronto Medical School, Toronto, Ontario, Canada and Asheville Veterans Administration Medical Centre, Duke University, Asheville, North Carolina.
Reprint requests should be addressed to Herbert F. Hope-Gill, M.D., Asheville Veterans Administration Medical Center, Duke University, Asheville, North Carolina 28805.
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cemia, obesity, and insulin resistance persist even when insulin levels are reduced by streptozotocin administration. These findings suggested the presence of an underlying tissue abnormality in insulin responsiveness independent of deficiencies in available receptor number.

To reconcile these apparent discrepancies we observed the effects of insulin and procaine HCl on hexose metabolism in adipose tissue of *ob/ob* mice and their lean littermates. Procaine, at a concentration of 0.1 mM, has been shown to stimulate and enhance the effects of insulin on glucose uptake, glycogenesis, lipogenesis, pentose shunt activity, and antilipolysis in rat adipose tissue.[20–22] This agent, like insulin, was also found to have antilipolytic effects on adipose tissue from lean littermates of *ob/ob* mice similar to the effects of insulin, yet neither agent exerted an antilipolytic effect in tissue of *ob/ob* mice without preincubation of tissue in an insulin-free medium.[3]

The effects of procaine did differ from those of insulin in that, unlike insulin, procaine exerted its antilipolytic effects on rat adipocytes treated with trypsin;[23] since trypsin has been shown to destroy the insulin receptor,[24] these findings suggested that procaine acts independently of this receptor. Moreover, evidence is presented in this paper that the insulin-like effects of procaine cannot be a nonspecific membrane perturbation but rather an effect utilizing a mechanism similar to that used by insulin. Failure to respond to this agent would therefore suggest an abnormality in transmission of the receptor-initiated signal, i.e. a postreceptor defect.

Studies have shown that preincubation of adipose tissue in an insulin-free medium leads to a return of sensitivity of adipose tissue of *ob/ob* mice to insulin; this presumably occurs by a resulting increase in the number of available receptors, by removal of bound insulin,[3,7,25] and, since once cells are removed from a hyperinsulinemic environment they increase their number of receptors,[14] by regeneration of insulin receptors. Thus, prewashing of adipose tissue of *ob/ob* mice resulted in a reduction of the previously elevated basal lipolysis and a complete return of the antilipolytic effect of insulin.[3] In addition, the studies of Loten and Jeanrenaud[25] and of Winand, Dehaye, and Christophe[7] showed that, when adipose tissue of *ob/ob* mice was prewashed for 1–2 h, a significant effect of insulin on glucose metabolism appeared. There was, virtually, no difference in the findings after 1 or 2 h of prewashing so the principal effect of preincubation presumably occurred during the first hour. In this study, a preincubation time of 1 h was therefore used so that comparison with effects on lipolysis[3] could be made.

Accordingly, in the present study we observed the effects of insulin and procaine on fructose and glucose metabolism in *ob/ob* mice and their lean littermates both before and after preincubation to examine effects of these agents on insulin resistance of adipose tissue from *ob/ob* mice following an increase of available insulin receptors.

MATERIALS AND METHODS

[U-^{14}C]glucose, [1-^{14}C]glucose, [6-^{14}C]glucose, and [U-^{14}C]fructose were purchased from New England Nuclear. Procaine hydrochloride was obtained from BDH Pharmaceuticals Ltd. All other reagents were of Analar grade. Insulin, purified by column chromatography, was obtained from Connaught Laboratories Ltd., Toronto. Animals used in this study were male C57BL/6J +/+ lean mice and C57BL/6J *ob/ob* obese mice purchased from Jackson Memorial Laboratories, Bar Harbor, Maine. They were fed laboratory chow ad libitum.

Incubation conditions. Animals were killed by light ether anesthesia and cervical dislocation and the epididymal fat pads were removed and cut into 50-mg pieces. Adipose tissue fragments were placed in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.5% bovine serum albumin (which had been dialyzed overnight against 500 vol of water) and 5.55 mM glucose or fructose at 37 °C in an atmosphere of 95% O_2 and 5% CO_2 for 1 h. The paired preincubated samples and the paired nonpreincubated samples were placed in fresh buffer containing 5.55 mM radiolabeled [U-^{14}C]glucose, [1-^{14}C]glucose, [6-^{14}C]glucose or [U-^{14}C]fructose in the presence or absence of added insulin or procaine as indicated in RESULTS. Incubations were carried out in an atmosphere of 95% O_2 and 5% CO_2 at 37 °C for 1 h.

Incubations were performed in polystyrene tubes stoppered with rubber caps supporting a hanging well, 1.5 × 1 cm. After incubation, 0.5 ml of 1 M hyamine was introduced into the center well through a rubber cap and 0.2 ml of 0.1 N sulfuric acid was then introduced via the side arm into the main compartment of the incubation tube. This stopped the reaction completely and ensured complete liberation of the CO_2. Incubation tubes were returned to the metabolic shaker for a further 1 h to ensure complete transport of CO_2 to hyamine.

Assay methods. The CO_2 production from labeled glucose metabolism was determined by transferring the center well, containing hyamine, to a counting vial with 5 ml of toluene polyphenylene oxide (PPO) and phenyl-oxazolyl-phenyl-oxazolyl-phenyl (POPOP). After incubation, adipose tissue fragments were removed, rinsed several times in cold saline, weighed, and then placed in 5 ml chloroform: methanol (2:1) overnight. Lipids were further extracted in 15 ml of chloroform:methanol according to the method of Folch, Lees, and Sloane-Stanley.[26] The washed extract was dried and then dissolved in 5 ml of ethanol: 1 ml was removed and counted in scintillation fluid PPO/POPOP. The remaining solution was saponified in alcoholic KOH at 60 °C for 1 h. After saponification the glyceride glycerol and fatty acids were isolated and determined according to the method of Denton and Randle.[27] The radioactive glycogen was isolated from the delipidated tissue using the method of Stetten, Katzen, and Stetten;[28] 20 mg of nonradioactive glycogen was added to each tube to serve as a carrier. After isolation, the purified glycogen was dissolved in 1 ml of water and counted in Instagel. Corrections for quenching were performed using internal standards.

The relative activity of the hexose monophosphate shunt was estimated using C-1– and C-6–labeled glucose according to the method of Katz, Landau, and Bartsch[29] using the formula:

$$\text{Pentose Shunt Activity (PC)} = \frac{S}{3 - 2S}$$

in which

$$S = \frac{1\text{-}CO_2 - 6\text{-}CO_2}{1 - 6\text{-}CO_2}.$$

TABLE 1
Effect of insulin and procaine on glucose transport in epididymal fat pads of lean and *ob/ob* mice after 1 h of preincubation

Animals	Additions to the medium	Glucose transport Rate of conversion of [1-^{14}C]glucose to $^{14}CO_2$ (μg C atoms/g tissue/h) 0.25 mM glucose	P value	5.5 mM glucose	P value
Lean mice	Controls	0.16 ± 0.03 (15)		64.94 ± 6.70 (10)	
	Insulin	0.31 ± 0.05 (15)	<0.0025	142.44 ± 10.23 (10)	<0.0005
	Procaine	0.27 ± 0.04 (15)	<0.025	111.11 ± 4.20 (10)	<0.0005
ob/ob mice	Controls	0.22 ± 0.06 (8)		29.09 ± 2.12 (8)	
	Insulin	0.21 ± 0.05 (8)	NS	29.69 ± 1.40 (8)	NS
	Procaine	0.21 ± 0.07 (8)	NS	29.31 ± 1.87 (8)	NS

After a 1 h preincubation in Krebs-Ringer bicarbonate buffer containing unlabeled glucose, adipose tissue fragments from lean and *ob/ob* mice were incubated for 1 h in Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.5% bovine serum albumin, 5.55 or 0.25 mM glucose with 0.1 μCi of [1-^{14}C]glucose, sodium fluoride 10 mM, and PMS 20 μM with or without added insulin 1 mU/ml or procaine 0.1 mM as described in METHODS. Results are expressed as mean ± SEM. Numbers in parentheses denote the number of paired samples. Significances of differences were calculated using the paired *t* test.

Measurement of glucose uptake. Glucose uptake was measured according to the method of Taylor, Mak, and Halperin[30] by determination of $^{14}CO_2$ production from [1-^{14}C]-glucose in the presence of phenazine methosulfate (PMS) and fluoride. Epididymal fat pads were incubated for 1 h in Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.5% bovine serum albumin, 5.55 mM or 0.25 mM glucose containing [1-^{14}C]glucose 0.1 μCi per tube, sodium fluoride 10 mM, and PMS 20 μM. Incubations were terminated after 1 h by the addition of 0.2 ml of 0.1 N sulfuric acid. The $^{14}CO_2$ was trapped in 0.5 ml of hyamine contained in the suspended center wells.

RESULTS

Glucose transport. As shown in Table 1, both insulin 1 mU/ml and procaine 0.1 mM induced increases in glucose transport in epididymal fat pads of lean mice that had been preincubated for 1 h. These agents induced respective increases in glucose uptake of 119 and 71% in the presence of 5.55 mM glucose and of 94 and 69% using 0.25 mM glucose as compared to their corresponding controls. However, in adipose tissue fragments of obese *ob/ob* mice that were prewashed for 1 h, no significant change in glucose transport was observed in samples treated with either insulin or procaine.

Incorporation into total lipids. Without preincubation of tissue, both insulin 1 mU/ml and procaine 0.1 mM significantly increased incorporation of [U-^{14}C]fructose into total lipids in adipose tissue fragments of lean mice by 52 and 51%, respectively (Table 2A). However, no significant effect of insulin or procaine was observed on incorporation of [U-^{14}C]fructose into total lipids in the unwashed tissue of *ob/ob* littermates.

After preincubation of the adipose tissue in an insulin-free medium for 1 h, both insulin 1 mU/ml and procaine 0.1 mM produced increases in incorporation of [U-^{14}C]fructose by 79 and 128%, respectively, in tissue of lean mice (Table 2B). However, in adipose tissue of *ob/ob* mice, a significant increase in incorporation of [U-^{14}C]fructose into total lipids of 47% was observed in the samples treated with insulin, whereas no significant effect of procaine was evident after preincubation.

Regarding effects on incorporation of radiolabeled glucose into total lipid following prewashing of tissue, insulin 1 mU/ml increased incorporation of [U-^{14}C]glucose, [1-^{14}C]glucose, and [6-^{14}C]glucose by 236, 135, and 278%, respectively, in tissue of lean mice (Tables 3 and 4). Procaine 0.1 mM induced respective increases of 97, 71, and 65% in the adipose tissue of lean mice. In contrast, in tissue of *ob/ob* mice, insulin 1 mU/ml increased synthesis from these three, radiolabeled glucoses to a lesser degree of only 44, 38, and 73%, respectively, whereas procaine 0.1 mM had no significant effect on incorporation of radiolabeled glucose into total lipid in fat pads of these mice (Tables 3 and 4).

Incorporation into glyceride glycerol and glyceride fatty acid. As with total lipids, basal rates of incorporation of radiolabeled fructose and glucose were both lower in the tissues of *ob/ob* mice compared with those in their lean littermates (Tables 2–4).

After preincubation, insulin 1 mU/ml increased incorporation of [U-^{14}C]fructose into glycerol by 96% and into fatty acid by 97% in adipose tissue fragments of lean mice (Table 2B). Lesser increases of incorporation into glycerol (59%) and fatty acid (27%) were observed in tissue of *ob/ob* littermates.

Procaine increased incorporation of [U-^{14}C]fructose into glycerol by 151% and into fatty acid by 188% in prewashed adipose tissue of the same lean mice (Table 2B). However, in contrast to the apparent but reduced degree of sensitivity of adipose tissue of *ob/ob* mice to insulin, procaine induced no increase in incorporation of [U-^{14}C]fructose into glycerol or fatty acid in these mice (Table 2).

Regarding incorporation of radiolabeled glucose in prewashed tissue of lean mice, insulin increased incorporation of [U-^{14}C]glucose, [1-^{14}C]glucose, and [6-^{14}C]glucose into glycerol by 123, 139, and 247%, respectively; incorporation into fatty acid was increased by a greater extent (246, 319, and 478%, respectively) (Tables 3 and 4). In tissue of *ob/ob* mice, insulin induced lesser respective increases of incorporation into glycerol (82, 125, and 90%) and into fatty acid (146, 146, and 149%).

In parallel to the findings regarding effects of procaine

TABLE 2
Effect of insulin and procaine on the metabolism of [U-^{14}C]fructose by the epididymal adipose tissue of lean and *ob/ob* mice

	Additions to the medium	Lean mice (μg C atoms/ g tissue/h)	P value	*ob/ob* mice (μg C atoms/ g tissue/h)	P value
A Without Preincubation					
Fructose incorporation into total lipids	Controls	16.86 ± 1.97 (8)		7.99 ± 0.48 (8)	
	Insulin	25.67 ± 2.98 (8)	<0.025	6.49 ± 0.73 (8)	NS
	Procaine	25.47 ± 2.96 (8)	<0.025	8.04 ± 1.03 (8)	NS
Fructose incorporation into glycogen	Controls	0.17 ± 0.02 (8)		0.28 ± 0.03 (8)	
	Insulin	0.30 ± 0.06 (8)	<0.05	0.23 ± 0.02 (8)	NS
	Procaine	0.35 ± 0.03 (8)	<0.0025	0.23 ± 0.02 (8)	NS
B With Preincubation					
Fructose incorporation into total lipids	Controls	18.41 ± 3.22 (4)		5.54 ± 0.17 (4)	
	Insulin	32.94 ± 5.45 (4)	<0.025	8.14 ± 0.70 (4)	<0.01
	Procaine	42.04 ± 6.01 (4)	<0.005	6.06 ± 0.70 (4)	NS
Fructose incorporation into glyceride glycerol	Controls	15.37 ± 2.72 (4)		4.50 ± 0.75 (4)	
	Insulin	30.18 ± 6.07 (4)	<0.025	7.16 ± 0.80 (4)	<0.0125
	Procaine	38.62 ± 6.14 (4)	<0.005	3.96 ± 1.16 (4)	NS
Fructose incorporation into glyceride fatty acid	Controls	0.34 ± 0.03 (4)		0.22 ± 0.02 (4)	
	Insulin	0.67 ± 0.04 (4)	<0.0005	0.28 ± 0.03 (4)	<0.025
	Procaine	0.98 ± 0.12 (4)	<0.0005	0.22 ± 0.02 (4)	NS
Fructose incorporation into glycogen	Controls	0.20 ± 0.04 (4)		0.30 ± 0.02 (4)	
	Insulin	0.31 ± 0.03 (4)	<0.025	0.54 ± 0.08 (4)	<0.0005
	Procaine	0.44 ± 0.06 (4)	<0.0025	0.31 ± 0.05 (4)	NS

After a 1 h preincubation in Krebs-Ringer bicarbonate buffer containing unlabeled fructose, adipose tissue fragments from lean and *ob/ob* mice were incubated for 1 h in Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.5% bovine serum albumin, and 5.55 mM [U-^{14}C]fructose with or without added insulin 1 mU/ml or procaine 0.1 mM as described in METHODS. Results are expressed as mean ± SEM. Numbers in parentheses denote the number of paired samples. Significances of differences were calculated using the paired *t* test.

on disposition of [U-^{14}C]fructose, this agent induced respective increases in incorporation of [U-^{14}C]glucose, [1-^{14}C]glucose, and [6-^{14}C]glucose into glycerol by 88, 44, and 70% and into fatty acid by 124, 452, and 478% in prewashed tissue of lean mice (Tables 3 and 4). In contrast to the apparent but reduced sensitivity to insulin of incorporation of radiolabeled glucose into glycerol and fatty acid by prewashed tissue of *ob/ob* mice, procaine induced no significant increase in synthesis of these compounds in tissue of these animals.

Pentose shunt activity. Both insulin 1 mU/ml and procaine 0.1 mM significantly increased hexose monophosphate shunt activity by 114 and 291%, respectively, in the epididymal fat pads of lean mice as compared with

TABLE 3
Effect of insulin and procaine on the metabolism of [U-^{14}C]glucose by the epididymal adipose tissue of lean and *ob/ob* mice after 1 h preincubation

	Additions to the medium	Lean mice (μg C atoms/ g tissue/h)	P value	Obese mice (μg C atoms/ g tissue/h)	P value
Glucose incorporation into total lipids	Controls	28.59 ± 5.18 (8)		10.11 ± 1.66 (8)	
	Insulin	95.94 ± 30.42 (8)	<0.025	14.71 ± 2.06 (8)	<0.0005
	Procaine	56.42 ± 18.42 (8)	<0.05	10.13 ± 2.09 (8)	NS
Glucose incorporation into glyceride glycerol	Controls	13.41 ± 4.39 (4)		6.14 ± 1.86 (4)	
	Insulin	29.88 ± 8.65 (4)	<0.005	11.21 ± 1.86 (4)	<0.05
	Procaine	25.27 ± 4.59 (4)	<0.05	5.40 ± 1.18 (4)	NS
Glucose incorporation into glyceride fatty acids	Controls	11.65 ± 9.87 (4)		1.21 ± 0.44 (4)	
	Insulin	42.42 ± 6.35 (4)	<0.05	2.97 ± 0.18 (4)	<0.025
	Procaine	26.10 ± 2.93 (4)	<0.05	1.39 ± 0.54 (4)	NS
Glucose incorporation into glycogen	Controls	1.17 ± 0.09 (8)		1.47 ± 0.11 (8)	
	Insulin	3.13 ± 0.67 (8)	<0.005	1.91 ± 0.13 (8)	<0.005
	Procaine	2.33 ± 0.57 (8)	<0.05	1.31 ± 0.11 (8)	NS

After a 1 h preincubation in Krebs-Ringer bicarbonate buffer containing unlabeled glucose, adipose tissue fragments from lean and *ob/ob* mice were incubated for 1 h in Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.5% bovine serum albumin, and 5.55 mM [U-^{14}C]glucose with or without added insulin 1 mU/ml or procaine 0.1 mM as described in METHODS. Results are expressed as mean ± SEM. Numbers in parentheses denote the number of paired samples. Significances of differences were calculated using the paired *t* test.

TABLE 4
Effect of insulin and procaine on the metabolism of [1-^{14}C]glucose and [6-^{14}C]glucose by the epididymal adipose tissue of lean and *ob/ob* mice after 1 h preincubation

	Additions to the medium	[1-^{14}C]glucose				[6-^{14}C]glucose			
		Lean mice	P value	*ob/ob* mice	P value	Lean mice	P value	*ob/ob* mice	P value
Glucose incorporation into total lipids	Controls	32.55 ± 5.53 (12)		13.45 ± 0.60 (12)		29.41 ± 8.11 (12)		9.69 ± 0.52 (12)	
	Insulin	76.58 ± 13.62 (8)	<0.005	18.62 ± 1.61 (8)	<0.005	111.09 ± 28.20 (8)	<0.005	16.80 ± 0.98 (8)	<0.0005
	Procaine	55.68 ± 8.42 (4)	<0.025	11.80 ± 1.79 (4)	NS	48.48 ± 3.76 (4)	<0.025	8.91 ± 0.43 (4)	NS
Glucose incorporation into glyceride glycerol	Controls	20.06 ± 12.93 (12)		6.87 ± 0.60 (12)		15.03 ± 3.40 (12)		7.26 ± 0.49 (12)	
	Insulin	48.03 ± 6.04 (8)	<0.0005	15.50 ± 1.07 (8)	<0.0005	52.22 ± 5.42 (8)	<0.0005	13.86 ± 0.72 (8)	<0.005
	Procaine	28.99 ± 4.13 (4)	<0.05	7.04 ± 0.78 (4)	NS	25.62 ± 0.90 (4)	<0.025	5.92 ± 0.85 (4)	NS
Glucose incorporation into glyceride fatty acids	Controls	3.12 ± 0.86 (12)		0.71 ± 0.21 (12)		7.17 ± 2.98 (12)		0.85 ± 0.17 (12)	
	Insulin	13.06 ± 2.14 (8)	<0.0005	1.75 ± 0.25 (8)	<0.005	41.43 ± 11.17 (8)	<0.005	2.13 ± 0.45 (8)	<0.01
	Procaine	17.21 ± 4.12 (4)	<0.005	0.70 ± 0.16 (4)	NS	19.12 ± 2.82 (4)	<0.0025	0.61 ± 0.12 (4)	NS

After a 1 h preincubation in Krebs-Ringer bicarbonate buffer containing unlabeled glucose, adipose tissue fragments from lean and *ob/ob* mice were incubated for 1 h in Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.5% bovine serum albumin, and 5.55 mM [1-^{14}C]glucose or [6-^{14}C]glucose with or without added insulin 1 mU/ml or procaine 0.1 mM as described in METHODS. Results are expressed as mean ± SEM. Numbers in parentheses denote the number of paired samples. Significances of differences were calculated using the paired *t* test. Results are expressed as micrograms of C atoms per gram of tissue per hour.

controls (Table 5). However, no significant stimulation of hexose monophosphate shunt activity was found in the epididymal fat pads of *ob/ob* mice treated with insulin or procaine as compared with controls.

Glycogen synthesis. Without preincubation of tissue, both insulin 1 mU/ml and procaine 0.1 mM increased incorporation of [U-^{14}C]fructose into glycogen by 75 and 105%, respectively, in adipose tissue of lean mice (Table 2A). Following preincubation of tissue, similar respective increases of 55 and 120% were observed in adipose tissue of these lean mice (Table 2B).

In contrast, in adipose tissue of *ob/ob* mice, insulin did not increase synthesis of glycogen from [U-^{14}C]fructose when the tissue was not preincubated. However, following preincubation, an 80% increase in incorporation of [U-^{14}C]fructose into glycogen was observed in the insulin-treated samples of *ob/ob* mice (Tables 2A and 2B).

Regarding incorporation of radiolabeled glucose, insulin increased incorporation of [U-^{14}C]glucose into glycogen by 167% in fat pads of lean mice. Procaine induced lesser increases of 99% in tissue of the same mice (Table 3). In prewashed tissue of *ob/ob* littermates, insulin induced a lesser increase of 30%.

Although procaine 0.1 mM had the above significant effects on adipose tissue of lean mice, it had no significant effect on glycogen synthesis from either [U-^{14}C]fructose or [U-^{14}C]glucose in adipose tissue of *ob/ob* mice.

DISCUSSION

Prior studies have shown that adipose tissue of *ob/ob* mice is completely resistant to the effects of insulin on glucose metabolism and that preincubation results in a return of the stimulatory effects of insulin.[4,7,25] The present study illustrates that, without preincubation, fructose metabolism in tissue of these mice is also unresponsive to insulin and that prewashing results in a return of responsiveness of fructose as well as glucose metabolism to insulin. This return of sensitivity to insulin following preincubation presumably occurs because of a resulting increase in the number of available insulin receptors according to the mechanisms[3,7,10–15] discussed in the beginning of this paper.

However, although insulin induced respective increases in incorporation of glucose and fructose into total lipids in adipose tissue of lean littermates following preincubation of 236 and 79%, it induced respective increases in incorporation of these sugars in adipose tissue of *ob/ob* mice of only 44 and 47% under the same conditions. These findings contrast with the effects of prewashing of adipose tissue of *ob/ob* mice on lipolysis in which preincubation results in a reduction of the previously elevated basal levels of lipolysis to levels similar to those seen in tissue of lean littermates and in which a complete return of the antilipolytic effect of insulin occurs.[3] The failure of the lipogenic effect of insulin to return completely even though the antilipolytic effect of insulin returned completely under the same conditions suggests continued underlying resistance to insulin regarding hexose metabolism separate from abnormalities in available receptor number.

As discussed in the beginning of this paper, in view of the many insulin-like effects of procaine[20–22] and in view of

TABLE 5
Effects of insulin and procaine HCl on pentose shunt activity in the epididymal fat pads of lean and *ob/ob* mice after 1 h of preincubation

	Pentose shunt activity (%)		
	Controls	Insulin (1 mU/ml)	P value
Lean mice	6.32 ± 2.25 (8)	13.52 ± 1.48 (8)	<0.025
ob/ob mice	9.15 ± 1.48 (8)	11.51 ± 1.80 (8)	NS
		Procaine (0.1 mM)	
Lean mice	3.30 ± 1.75 (4)	12.90 ± 1.70 (4)	<0.005
ob/ob mice	3.01 ± 0.51 (4)	3.86 ± 1.21 (4)	NS

After a 1 h preincubation in Krebs-Ringer bicarbonate buffer containing unlabeled glucose, adipose tissue fragments from lean and *ob/ob* mice were incubated for 1 h in Krebs-Ringer bicarbonate buffer pH 7.4 containing 0.5% bovine serum albumin, and 5.55 mM [1-^{14}C]glucose or [6-^{14}C]glucose with or without added insulin 1 mU/ml or procaine 0.1 mM as described in METHODS. Results are expressed as mean ± SEM. Numbers in parentheses denote the number of paired samples. Significances of difference were calculated using the paired *t* test.

findings that this agent, unlike insulin, exerts its effects on adipocytes and adipocyte membranes pretreated with trypsin[28] (which destroys the insulin receptor[24]), failure to respond to this agent would suggest a postreceptor defect. The present findings illustrate that, although procaine had effects similar to insulin on glucose transport and pentose shunt activity as well as on glycogenesis and lipogenesis from fructose and glucose in adipose tissue of lean littermates, it had no insulin-like effects on hexose metabolism of adipose tissue of *ob/ob* mice. In contrast, prior studies have shown that the antilipolytic effect of both insulin and procaine are completely restored by prewashing of adipose tissue of *ob/ob* mice.[3] These findings suggest that there is some postreceptor defect in control of hexose metabolism but not in control of antilipolysis in adipose tissue of *ob/ob* mice independent of receptor abnormalities. Attempts were therefore made in these experiments to localize at what point in hexose metabolism this proposed postreceptor defect might be.

Glucose transport itself was measured according to the method of Taylor, Mak, and Halperin.[30] In adipose tissue of lean controls, insulin and procaine initiated increases in transport measured in this manner by 119 and 71%, respectively, in the presence of 5.55 mM glucose. Yet no effect of either agent was evident in prewashed tissue from *ob/ob* mice at either concentration of glucose. Other findings, that the effect of insulin on lipogenesis in adipose tissue of *ob/ob* mice from glucose was itself partially reversible by prewashing, illustrate that the partial return of the effects of insulin on glucose metabolism cannot be attributed to a return of sensitivity of glucose transport to insulin. This observation suggests that there is an irreversible defect in responsiveness of glucose transport in adipose tissue of *ob/ob* mice. Moreover, since procaine had effects similar to insulin on glucose uptake in adipose tissue of lean mice and since neither agent exerted an effect in *ob/ob* mice, the effect of procaine on glucose uptake cannot be a nonspecific membrane effect but rather an effect utilizing a mechanism similar to that used by insulin. Failure to respond to this agent supports the presence of an irreversible postreceptor defect in glucose transport in their tissue.

As with glucose transport, no effect of either insulin or procaine was evident on pentose shunt activity even after prewashing of tissue. This finding suggests an additional absolute resistance of this metabolic pathway in adipose tissue of *ob/ob* mice independent of abnormalities in available receptor number.

Lipogenesis, independent of effects on active substrate transport, was studied by observation of the effects of insulin and of procaine on lipid synthesis from [U-^{14}C]fructose. Since fructose entry is not stimulated by insulin, any effect of insulin on metabolism of this hexose should reflect intracellular effects. After prewashing, insulin increased incorporation of fructose into total lipids by only 47% in adipose tissue of *ob/ob* mice compared with a 79% increase in adipose tissue of lean littermates. Moreover, although insulin increased incorporation of [U-^{14}C]glucose into total lipids by 236% in lean mice, its effect in prewashed adipose tissue of *ob/ob* mice was an increase of only 44%, an increase similar to that seen with fructose. These findings further suggest that the partial return of sensitivity of lipogenesis from glucose in adipose tissue of *ob/ob* mice can be attributed to effects independent of those on glucose transport itself.

Of further interest is the finding that the stimulatory effect of procaine on lipogenesis from fructose or glucose in lean mice was not evident before or after prewashing of adipose tissue of *ob/ob* mice. This finding plus the observations of a partial restoration of the effects of insulin by prewashing suggest that a portion of the resistance to insulin, that of activation of the enzymes of lipid synthesis, is reversible by removal of bound insulin and by increasing available receptor number. However, since procaine failed to correct the abnormality, the findings further suggest that, without binding of insulin to these newly available receptors, the underlying resistance of the enzymes of lipid synthesis remains.

It has been shown previously that insulin can activate the key enzymes of fatty acid synthesis, pyruvate dehydrogenase and acetyl CoA carboxylase, in adipose tissue of *ob/ob* mice after a period of prewashing.[25] Assuming that glycerophosphate production is linked to fatty acid and triglyceride synthesis, activation of these enzymes explains the stimulation of lipid synthesis by insulin in their tissues after preincubation. That the degree of stimulation was less than that seen in tissue of lean littermates can be explained by the lesser stimulation of these enzymes in tissue of *ob/ob* mice[25] and by the absolute resistance to insulin of pentose shunt activity observed in this study. The additional absolute resistance to insulin of glucose uptake in their tissue contributed further to the failure of insulin to stimulate incorporation of labeled glucose into total lipid to a degree seen in tissue of lean littermates, such that the observed increase was similar to that seen with fructose.

Effects of insulin and procaine on glycogenesis paralleled those on lipogenesis. No effect of insulin or procaine on incorporation of [U-^{14}C]fructose was evident in adipose tissue of *ob/ob* mice without preincubation. However, after prewashing, stimulation by insulin of incorporation of both [U-^{14}C]glucose and [U-^{14}C]fructose was observed although the effect was less than that seen in tissue of lean littermates. Again, procaine had no effect in adipose tissue of *ob/ob* mice with or without prewashing. As with lipogenesis, these results suggest that resistance of glycogen synthesis to activation by insulin is partially reversible by increased availability and occupancy of insulin receptors but that there is a continued and underlying postreceptor resistance to insulin.

Further studies are required concerning the nature of these proposed postreceptor defects. Since insulin is thought to act by reduction of cellular cyclic AMP levels,[31,33,34] by induction of monovalent ion flux,[33,34] and by altering membrane and cellular handling of calcium,[20–23,33–36] a disturbance of responsiveness of one or more of these modes of action might be involved.

In conclusion, these studies support those of LeMarchand et al.[18] and of Boozer and Mayer,[19] which showed that there are underlying tissue defects in adipose tissue of *ob/ob* mice independent of hyperinsulinemia and abnormalities in available receptor number. In addition, the findings suggest that the defects regarding lipogenesis and glycogenesis can be partially overcome by increasing num-

ber and occupancy of insulin receptors. However, resistance to the effects of insulin on glucose uptake and pentose shunt activity appears to be absolute and irreversible by insulin, at least on a short-term basis. These postreceptor defects might account for a large portion of the insulin resistance seen in adipose tissue of *ob/ob* mice. If postreceptor resistance independent of hyperinsulinemia also exists in liver of *ob/ob* mice, as suggested by the studies of LeMarchand et al.,[18] postreceptor defects might be initiating events that result in elevated insulin levels. The resulting hyperinsulinemia might then cause the observed further insulin resistance of muscle (which, as LeMarchand et al. demonstrated, is reversible by correction of hyperinsulinemia[18]), as well as additional resistance of adipose tissue and liver through induction of a decrease in available insulin receptors, according to the mechanisms discussed previously.[3,7,10–14,15]

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