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Effects of Thyroid Hormone on the Gluconeogenic Capacity of Lipemic BHE Rats[1] (41544)

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Abstract. Gluconeogenesis and ketogenesis from a variety of substrates by isolated hepatocytes from thyroxine-treated and control 40-hr starved BHE rats were determined. Thyroid hormone treatment stimulated glucose production from lactate and inhibited glucose production from alanine, lactate plus NH_4Cl, or glutamate. β-Hydroxybutyrate production by cells incubated with glycerol was decreased in the cells from thyroxine-treated rats. Glycerol had a greater antiketogenic effect in the thyroxine-treated rats than in the control rats. Acetoacetate production from 10:1 lactate:pyruvate, lactate plus NH_4Cl, lactate plus lysine, and lactate plus ethanol was also less in the hepatocytes from the thyroxine-treated rats. Acetoacetate production from alanine was increased by thyroxine treatment. From these results it can be suggested that the metabolic abnormalities previously reported in the BHE rats can, in part, be attributable to abnormalities in endogenous thyroid hormone action and in part due to some as yet unknown regulatory process within the liver cell.

Gluconeogenesis, or the synthesis of glucose, has been shown to be controlled in part by the activity of the malate–aspartate shuttle (1–3) and in part by hormones such as glucagon (4–8), epinephrine (4–7), cortisol (9), and thyroxine (10–14). The thyroid hormone not only stimulates gluconeogenesis but also affects metabolism in other ways as well. It increases the activity of the α-glycerophosphate shuttle (15, 16), mitochondrial $Ca^{2+}Mg^{2+}$ ATPase (17), mitochondrial oxygen consumption (14, 16, 18–20), the activity of adenine nucleotide translocase (20, 21), calcium ion movement (22–24), and lowers the phosphorylation state of the hepatic cell (25). All of these actions result in an increased turnover of metabolic fuels, particularly glucose, within the hepatic cell.

In a previous paper we reported that the normally "slower" mitochondrial activity of the BHE rat[2] could be increased by thyroxine treatment (26). Both the α-glycerophosphate and the malate–aspartate shuttles were affected as was the $Ca^{2+}Mg^{2+}$ ATPase activity. We have also reported that isolated hepatocytes from BHE rats synthesized more glucose from a variety of substrates than did hepatocytes from Wistar rats (27). Since the thyroid hormones are so important in the regulation of mitochondrial activity which, in turn, is involved in the regulation of gluconeogenesis, studies of the gluconeogenic response to this hormone by the BHE rat might assist in our understanding of how the hormone functions in the regulation of this process. Secondarily, the results of these studies would expand our understanding of how the lipemic/glycemic BHE rat differs from its normal counterpart. Thus, this paper reports on the effects of thyroxine administration on the gluconeogenic capacity of hepatocytes isolated from 40-hr starved young BHE male rats.

Materials and Methods. Two groups of male 50- to 60-day-old BHE rats[2] were used. One group was injected, sc, with 10 μg thyroxine/100 g body weight while the other group was injected with solvent only and served as the control group. The dose of thyroxine was divided into two portions administered at 12-hr intervals. Thyroxine was dissolved in 5 m*M* NaOH to a concentration of 1 mg/ml. The solution was made fresh daily.

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[2] The BHE strain originated from a cross between the Osborne–Mendel and the Pennsylvania State College strains. A colony of these rats is maintained at the University of Georgia. The rats are characterized by a genetic tendency toward maturity onset diabetes and lipemia, renal disease, and cardiovascular lesions similar to a type IV lipemic. Several recent reviews have been published which describe their biochemical characteristics (52, 53).

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TABLE I. GLUCOSE PRODUCTION BY ISOLATED HEPATOCYTES OF THYROXINE-TREATED AND CONTROL 40-hr STARVED BHE RATS[a]

Incubation medium	Treatment	
	Control	10 μg thyroxine/100 g/day
No additions, 0 time	3.08 ± 0.28 (12)[b]	1.69 ± 0.12 (12)*
No additions, 60 min	8.63 ± 0.58 (14)	4.46 ± 0.17 (10)*
Lactate, 10 m*M*	41.0 ± 4.1 (12)	52.0 ± 3.7 (12)*
Pyruvate, 10 m*M*	38.8 ± 3.8 (14)+	30.8 ± 2.5 (11)+
Lactate, 10 m*M* + pyruvate, 1 m*M*	57.5 ± 4.8 (14)+	68.7 ± 3.4 (12)+
Lactate, 10 m*M* + NH_4Cl, 5 m*M*	63.5 ± 6.3 (14)+	41.7 ± 3.4 (12)*,+
Lactate, 10 m*M* + lysine, 2 m*M*	57.8 ± 5.9 (14)+	66.4 ± 2.14 (11)+
Lactate, 10 m*M* + ethanol, 10 m*M*	23.2 ± 3.3 (13)+	11.8 ± 0.28 (11)+
Alanine, 10 m*M*	24.3 ± 3.1 (10)+	9.10 ± 1.81 (10)*,+
Glutamate, 10 m*M*	19.3 ± 5.2 (14)+	8.08 ± 0.28 (12)*,+
Glycerol, 10 m*M*	28.8 ± 2.0 (14)+	48.8 ± 1.92 (11)*
Dihydroxyacetone, 10 m*M*	121.5 ± 2.1 (14)+	115.2 ± 9.85 (12)+

[a] Cells were incubated 60 min, 37° in Krebs Henseleit buffer containing 2.5% fraction V albumin with or without added substrates in a total volume of 4 ml.

[b] Results are μmole glucose/hr/g cell wet weight, mean ± SEM. Number of observations in parentheses. Significant ($P < 0.05$) hormone treatment differences are indicated by *; significant ($P < 0.05$) differences attributable to substrate addition to or substitution of lactate are indicated by +.

After 2–3 days of treatment oxygen consumption was determined using a whole body respirometer to ascertain the effectiveness of the treatment.

After 7 days of thyroxine injection, the animals were starved for 40 hr. Isolated hepatocytes were prepared according to the Krebs–Cornell modification of the Berry and Friend technique (28, 29). Viability of the cells were ascertained by trypan blue exclusion. Preparations were considered acceptable if 90–95% of the cells excluded the dye. Cells (75 mg wet weight) were suspended in Krebs–Henseleit buffer containing 2.5% fraction V bovine serum albumin. The albumin had been dialyzed with buffer to remove the excess citrate. Cells were incubated without added substrates or with the addition of 10 m*M* lactate, 10 m*M* pyruvate, 10 m*M* alanine, 10 m*M* glutamate, 10 m*M* glycerol, 10 m*M* dihydroxyacetone or 10 m*M* lactate plus 1 m*M* pyruvate, 5 m*M* NH_4Cl, 2 m*M* lysine or 10 m*M* ethanol in a total volume of 4 ml. After 60 min of incubation at 37°, the cells were killed by the addition of 0.2 ml 60% PCA. An aliquot of the cells prior to incubation was treated with PCA to provide the initial values for glucose, ketones, and ATP. The ATP content was used as another criterion of cell viability. Preparations having initial ATP values of less than 2 μmole ATP/g wet weight of cells were discarded. Ideally, isolated hepatocytes should contain between 2 and 3 μmole of ATP/g wet weight.[3] After KOH neutralization, the glucose (30), ketone (31), and ATP (32) content of the cells were determined. Glucose, β-hydroxybutyrate and acetoacetate production were calculated as micromoles product formed per hour per gram wet weight of cells. ATP content was calculated as micromoles per gram wet weight of cells. Significant differences attributable to thyroxine treatment or to the addition to or substitution for lactate were ascertained using a *t* test for groups of unequal number (33).

Results. Most investigators have used 48-hr starved rats for their studies of gluconeogenesis. However, our thyroxine-treated BHE rats could not withstand 48 hr without food and we were thus forced to use a shorter starvation period. Nonetheless, the values obtained for glucose production compare favorably with those in the literature. Glucose production from lactate or pyruvate (Table I) was slightly higher than that reported by Cornell *et al.* for female 48-hr starved Wistar rats (28) and significantly higher than those values

[3] N. Cornell, personal communication.

reported previously (27) for 40-hr starved male Wistar rats. The effects of lactate plus pyruvate, lysine or NH_4Cl on glucose production by hepatocytes from control 40-hr starved rats likewise are consistent with those results reported by Cornell *et al.* (28). The inhibition of gluconeogenesis by ethanol when added to lactate was similar to that reported by Krebs *et al.* (34) who used a perfused liver system rather than isolated hepatocytes.

Treatment with thyroxine appeared to "normalize" glucose production from some but not all of the substrates used. For example, glucose production was lower in the thyroxine-treated rats when NH_4Cl, alanine, or glutamate were added to the lactate-containing incubation media but was stimulated when lactate alone or glycerol were used. No other thyroxine treatment effects were observed. The effect of ethanol on glucose production from lactate was more pronounced in hepatocytes from the thyroxine-treated rats than from the nontreated rats. The values for the hepatocytes from the thyroid-treated rats were very similar to those reported previously for Wistar rats (27).

Consistent with our previous reports of elevated β-hydroxybutyrate:acetoacetate ratios in frozen clamped liver (35) and elevated ketone levels in the blood of starved rats (36), ketone production (Table II) by hepatocytes from both control and thyroxine-treated rats was higher than that reported by others (37, 38) and higher than that reported for 40-hr starved Wistar rats of the same sex and age (27).

β-Hydroxybutyrate production (Table II) was unaffected by thyroxine treatment except when glycerol was used as the substrate. With this substrate, β-hydroxybutyrate production was reduced. This was in contrast to the production of glucose which was increased in the hepatocytes from the thyroxine-treated rats compared to the control rats. β-Hydroxybutyrate production was decreased when NH_4Cl was added to the lactate-containing medium and when alanine, glycerol, or dihydroxyacetone were used as substrates. β-Hydroxybutyrate production was increased when ethanol was added to the lactate-containing medium. Note again the reciprocal relationship between glucose and β-hydroxybutyrate production.

TABLE II. KETONE PRODUCTION BY ISOLATED HEPATOCYTES OF THYROXINE-TREATED AND CONTROL 40-hr STARVED BHE RATS[a]

Incubation medium	Treatment			
	β-Hydroxybutyrate		Acetoacetate	
	Control	Thyroxine	Control	Thyroxine
No addition, 0 time	6.61 ± 0.30 (14)[b]	4.92 ± 0.26 (12)*	13.5 ± 0.3 (14)	11.7 ± 0.74 (12)
No additions, 60 min	11.3 ± 0.6 (14)	9.27 ± 0.40 (10)	28.2 ± 0.9 (14)+	25.1 ± 0.4 (10)+
Lactate, 10 m*M*	12.7 ± 0.9 (12)	11.6 ± 1.71 (12)	12.1 ± 0.2 (12)	8.89 ± 0.44 (12)
Pyruvate, 10 m*M*	12.3 ± 0.9 (14)	14.3 ± 1.5 (11)	21.9 ± 0.6 (14)+	19.0 ± 0.6 (11)+
Lactate, 10 m*M* + pyruvate, 1 m*M*	12.3 ± 1.0 (14)	11.4 ± 1.31 (12)	12.1 ± 0.2 (14)	9.28 ± 0.42 (12)*+
Lactate, 10 m*M* + NH_4Cl, 5 m*M*	7.32 ± 0.9 (14)+	9.14 ± 0.80 (12)	19.3 ± 0.8 (14)+	10.6 ± 1.8 (12)*+
Lactate, 10 m*M* + lysine, 2 m*M*	14.2 ± 1.3 (14)	12.7 ± 1.8 (11)	14.0 ± 0.4 (14)	10.1 ± 0.6 (11)*+
Lactate, 10 m*M* + ethanol, 10 m*M*	26.2 ± 0.6 (13)+	25.0 ± 2.1 (11)+	4.30 ± 0.20 (14)+	2.46 ± 0.10 (11)+*
Alanine, 10 m*M*	6.61 ± 0.54 (10)+	4.89 ± 1.41 (11)+	16.3 ± 0.6 (12)+	20.2 ± 0.6 (9)+*
Glutamate, 10 m*M*	11.0 ± 0.3 (14)	11.6 ± 1.1 (12)	22.6 ± 0.3 (14)+	21.8 ± 0.9 (12)+
Glycerol, 10 m*M*	5.04 ± 1.25 (14)+	2.21 ± 0.17 (11)+*	18.7 ± 0.1 (14)+	16.4 ± 0.6 (11)+
Dihydroxyacetone, 10 m*M*	4.44 ± 0.16 (14)+	3.68 ± 0.66 (11)+	18.7 ± 0.2 (14)+	17.3 ± 1.0 (12)+

[a] Cells were incubated 60 min, 37°, in Krebs–Henseleit buffer containing 2.5% fraction V albumin with or without the indicated substrates in a total volume of 4 ml.
[b] Results are μmole ketone/hr/g cell wet weight, mean ± SEM. Number of observations in parentheses. Significant ($P < 0.05$) differences attributable to thyroxine treatment are indicated by *; significant ($P < 0.05$) differences attributable to substrate addition to or substitution for lactate are indicated by +.

Acetoacetate production was lower in hepatocytes from thyroxine-treated rats than in hepatocytes from control rats when pyruvate, NH_4Cl, lysine, or ethanol were added to the lactate-containing media and higher when alanine was the substrate used. Differences in acetoacetate production with the different additions to the incubation media were observed. All but pyruvate, alanine, and glutamate lowered the acetoacetate production by the hepatocytes. The medium having lactate as a substrate resulted in a reduction (from the basal) in acetoacetate production. Glycerol or dihydroxyacetone addition did not lower acetoacetate production to the same extent as did the addition of lactate to the incubation media.

The hepatocyte content of ATP at the beginning and end of the incubation period differed little. Thyroid treatment likewise did not affect hepatocyte ATP levels. Of the various substrates used, only glycerol affected the ATP content of these cells. Cells incubated with glycerol had less ATP (0.88 ± 0.03 vs 2.10 ± 0.11), and thyroid treatment resulted in a partial restoration in the ATP content of these hepatocytes incubated with glycerol (1.65 ± 0.09 vs 0.88 ± 0.03). The difference attributable to the thyroid treatment was probably due to the effect of this hormone on the α-glycerophosphate dehydrogenase in the mitochondria and the mitochondrial α-glycerophosphate shuttle (15, 16, 26, 40–42). While thyroid treatment affected the ATP content of the cells incubated with glycerol, it also resulted in an increase in glucose production by the hepatocytes from these rats. Since glucose production from glycerol uses ATP in the initial glycerokinase step, these observations suggest that the ADP thus produced, is, under the influence of the thyroid hormone, more rapidly translocated into the mitochondria and rephosphorylated via oxidative phosphorylation. Thyroid hormone has been shown to affect adenine nucleotide translocase (21) and probably explains why the above occurs. It would also appear that under the influence of the thyroid hormone, less "futile" cycling, i.e., fructose 6-phosphate to fructose 1,6-diphosphate to fructose 6-phosphate, occurs which would reduce the amount of ATP utilized in the production of glucose such that the cellular ATP content would increase. Soling and Kleineke (43) have proposed such an explanation for the observed species differences in the energy costs and regulation of gluconeogenesis.

Discussion. The results of the present work indicate that although thyroid hormone can stimulate glucose synthesis in BHE rats, it does so selectively rather than accelerating glucose production from all substrates uniformly. Reports in the literature indicate that thyroxine induces an increase in the activity of a number of gluconeogenic enzymes (44) as well as an increase in the conversion of alanine to glucose (11), in lactate utilization for glucose formation (10), and in the conversion of pyruvate to glucose (46). Contrary to Singh and Snyder's work (11) with Sprague–Dawley rats, thyroid hormone administered to the BHE rats inhibited alanine conversion to glucose. In addition, it inhibited the stimulatory effect of NH_4Cl on lactate conversion to glucose and the utilization of glutamate for glucose production while not affecting the stimulatory effect of lysine on lactate utilization. These differences from Wistar and Sprague–Dawley rats together with our previous report (27) of increased glucose synthesis from these same substrates above that observed in Wistar rats, suggests that injections of thyroid hormone served to "normalize" (rather than induce) the gluconeogenic process in the BHE hepatocytes. It also suggests that when thyroid-treated BHE rats are starved they will be more like the Wistar rats in their choice of body tissues catabolized for fuel. That is, instead of losing more body protein and less body fat than Wistar rats (46), the reverse will be true. These differences in gluconeogenesis from nitrogen-containing substrates lend further support to the notion that the control of both urea synthesis and glucose synthesis rests with the functional capacity of the mitochondrial membrane to exchange metabolites, ions and adenine nucleotides via the various shuttle and translocase systems. While no studies have been conducted on the hepatic ureogenic capacity of BHE rats, it has been shown that ammonia levels (in relation to urea levels) in frozen clamped tissue are higher in these rats than in Wistar rats (35). While no evidence has been obtained that anion transport is rate limiting for gluconeogenesis and ureogenesis, the intracellular calcium ion movement may

well serve to regulate both processes. Calcium ion activates mitochondrial fatty acid oxidation (47), stimulates gluconeogenesis (48–50), and in general acts as a second messenger for a variety of hormones including the thyroid hormone. Thyroid hormone enhances mitochondrial calcium uptake (22–24) and in so doing apparently increases the exchange of adenine nucleotides by the adenine nucleotide translocase (21). The first rate-limiting step of ureogenesis is the carbamyl phosphate synthetase step. In the mitochondria, this enzyme competes with the translocase for ATP and if the affinity of the translocase is high, the competition will not be as successful as if the affinity of the translocase is low. The calcium ion may well affect this affinity and hence may well determine the rate of urea synthesis. If, in the BHE rat, there was an impairment in the calcium ion movement either into or out of the mitochondrial compartment the coordination of gluconeogenesis and ureogenesis, described Meijer *et al.* (51), would not be as efficient. If this is true, then one might predict an increase in the rate of gluconeogenesis accompanied by an increase in the levels of ammonia in the cell. Detailed studies will be needed to support such an hypothesis.

Although thyroid hormone decreased gluconeogenesis from substrates containing nitrogen, it did not affect gluconeogenesis from lactate. In fact, thyroid hormone-treated rats had even greater rates of gluconeogenesis than their controls who, in turn, synthesized nearly 50% more glucose from lactate than Wistar rats. While thyroid hormone enhancement of gluconeogenesis from lactate is consistent with the literature on thyroid hormone action, the differences due to strain suggests that while part of the abnormality in the BHE rat which results in a maturity onset lipemia, glycemia, degenerative tissue changes, and a shortened lifespan may be attributable to some abnormality in endogenous thyroid hormone action in these rats and correctable by exogenous thyroid hormone administration, there still remains a part which has yet to be elucidated. That part seems to be intimately related to the interplay between the regulation of the redox state and the phosphorylation state of the cytosolic and mitochondrial compartments. This suggestion arises from the observations of raised redox and phosphorylation states in frozen clamped livers (35) and from the current work which showed elevations in glucose production when the lactate:pyruvate ratio was manipulated. Future work will need to focus on this aspect of intermediary metabolism in the BHE rat compared to the Wistar rat.

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