

Effect of Thyroid Status on Insulin Action in Rat Adipocytes and Skeletal Muscle

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ABSTRACT Isolated adipocytes and soleus muscles prepared from mature rats, rendered hypothyroid by a low iodine diet and propylthiouracil, markedly resisted the ability of insulin to increase glucose utilization. In adipocytes, the sum of basal D-(1-¹⁴C)-glucose conversion to CO₂, glyceride-glycerol, and fatty acid was unaltered by hypothyroidism, although conversion to fatty acid was decreased. The response of each of these metabolic pathways to insulin at all concentrations tested was greatly diminished in hypothyroid rat adipocytes. 3-O-Methylglucose transport rates in the presence of insulin were not significantly different in adipocytes from hypothyroid as compared with euthyroid rats, although basal transport rates were significantly higher in the hypothyroid state. Lipolysis and cyclic AMP accumulation in adipocytes from hypothyroid rats in response to theophylline were markedly diminished compared with euthyroid controls, but insulin was about as effective in inhibiting lipolysis in these cells as in those derived from euthyroid animals. The binding of ¹²⁵I-insulin to adipocytes at several hormone concentrations was also shown to be unaffected by hypothyroidism.

In soleus muscle, basal glucose conversion to H₂O and glycogen was unaltered in the hypothyroid state, whereas insulin action on these pathways was markedly inhibited. The decrease in muscle insulin responsiveness was less marked than that observed in adipocytes. Uptake of either 2-deoxyglucose or L-arabinose in the presence or absence of insulin was similar in soleus muscles derived from euthyroid vs. hypothyroid rats. Similarly, insulin action on the conversion of soleus muscle glycogen synthase *D* to the *I* form in the absence of glucose was unaltered

by hypothyroidism. We conclude that (a) hypothyroidism in mature rats leads to a marked decrease in the responsiveness of glucose metabolism in adipocytes and skeletal muscle to insulin; (b) no detectable impairment of the membrane insulin effector systems that mediate the regulation of adipocyte hexose transport and glycogen synthase is caused by hypothyroidism in this animal model; and (c) the cellular defect that leads to apparent insulin resistance of adipocyte and soleus muscle glucose utilization resides at the level of one or more intracellular enzymes involved in glucose catabolism.

INTRODUCTION

Recent studies by Danforth et al. (1, 2) have uncovered a positive relationship between carbohydrate intake and circulating levels of triiodothyronine in humans. These data are consistent with the possibility that thyroid hormone regulates the capacity of tissues to use carbohydrate. Little information is available on the influence of thyroid hormone on carbohydrate metabolism in the peripheral, insulin-sensitive tissues, fat and muscle.

The present studies were thus designed to investigate a possible effect of thyroid hormone on glucose use in these tissues. In addition, the influence of thyroid status on the responsiveness of these tissues to insulin action on hexose transport and metabolism was assessed. The data demonstrate a striking deficit in the capacity of fat and muscle derived from hypothyroid rats to use glucose in the presence of insulin. This decreased response of glucose use to insulin in hypothyroid rats appears to be related to decreased activity of intracellular enzymes involved in glucose catabolism rather than a defective insulin effector system.

METHODS

Animals. Female Sprague-Dawley rats (Charles River CD strain, Charles River Breeding Laboratories, Inc., Wilmington,

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Mass.) were used in this study. Rats (150–175 g at start) were rendered hypothyroid by maintenance on an iodine-deficient diet 17700, United States Biochemical Corp., Cleveland, Ohio), and drinking water contained 0.00625% 6-*N*-propyl-2-thiouracil for 21–24 d. This protocol has been shown to lead reproducibly to a hypothyroid state in rats as determined by circulating thyroid hormone measurements (3–5). Control rats were littermates or rats of the same weight as the experimental rats and were maintained on tap water and the same test diet, to which normal iodine had been added by the commercial supplier. At sacrifice, control rats weighed 258 ± 4 g and hypothyroid rats weighed 231 ± 5 g. An additional group of rats was rendered hypothyroid as above and then given 30 μg triiodothyronine/100 g body wt daily for 4 d while remaining on the iodine-deficient diet and the 6-*N*-propyl-2-thiouracil. Fat cell size was unaltered by any of the conditions used here.

Isolation of fat cells. White fat cells were obtained by enzymatic digestion of parametrial adipose tissue according to the procedure of Rodbell (6). Pooled adipose tissue from one to three rats was minced with scissors and placed in small plastic bottles. Each bottle, with 3–10 g of tissue and 10 ml of Krebs-Ringer phosphate buffer containing 3% albumin and 1 mg/ml of crude collagenase (clostridium histolyticum, Worthington Biochemical Corp., Freehold, N. J., lot CLS 46E168P), was incubated for 60 min at 37°C. At the end of 60 min digestion, cells were filtered through one layer of nylon chiffon and washed twice with the albumin buffer. The number of fat cells was estimated as described by Gliemann (7).

Cyclic AMP assay. Cyclic AMP accumulation was measured in cells plus medium after a 0.2-ml aliquot of cells plus medium, in duplicate, was extracted from 1 ml of incubation volume and added to tubes on ice containing 20 μl of 2 N HCl. The tubes were then placed in a boiling water bath for 1 min. The tubes were allowed to cool before 10 μl of 4 N NaOH was added. The contents of the tubes were mixed and centrifuged before removal of 20- μl aliquots for determination of cyclic AMP. In each experiment, no more than 50 mg of fat cells were incubated per milliliter of medium, which means that the 20- μl aliquots taken for cyclic AMP analysis represented <1 mg of fat cells. Cyclic AMP release to the medium at the end of the incubation was determined just before removing the 0.2-ml aliquots of cells plus medium. The cyclic AMP standards were prepared in incubation medium containing albumin, which was treated in the same manner as the unknown samples, by adding acid and then boiling and neutralizing. The assay for cyclic AMP was done by a modification of the Gilman (8) protein kinase binding procedure. The cyclic AMP binding protein used in this assay was from the 10,000 g_{max} supernatant fraction of homogenized bovine adrenal glands and the assay was conducted as described by Brown et al. (9) to eliminate possible interference by ATP.

Assay of fat cell hexose transport. Isolated adipocytes ($1-3 \times 10^5$ cells/tube) were incubated in plastic culture tubes containing 0.2 ml Krebs-Ringer phosphate buffer with 4% albumin at 37°C. Insulin was present at the indicated concentrations or was absent from the incubation medium. Transport was initiated with the addition of 10 μl of a solution containing [3- ^3H]methylglucose (4–6 μCi /tube). The labeled hexose was dissolved in isotonic saline before addition. The tubes were immediately shaken vigorously by hand and incubated at 37°C. After 10 s, transport was stopped by addition of 3 ml of ice-cold Krebs-Ringer phosphate buffer containing 0.1% albumin to the cells, then poured onto the center of the glass fiber filters under vacuum. It was crucial that the filters first be wetted (routinely 10 s before cells are poured) with albumin buffer (0.3–0.5% albumin) and that the

cold buffer containing cells and labeled hexose be aspirated through the filter before spreading to its edges and contaminating the glass of the filter apparatus (Millipore Corp., Bedford, Mass.). The cells were immediately washed by rapidly decanting 6 ml of ice-cold Krebs-Ringer phosphate buffer containing 0.1% albumin onto the filters. The total time taken to filter and wash the cells was <15 s. Dried filters were subjected to liquid scintillation spectrophotometry in 4 ml of scintillant containing 33% vol/vol Triton X-100 in toluene with 4 g/liter of Omnifluor (New England Nuclear, Boston, Mass.). Net uptake of label is the amount of radioactivity accumulated at a given time minus the radioactivity bound on filters containing cells to which labeled 3-*O*-methylglucose and 3 ml of cold buffer were added together.

Assay of fat cell glucose utilization and glycerol release. Isolated fat cells (3×10^5 cells/tube) were incubated in plastic culture tubes containing Krebs-Ringer phosphate buffer with 4% albumin at 37°C. Insulin was present at the indicated concentrations or was absent from the incubation medium. The reaction was begun by addition of D-[1- ^{14}C]glucose (0.2 μCi /tube) and stopped after 60 min by the addition of 0.2 ml of 0.5 M H_2SO_4 . Glucose conversion to CO_2 and glycerol release were determined as described by Fain et al. (10).

Assay of ^{125}I -insulin binding to fat cells. Fat cells (5×10^5 cells/tube) were incubated in plastic culture tubes containing 0.4 ml Krebs-Ringer phosphate buffer with 4% albumin at 25°C. Unlabeled and ^{125}I -insulin (New England Nuclear) were added at the indicated total concentrations for the 40-min incubation. At the end of this period, total bound insulin was assayed by the same filtration procedure described for the assay of 3-*O*-methylglucose uptake. Nonspecific insulin binding was estimated from levels of ^{125}I -insulin remaining bound to the fat cells in the presence of a supermaximal dose (10 μg /ml) of unlabeled insulin. These values were subtracted from other experimental values to determine specific binding.

Soleus muscle isolation and assay of glucose metabolism and transport. Soleus muscles were removed from decapitated rats as previously described in detail by LeMarchand et al. (11). The muscle was tied taut across a miniature device shaped like a horseshoe constructed with stainless steel wire, and tied muscles were incubated in the bottom of flat-bottomed vessels containing 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2% bovine serum albumin. The muscles were continually gassed ($\text{O}_2:\text{CO}_2$, 95:5) through rubber stoppers that sealed the vessels. In a typical experiment, the muscles were incubated with or without the indicated concentration of insulin for 30–40 min before addition of 5 mM D-[5- ^3H]glucose. The muscles were incubated for an additional 15 min and then assayed for labeled glycogen (12). Incorporation of label into H_2O in the medium was assayed as an index of glycolytic flux as previously described (10). The activity of the initial steps in glucose utilization—transport and phosphorylation—were estimated using 2-deoxyglucose. Soleus muscles were incubated with 2-[^3H]deoxyglucose at 0.2 mM for 15 min subsequent to incubation in the presence of 2 mM pyruvate with or without insulin for 30 min. The muscles were then quickly rinsed in ice-cold Krebs-Ringer bicarbonate buffer and dissolved in 0.5 ml of 1 N NaOH before monitoring an aliquot by liquid scintillation spectrophotometry. A similar assay condition was used for the assay of arabinose uptake.

RESULTS

Fig. 1 illustrates the pattern of D-[1- ^{14}C]glucose metabolism in isolated fat cells prepared from normal

and hypothyroid rats. In both groups of cells, production of labeled glyceride-glycerol and CO_2 greatly exceeded fatty acid synthesis. No significant difference between cell types was found in basal D-[1- ^{14}C]glucose conversion to CO_2 and glyceride-glycerol, but a significant decrease in labeled fatty acid formation in cells from hypothyroid rats was observed. A key observation was the markedly decreased effects of insulin to stimulate D-[1- ^{14}C]glucose to all these metabolites tested. This difference was significant ($P < 0.05$ for all values) at all concentrations of hormone tested (Fig. 1).

To evaluate whether the decreased responsiveness of fat cells from hypothyroid rats was due to one or more intracellular defects or to alterations in the insulin effector system, glucose metabolism was determined at various glucose concentrations. It is thought that fat cell glucose utilization rates increasingly reflect intracellular enzyme activity, rather than hexose transport activity, as the medium glucose concentration is increased (14, 15). A defect in hexose transport activa-

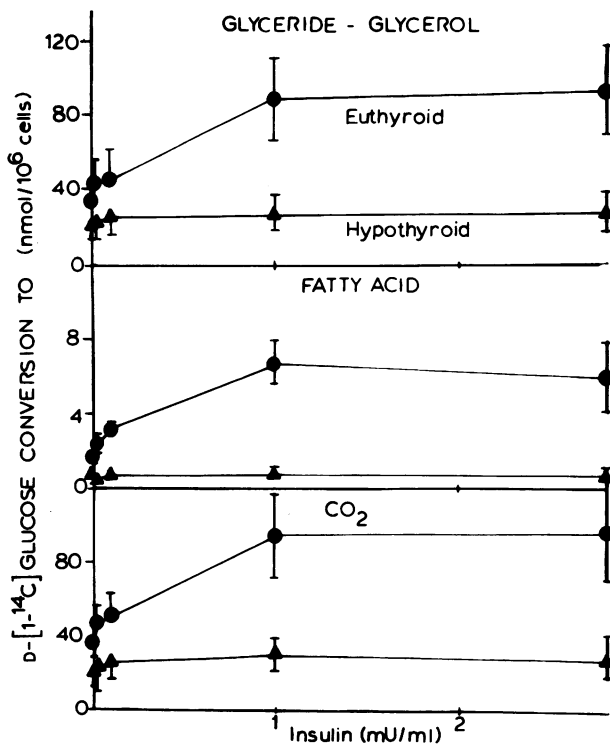


FIGURE 1 Response of glucose metabolism in euthyroid and hypothyroid rat fat cells to submaximal and maximal doses of insulin. Fat cells from euthyroid and hypothyroid rats were incubated in 1 ml Krebs-Ringer phosphate buffer at 37°C containing 4% albumin and in the presence of the indicated concentrations of insulin for 1 h. D-[1- ^{14}C]glucose was present at a final concentration of 270 μM and its conversion to carbon dioxide (CO_2), fatty acids, and glyceride-glycerol was measured. The values for CO_2 , fatty acid, and glyceride-glycerol represent the nanomoles of glucose incorporated \pm SE in four paired experiments performed on different days.

tion by insulin might therefore be less markedly expressed when cells are incubated at 20 mM glucose compared with 0.5 mM glucose. Table I shows that basal D-[1- ^{14}C]glucose conversion to total lipids was not significantly different between cell types at all glucose concentrations tested, in accord with the data in Fig. 1. However, the nanomoles of labeled glucose converted into lipids in the presence of insulin were greatly decreased in cells from hypothyroid rats compared with control cells at all concentrations of medium glucose. No significant increase in glucose conversion to total lipids due to insulin was observed in the former cells at medium glucose concentrations of 5 mM (Table I). The decrease in glucose metabolism observed in adipocytes from hypothyroid rats at both the low and high glucose concentrations in the presence of insulin was significant ($P < 0.05$). Similar observations were made for glucose oxidation to CO_2 (not shown).

These data are consistent with the concept that intracellular metabolic capacity is already saturated in these cells in the absence of insulin. To test this hypothesis directly, estimated initial [3-O- ^3H]methylglucose uptake rates were monitored in fat cells from euthyroid or hypothyroid rats (Fig. 2). In contrast to the lack of effect of the hypothyroid state on basal fat cell glucose use basal [3-O- ^3H]methylglucose transport during a 10-s period was significantly increased in fat cells from hypothyroid rats. Insulin increased [3-O- ^3H]methylglucose transport in both cell types in a dose-dependent manner that was maximal at $\sim 100 \mu\text{U/ml}$ insulin. No difference in the insulin-stimulated rates of hexose transport activity could be observed between cell types when maximal insulin concentrations were employed. Thus, D-glucose transport system activity of cells from hypothyroid rats was equal to or greater than that from euthyroid rats under all conditions tested, but glucose utilization in the former cell type was markedly decreased in the presence of insulin (Fig. 1 and Table I).

It is not clear from the data presented in Fig. 2 whether insulin action itself is impaired in the hypothyroid state. The apparent decreased percent activation of hexose transport by insulin in fat cells from hypothyroid animals may simply be due to an "insulin-like" effect of the thyroid hormone deficiency. Alternatively, hypothyroidism might be increasing the number of insulin-responsive hexose transport systems per cell as well as impairing insulin responsiveness. To evaluate this question, we studied two other aspects of insulin action in fat cells from euthyroid and hypothyroid rats: (a) insulin-receptor interaction and (b) inhibition of lipolysis. Table II illustrates the binding of ^{125}I -insulin to such fat cells at concentrations of the hormone that range from 0.2 to 1.6×10^3 nM. No significant differences in the binding of ^{125}I -insulin to these cell types was observed.

The data in Table III illustrate the effectiveness

TABLE I
*Effects of Increasing Glucose Concentrations on Decreased
Glucose Metabolism Due to Hypothyroid State*

Condition	Glucose concentration	D-[1- ¹⁴ C]Glucose conversion to total lipids		
		Euthyroid	Hypothyroid	Mean increment due to hypothyroid state
		<i>mM</i>	<i>nmol/g fat cells</i>	
Control	0.5	132±29	168±54	36±25
+Insulin (2.4 mU/ml)	0.5	319±33	236±34	-82±4
Control	5	218±36	222±48	4±15
+Insulin (2.4 mU/ml)	5	538±131	278±42	-261±80
Control	20	321±26	234±72	94±50
+Insulin (2.4 mU/ml)	20	616±133	246±28	-369±107

Fat cells from euthyroid and hypothyroid rats were incubated in 1 ml of Krebs-Ringer phosphate buffer containing 4% serum albumin. Incubations were performed with insulin concentrations of 2.4 mU/ml for each of the glucose concentrations indicated. The incubation was initiated by the addition of the fat cells to medium containing the above agents and was terminated 1 h later by the addition of 0.2 ml of 2 N HCl. The values presented are the means of three experiments performed in duplicate on different days.

of insulin to inhibit theophylline-stimulated lipolysis in isolated fat cells from euthyroid and hypothyroid rats. As reported previously (16), the cells from the latter rats were poorly responsive to the lipolytic agent. Rates of glycerol release from these cells treated with 2 and 5 mM theophylline were similar to the glycerol release observed in control cells incubated with 0.25 and 0.5 mM theophylline, respectively. Control cells exhibited maximal lipolytic rates at 5 mM theophylline compared with the 2 mM condition. Although these differences complicate interpretations somewhat, it can be seen that insulin effectively inhibited glycerol release in both cell types (Table III). No significant difference in the decrement of glycerol release due to insulin was observed between control cells treated with 0.25 mM theophylline and cells from hypothyroid rats incubated with 2 mM theophylline. Other experiments indicated that sensitivity to lower insulin concentrations was also largely intact (not shown). The level of cyclic AMP in these cells at 2 min of incubation were also studied (Fig. 3). Control cells exhibited higher cyclic AMP levels under all conditions, compared with cells derived from hypothyroid rats ($P < 0.05$). Insulin had no significant effect on cyclic AMP levels except in control cells incubated with 2 mM theophylline, where a significant decrease due to insulin was observed ($P < 0.05$). Most striking, however, was the lack of correlation between cyclic AMP levels and lipolytic rates in the two cell types. Thus, cyclic AMP levels were lower in cells from hypothyroid rats, which released 3.4 mmol glycerol/10⁶ cells, than in control cells, which exhibited 0.28 mmol glycerol/10⁶ cells.

The pattern of glucose metabolism in isolated soleus muscles was studied in order to gain insight into the effects of thyroid status on skeletal muscle metabolism. It has been demonstrated that conversion of D-[5-³H]glucose to ³H₂O represents a useful index of glycolytic flux in such muscles (13, 17). Table IV illustrates that the conversion of D-[5-³H]glucose to both labeled glycogen and H₂O are similar in muscles derived from euthyroid and hypothyroid rats when incubated in the absence of insulin. A supermaximal concentration of insulin provoked a ninefold increase in labeled glycogen production as well as a much smaller effect on ³H₂O production in control soleus muscles. In contrast, no stimulation of ³H₂O production by insulin was observed in muscles derived from hypothyroid rats. A significant decrease in soleus muscle [³H]glycogen production in the presence of insulin was also observed, compared with that found in muscles from euthyroid rats. Similar defective responses to lower concentrations of insulin (300 μU/ml and 2 mU/ml) were also observed (not shown).

To determine whether the blunted responsiveness to insulin observed in soleus muscles from hypothyroid rats was due to a defect in the insulin effector system, insulin action on muscle hexose transport system activity was monitored using both 2-deoxyglucose and L-arabinose as substrates. Table V illustrates the uptake of 2-[³H]deoxyglucose in isolated muscles over a 15-min period in the presence and absence of a submaximal and maximal dose of insulin. No significant difference in deoxyglucose uptake could be observed between muscle types in the absence of insulin. In-

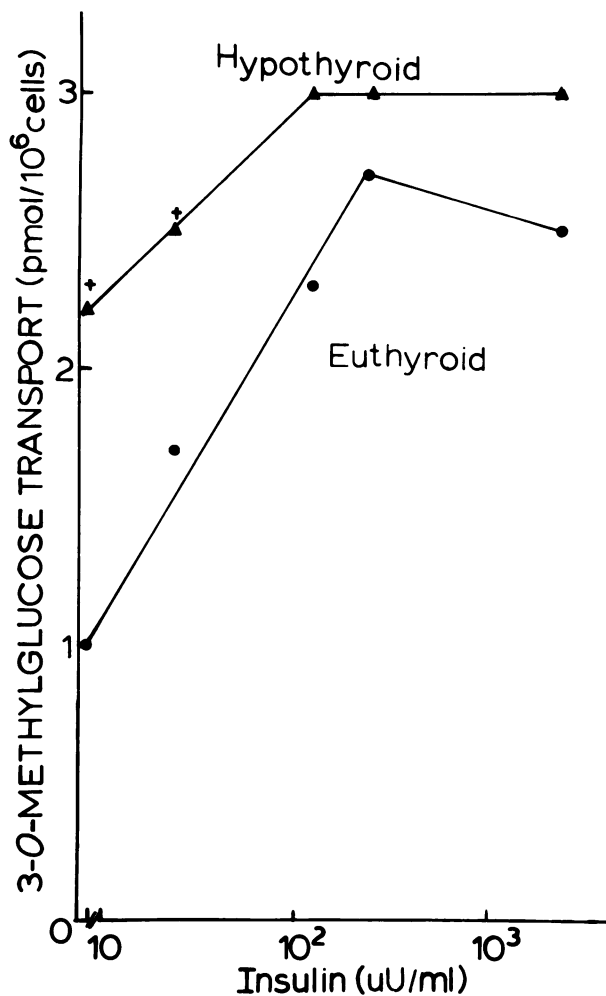


FIGURE 2 Insulin action on 3-O-methylglucose transport in fat cells derived from normal and hypothyroid rats. Fat cells ($1-4 \times 10^6$ cells/tube) were incubated with or without the indicated concentrations of insulin for 10 min in Krebs-Ringer phosphate buffer at 37°C before addition of 50 μ M [3-O-³H]methylglucose and assay of uptake for 10 s. The amount of hexose uptake observed in the presence of 50 μ M cytochalasin B was taken as diffusion and was subtracted from the other values obtained. The data presented are the means of four experiments performed in triplicate on different days. Asterisks denote that difference between hypothyroid and control values is statistically significant ($P < 0.05$).

Insulin markedly enhanced deoxyglucose uptake by soleus muscles derived from both groups of animals. However, the mean percent increments in deoxyglucose uptake due to 1.08 or 108 mU/ml insulin were not significantly different between muscles from euthyroid or hypothyroid rats. Studies not illustrated indicated deoxyglucose uptake was linear in soleus muscles for over 15 min.

To corroborate this result using another hexose analogue, L-[¹⁴C]arabinose was used in similar studies. Fig. 4 illustrates the time-course of L-[¹⁴C]arabinose

TABLE II
Binding of ¹²⁵I-Insulin to Adipocytes from Euthyroid and Hypothyroid Rats

¹²⁵ I-Insulin concentration	¹²⁵ I-Insulin bound	
	Euthyroid	Hypothyroid
	<i>fmol/10⁶ cells</i>	
0.2 nM	0.023±0.003	0.024±0.005
1.8 nM	0.17±0.03	0.18±0.03
16 nM	1.2±0.2	1.2±0.2
1.6 μ M	74±12	84±9

¹²⁵I-Insulin tracer (2×10^{-10} M) was incubated with fat cells (2×10^5 , 0.5 ml/tube) in the presence or absence of unlabeled insulin to the final insulin concentration indicated below. The incubation was terminated by the addition of 3.0 ml ice-cold Krebs-Ringer phosphate buffer containing 0.1% albumin and the mixture was rapidly filtered under vacuum through a Whatman GFA filter. The filter was quickly washed with 9.0 ml additional ice-cold buffer, dried, and counted. Each point was determined in triplicate. The data given above represent the mean±SE of three experiments performed on separate days.

uptake in isolated soleus muscles over a 90-min period. In control muscles, uptake of this hexose increased steadily over the entire period of incubation, while the known transport inhibitor cytochalasin B significantly decreased uptake. Insulin at 3 mU/ml markedly activated L-[¹⁴C]arabinose transport in soleus muscles, and this increased accumulation of label was observed throughout the experiment. Equilibration of the label thus did not occur in control and cytochalasin B-treated muscles, indicating that L-[¹⁴C]arabinose is a slowly transported glucose analogue in this system. Table VI depicts the results of a series of experiments where insulin action on L-[¹⁴C]arabinose was assessed in soleus muscles from euthyroid and hypothyroid rats. No difference due to thyroid status in hexose transport rates was observed in the presence or absence of hormone.

The stimulatory action of insulin on glucose conversion to glycogen reflects the capability of insulin to both activate hexose transport and to elicit the conversion of glycogen synthase D to the I form (18). The decreased flux observed in muscles from hypothyroid rats of glucose into glycogen in response to insulin in the face of unimpaired insulin action on hexose transport (Tables IV and VI) could then result from defective synthase activation. We therefore studied the effect of insulin on glycogen synthase activity in soleus muscles incubated in the absence of medium glucose (Table VII). Total glycogen synthase activity was estimated in muscle homogenates supplemented with 10 mM glucose-6-phosphate, and synthase I activity was monitored in the absence of this hexose phosphate. No differences in the percentage of synthase activity

TABLE III
Antilipolytic Effect of Insulin on Fat Cells Derived
from Euthyroid and Hypothyroid Rats

Theophylline concentration	Euthyroid		Hypothyroid	
	Without insulin	Mean decrement due to 2.4 mU/ml insulin	Without insulin	Mean decrement due to 2.4 mU/ml insulin
	mM	mmol/10 ⁶ cells	mmol/10 ⁶ cells	mmol/10 ⁶ cells
0	0.28±0.08	0.22±0.062	0.20±0.083	0.054±0.028
0.1	0.83±0.15	0.53±0.16	0.22±0.078	0.055±0.035
0.25	2.6±0.55	2.2±0.43	0.39±0.14	0.12±0.12
0.50	3.8±0.64	3.1±0.47	0.66±0.28	0.42±0.25
2.0	6.4±1.0	2.6±0.45	2.3±0.62	1.6±0.50
5.0	6.8±0.85	0.55±0.54	3.40±0.66	1.4±0.066

Fat cells were incubated in 1 ml of Krebs-Ringer phosphate buffer containing 4% bovine serum albumin for 2 h at 37°C. The indicated concentrations of theophylline were added at the start of the experiment in the presence or absence of 2.4 mU/ml insulin. At the end of the incubation period the fat cells were allowed to float, and 50- μ l aliquots of medium were taken for analysis of glycerol. The values presented are the means \pm standard error of total glycerol or the decrement due to insulin of three separate experiments performed in triplicate.

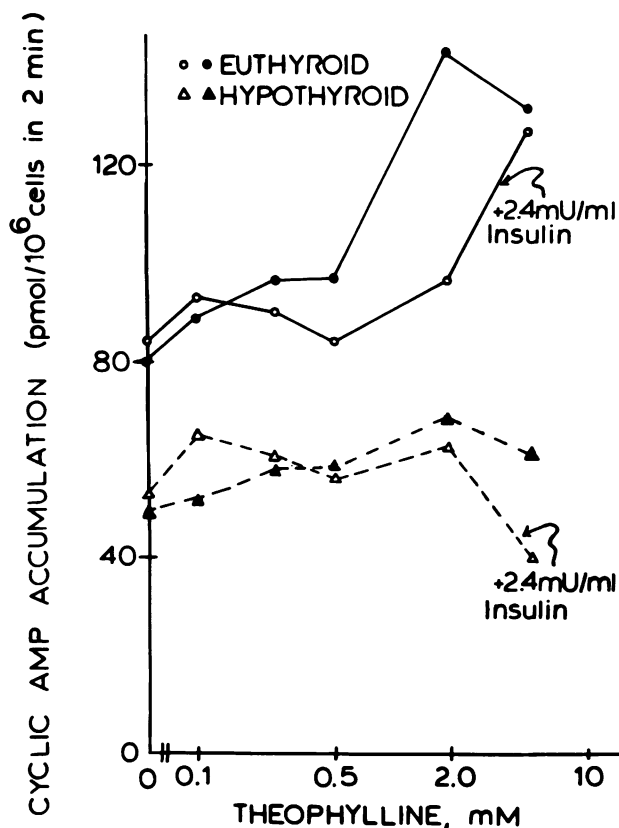


FIGURE 3 Effect of insulin on theophylline-stimulated cyclic AMP accumulation of fat cells from euthyroid and hypothyroid rats. Fat cells from euthyroid (circles) or hypothyroid (triangles) rats were incubated in 1 ml of Krebs-Ringer phosphate buffer containing 4% bovine serum albumin at 37°C

that was observed to be in the *I* form, or in insulin action on conversion of *D* to *I* form in soleus muscles could be observed due to thyroid status of the rats (Table VII).

DISCUSSION

The data presented in this report demonstrate a strikingly similar alteration in the carbohydrate utilization of both adipocytes and soleus muscles due to hypothyroidism in rats. This alteration in the hypothyroid state is expressed as a decreased insulin responsiveness of *D*-glucose utilization in these tissues under conditions where basal glucose metabolism is not significantly different from euthyroid values. However, all of the results obtained are consistent with the concept that the cellular insulin effector systems in these tissues are not defective in the hypothyroid state, but rather that one or more intracellular enzymes involved in glucose metabolism are diminished. The following data support this conclusion: (a) hexose transport system activity measured by nonmetabolizable hexose uptake in the presence of insulin is not different in fat cells or soleus muscles from euthyroid vs. hypothyroid

with the indicated concentrations of theophylline in the absence (filled symbols) or presence (open symbols) of 2.4 mU/ml of insulin. The incubation was initiated by the addition of the fat cells to medium containing the above agents and was terminated 2 min later by the addition of 0.1 ml of 2 N HCl. The tubes were then boiled for 1 min and cooled to room temperature in 20- μ l aliquot of cells plus medium. The values presented are the means of three separate experiments performed in triplicate.

TABLE IV
Effects of Insulin on D-[5-³H]Glucose Conversion to Glycogen and H₂O in Soleus Muscles of Euthyroid and Hypothyroid Rats

	[³ H]Glucose conversion to glycogen	Number of muscles studied	[³ H]Glucose conversion to H ₂ O	Number of muscles studied
	nmol/mg		nmol/mg	
Euthyroid				
Control	0.30±0.04	11	2.2±0.2	10
Insulin (77 mU/ml)	2.6±0.3	11	2.7±0.2	10
Hypothyroid				
Control	0.27±0.03	11	2.0±0.2	10
Insulin	1.6±0.2*	11	2.0±0.1	11

Soleus muscles were incubated with continuous oxygenation in 2.5 ml Krebs-Ringer bicarbonate buffer containing 2% bovine serum albumin for 30 min in the presence or absence of 77 mU/ml insulin. All vessels then received 4 mM D-[5-³H]glucose (1–2 μCi/ml); 15 min later, muscles were assayed for labeled glycogen, and 0.5 ml of medium was lyophilized to determine labeled H₂O. Values presented are the means±SE.

* Significantly different from euthyroid value (*P* < 0.01).

rats under conditions wherein glucose metabolism was substantially diminished in the hypothyroid state (Fig. 3 and Table VI); (b) insulin action on other cellular functions, such as adipocyte lipolysis (Table III) and soleus muscle glycogen synthase (Table VII) in the absence of medium glucose, was normal in the hypothyroid state; and (c) ¹²⁵I-insulin binding to cell sur-

face receptors was unimpaired in adipocytes derived from hypothyroid rats (Table II). Thus the data lead to the conclusion that the cellular locus responsible for the apparent insulin resistance observed in these studies is one or more intracellular enzymes involved in glucose metabolism.

It should be noted that the impact of the hypothyroid state on isolated adipocyte metabolism appears greater in magnitude than on the isolated soleus muscle system, inasmuch as the latter did not appear to be af-

TABLE V
2-Deoxyglucose Uptake in Soleus Muscles of Hypothyroid and Euthyroid Rats

Condition	Number of muscles studied	[³ H]Deoxyglucose uptake	Increment due to insulin
		pmol/mg muscle	%
Euthyroid			
Control	10	2.07±0.7	
Insulin (1.08 mU/ml)	5	4.46±0.8	115
Insulin (108 mU/ml)	6	7.73±1	273
Hypothyroid			
Control	11	1.33±0.4	
Insulin (1.08 mU/ml)	6	3.45±0.4	159
Insulin (108 mU/ml)	6	6.0±0.8	351

All muscles were incubated for 30 min at 37°C with continuous oxygenation in 2.5 ml Krebs-Ringer bicarbonate buffer containing 2% albumin and 1.6 mM pyruvate in the presence or absence of 1.08 or 108 mU/ml insulin. The addition of 0.16 mM 2-[³H]deoxyglucose was made to all vessels; 15 min later, muscles were rinsed twice in ice-cold buffer and assayed for accumulated label. All values were corrected for extracellular label by the subtraction of the amount of accumulated label in muscles exposed to 80 μM cytochalasin B. Values are the means±SE of the number of muscles indicated or the percent increments due to insulin.

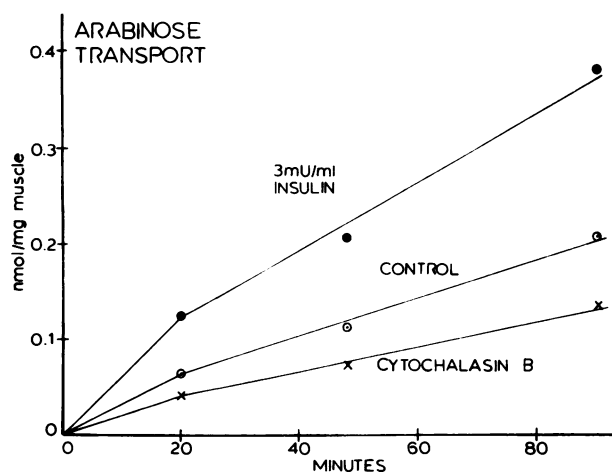


FIGURE 4 Cytochalasin B-sensitive arabinose uptake in isolated soleus muscles. Soleus muscles were incubated for 15 min at 24°C with continuous oxygenation in 2.5 ml Krebs-Ringer bicarbonate buffer containing 2% albumin in the presence or absence of 3 mU/ml insulin or 50 μM cytochalasin B. Addition of 0.5 mM L-[¹⁴C]arabinose was made to all vessels, and at the indicated times of incubation muscles were rinsed twice for 5 min each in ice-cold buffer and assayed for accumulation of label.

TABLE VI
Arabinose Uptake in Soleus Muscles of Hypothyroid and Euthyroid Rats

	Number of muscles studied	[¹⁴ C]Arabinose uptake	Mean increment due to insulin
		nmol/mg muscle	%
Euthyroid			
Control	7	0.025±0.006	280±80
Insulin (3 mU/ml)	7	0.086±0.009	
Hypothyroid			
Control	8	0.023±0.004	456±143
Insulin (3 mU/ml)	8	0.091±0.007	

Soleus muscles were incubated for 15 min at 24°C with continuous oxygenation in 2.5 ml Krebs-Ringer bicarbonate buffer containing 2% albumin in the presence or absence of 3 mU/ml insulin. Addition of 0.5 mM L-[¹⁴C]arabinose was made to all vessels; 45 min later, muscles were rinsed twice for 5 min each in ice-cold buffer and assayed for accumulation of label. All values were corrected for extracellular L-[¹⁴C]arabinose by subtracting the amount of label accumulated in the presence of cytochalasin B. Values presented are the means±SE number of muscles shown.

ected in respect to glucose transport rates. However, insulin responsiveness of glucose conversion to glycogen was inhibited by almost 50% in the hypothyroid state, while glycolytic flux stimulation by the hormone was completely inhibited. Supermaximal concentrations of insulin (77 mU/ml) were used to test maximal responsiveness of the system rather than insulin sensitivity. In experiments not presented, responsiveness to lower concentrations of insulin approaching the physiological range was also inhibited in this soleus muscle preparation. We do not yet know whether these observations are physiologically important in the intact animal. However, the diminished muscle insulin responsiveness reported here is highly statistically significant and parallels the adipocyte defect in hypothyroidism, indicating a potentially important physiological basis.

Our data documenting decreased glucose metabolism in adipocytes treated with insulin due to hypothyroidism conflict with the results of Correze et al. (19, 20), who found increased adipocyte glucose metabolism in thyroidectomized rats. Rats were rendered hypothyroid for periods similar to those employed in our studies before experiments were performed. However, a key difference in those studies is the very young age and hence weight (60–80-g rats) at which thyroidectomy was performed. Growth of the animals is markedly affected by hypothyroidism at this early stage of development, and interpretations of results obtained in such studies are complicated by this problem. In the experiments described in this report,

TABLE VII
Glycogen Synthase Activity in the Soleus Muscle of Hypothyroid and Euthyroid Rats

Condition	Glycogen synthase I	Number of muscles studied
	%	
Hypothyroid		
Control	29.3±2.9	8
Insulin (77 mU/ml)	38.7±2.4	9
Euthyroid		
Control	28.6±1.4	9
Insulin (77 mU/ml)	37.4±1.6	9

All muscles were incubated with continuous oxygenation for 30 min in Krebs-Ringer bicarbonate buffer with 2% albumin at 37°C in the presence or absence of 77 mU/ml insulin. The muscles were homogenized with Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) in 1 ml of ice-cold 100 mM KF and 10 mM EDTA, pH 7.0 for 20 s; homogenates were centrifuged for 30 min at 3,000 g. Supernatant aliquots of 30 μl were added to the substrate, incubated for 10 min at 30°C, and each sample was spotted on filter paper, washed, and assayed for label accumulation. Glycogen synthase activity is expressed as a percentage of total activity (measured under the same conditions except for the addition of 10 mM glucose-6-phosphate). Values are the means±SE of the number of muscles indicated.

only fully mature animals weighing 175–200 g were subjected to the regimen producing hypothyroidism. In addition, the effects reported here do not relate to extraneous effects of the diet and propylthiouracil treatment on rats, inasmuch as injection of triiodothyronine was capable of reversing the metabolic defects observed (not illustrated).

An interesting observation in our studies was an increased D-glucose transport system activity in fat cells from hypothyroid rats (Fig. 2). This phenomenon was not observed in soleus muscles tested for hexose transport activity using 2-deoxyglucose and L-arabinose uptake rates (Tables V and VI). The increased transport activity in adipocytes from hypothyroid rats does not appear to have significant physiological relevance because basal adipocyte glucose metabolism was not increased in parallel with the increased transport rate. This lack of correlation can be attributed to the postulated decreased capacity of intracellular enzymes to metabolize glucose due to the hypothyroidism. It is tempting to speculate, however, that thyroid hormone deficiency leads to the modulation of a common membrane transduction system that regulates hexose transport and is also responsive to insulin. Modulation of the transmembrane signaling between the β-receptor and the adenylyl cyclase has been recently proposed as the basis for the altered catecholamine-stimulated lipolytic response of fat cells from hypothyroid rats (16).

A series of studies by Schoenle et al. (21–23) have demonstrated increased adipocyte hexose transport activity in hypophysectomized rats. Administration of growth hormone to these animals reversed the elevated transport activity to levels observed in control adipocytes. Glucose transport activity in the presence of insulin was similar in adipocytes derived from both control and hypophysectomized rats, however. These data are remarkably similar to our observations with hypothyroid rats and thyroid hormone administration. Thyroid hormone administration to hypophysectomized rats did not decrease glucose transport in the studies of Schoenle et al. (21–23). However, it seems possible that thyroid hormone action may require the presence of growth hormone to act. Taken together, the available data indicate that both growth hormone and thyroid hormone play significant roles in the regulation of glucose transport and its modulation by insulin.

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