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# Rat Strain Differences in Gluconeogenesis by Isolated Hepatocytes[1] (41310)

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*Abstract.* An investigation of the glucose production by isolated hepatocytes from lipemic BHE and nonlipemic Wistar rats was conducted. Hepatocytes were isolated from 40-hr starved young rats. Glucose, β-hydroxybutyrate, and acetoacetate production and ATP content of the cells after 60 min incubation with a variety of substrates were determined. BHE hepatocytes produced more glucose and β-hydroxybutyrate than Wistar hepatocytes. No strain differences in ATP content were observed. It is suggested that, in part, the hyperglycemia observed in the mature BHE rat may have its origins in the increased hepatic gluconeogenic capacity of this strain.

In previous reports of the differences between BHE and Wistar rats, it was shown that the two strains differ in the regulation of their hepatic metabolism (1, 2). In particular, BHE rats synthesize more triglyceride and cholesterol than do Wistar rats (3) and have less active mitochondria in terms of shuttle activity and oxygen uptake (1, 2). Age augments these strain differences (4). As BHE rats age, lipemia, glycemia, and a variety of tissue degenerative changes develop (5–7). One aspect of metabolism heretofore uninvestigated in these two strains is the synthesis of glucose via the gluconeogenic pathway. This pathway can be stimulated by glucagon (8–12), epinephrine (8–11), and thyroxine (13, 14). Its activity is thought to be regulated by a number of factors including the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase (15–19), pyruvate kinase (10, 20, 21), the mitochondrial shuttles (22, 23), cortisol (24), phosphofructokinase and fructose 1,6-diphosphatase (8), free fatty acid level (25, 26), insulin level (11), as well as substrate and ATP levels (28). Previous work which showed a slower α-glycerophosphate and state 4 malate–aspartate shuttle, a raised phosphorylation state (2), and decreased glucagon responses to starvation (29, 30), suggested that gluconeogenic capacity may be impaired in BHE rats. However, an age-related increase in blood glucose levels (5, 6) and a decline in glucose tolerance (5) suggested just the opposite since diabetes has been reported to be characterized by an increase in gluconeogenic capacity (31). Thus, the present paper reports the results of work designed to determine hepatic gluconeogenic activity in BHE and Wistar rats. Using isolated hepatocytes from 40-hr starved rats, it was found that hepatocytes from BHE rats made more glucose from a variety of substrates than did hepatocytes from Wistar rats.

**Materials and Methods.** Male 50- to 60-day-old BHE and Wistar[2] rats were used. They were housed individually in hanging wire mesh cages in a temperature–humidity–light-controlled room. Water was always available. Prior to use, the rats were starved for 40 hr. Isolated hepatocytes were prepared according to the Krebs and Cornell modification of the Berry and Friend procedures (32). Viability of the cells was ascertained by methylene blue exclusion; preparations were considered acceptable if 90–95% of the cells excluded the dye. Cells (75 mg wet wt) were suspended in Krebs–Henseleit buffer containing 2.5% bovine fraction V serum albumin. The albumin had been dialyzed with buffer to remove the excess citrate.

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[2] Charles River, Wilmington, Mass.



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Cells were incubated without added substrates or with the addition of 10 m*M* lactate, 10 m*M* pyruvate, 10 m*M* lactate plus 1 m*M* pyruvate, 5 m*M* $NH_4Cl$, 1 m*M* oleate, 2 m*M* lysine, or 10 m*M* ethanol, 10 m*M* alanine, 10 m*M* glutamate, 10 m*M* glycerol or 10 m*M* dihydroxyacetone in a total volume of 4 ml. After 60 min incubation at 37°, the cells were killed by the addition of 0.2 ml 60% PCA. An aliquot of cells was also treated with PCA to provide initial values for glucose, ketones, and ATP. After KOH neutralization, the glucose (33), β-hydroxybutyrate (34), acetoacetate (34), and ATP (35) content of the cells were determined. Glucose and ketone production was calculated as micromoles product formed per hour per gram of wet weight of cells. ATP content was calculated as micromoles ATP per gram wet weight of cells. Glycogen content (36) and phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) (37) activity were determined in livers from 40-hr starved rats age-matched to those used for the preparation of the isolated hepatocytes. Significant strain differences were determined using a *t* test for groups of unequal number and variance (38).

**Results.** Glucose production was significantly greater in hepatocytes from BHE rats than in hepatocytes from Wistar rats with all substrates except glycerol (Table I). The initial glucose levels of the hepatocytes from both strains did not differ. This is consistent with our previous reports of hepatic glucose levels in freeze-clamped livers (2, 7). However, after 60 min of incubation either in the presence or absence of added substrates, hepatocytes from BHE rats had a greater gluconeogenic capacity than did the hepatocytes from the Wistar rats. The effects of the various additions on the glucose production varied within the strains. Lactate or pyruvate addition resulted in a fivefold increase in glucose production above that observed in hepatocytes incubated without added substrates. The results obtained with the Wistar hepatocytes are similar to those reported by Cornell *et al.* (39) for female Wistar rats while the results obtained from the BHE hepatocytes are higher than those reported by Cornell. When both lactate and pyruvate were added, a further stimulation in glucose production was observed in the hepatocytes from the BHE rats but not in the hepatocytes from the Wistar rats. This was also true for the additions of $NH_4Cl$ and lysine

TABLE I. GLUCOSE PRODUCTION BY ISOLATED HEPATOCYTES FROM 40-hr STARVED YOUNG MALE BHE AND WISTAR RATS

| Incubation media | Strain: BHE | Strain: Wistar |
|---|---|---|
| | μmole glucose/hr/g cells wet wt[a] | |
| No additions, 0 time | 3.08 ± 0.28 (12)[b] | 2.44 ± 0.40 (10)[d] |
| No additions, 60 min | 8.63 ± 0.58 (14)[d] | 5.49 ± 0.58 (10)[c] |
| Lactate, 10 m*M* | 41.0 ± 4.1 (12) | 26.1 ± 2.2 (10)[c] |
| Pyruvate, 10 m*M* | 38.8 ± 3.8 (14) | 24.9 ± 1.7 (10)[c] |
| Lactate, 10 m*M* + pyruvate, 1 m*M* | 57.5 ± 4.8 (14)[d] | 35.9 ± 3.5 (10)[c] |
| Lactate, 10 m*M* + $NH_4Cl$, 5 m*M* | 63.5 ± 6.3 (14)[d] | 32.0 ± 3.1 (10)[c] |
| Lactate, 10 m*M* + oleate, 1 m*M* | 14.3 ± 0.3 (13)[d] | 5.16 ± 0.9 (10)[c,d] |
| Lactate, 10 m*M* + lysine, 2 m*M* | 57.8 ± 5.9 (14)[d] | 33.1 ± 3.6 (10)[c] |
| Lactate, 10 m*M* + ethanol, 10 m*M* | 23.2 ± 3.3 (13)[d] | 10.9 ± 1.0 (10)[c,d] |
| Alanine, 10 m*M* | 24.3 ± 3.1 (10)[d] | 14.4 ± 0.9 (10)[c,d] |
| Glutamate, 10 m*M* | 19.3 ± 5.2 (14)[d] | 8.64 ± 0.68 (10)[c,d] |
| Glycerol, 10 m*M* | 28.8 ± 2.0 (14)[d] | 30.2 ± 1.1 (10) |
| Dihydroxyacetone, 10 m*M* | 121.5 ± 2.1 (14)[d] | 106.0 ± 5.7 (10)[c,d] |

[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] Mean ± SEM, number of observations in parentheses.
[c] Strain differences are significant ($P < 0.05$).
[d] Significantly different from cells incubated with the addition of lactate ($P < 0.05$).

to the lactate-containing media. The additive efffects of pyruvate, lysine, and $NH_4Cl$ are consistent with the earlier report of Cornell (39) on the effects of these compounds on glucose production. The inhibition of glucose production by ethanol is consistent with the report of Krebs *et al.* (40) who used a perfused liver system rather than isolated hepatocytes. Alanine in the current study was not as good a substrate for glucose production as was lactate. Glucose production from alanine was approximately 50% lower than when lactate was used as the substrate. Others (20) have reported that alanine stimulates gluconeogenesis, however, alanine was not used by itself but was added to a media containing lactate and pyruvate. Similarly, the use of glutamate or glycerol as substrates resulted in a decrease in glucose production. In the hepatocytes from the BHE rats, glycerol utilization for glucose production was significantly less than lactate utilization, whereas in the hepatocytes from the Wistar rats, lactate and glycerol appeared to be equivalent precursors of glucose. The failure of glutamate to significantly stimulate gluconeogenesis is probably due to the relative impermeability of the liver cell to this substrate (41). The addition of dihydroxyacetone to the incubation media resulted in a large increase in glucose production by hepatocytes from both strains.

The production of ketone bodies (Table II), β-hydroxybutyrate (βHB) and acetoacetate (AcAc), followed a pattern similar to that observed for glucose production. Without exception, hepatocytes from BHE rats produced more βHB than did hepatocytes from Wistar rats. Incubation of hepatocytes with lactate plus oleate resulted in the largest βHB production, whereas, incubation with lactate, pyruvate, lactate plus pyruvate or lysine, or with glutamate resulted in similar amounts of βHB being formed. The addition of $NH_4Cl$ to the lactate-containing media inhibited βHB production, whereas the addition of ethanol enhanced βHB production. Alanine, glutamate, and dihydroxyacetone additions had no effect on βHB production. The production of AcAc was inhibited by lactate, lactate plus pyruvate, lactate plus oleate, lactate plus lysine, lactate plus ethanol, alanine, glutamate, glycerol, and dihydroxyacetone in hepatocytes from both strains of rats. AcAc production was significantly lower in hepatocytes from BHE rats compared to hepatocytes from Wistar rats when either glutamate or dihydroxyacetone were added

TABLE II. KETONE FORMATION BY ISOLATED HEPATOCYTES FROM 40-HR STARVED MALE BHE AND WISTAR RATS

| | μmole/hr/g wet wt cells[a] | | | |
|---|---|---|---|---|
| | β-Hydroxybutyrate | | Acetoacetate | |
| Incubation media | BHE | Wistar | BHE | Wistar |
| No additions, 0 time | 6.61 ± 0.30 (14)[b,d] | 2.62 ± 0.14 (10)[c,d] | 13.5 ± 0.3 (14) | 14.2 ± 0.9 (10)[d] |
| No additions, 60 min | 11.3 ± 0.6 (14) | 6.58 ± 0.13 (10)[c] | 28.2 ± 0.9 (14)[d] | 34.0 ± 1.2 (10)[d] |
| Lactate, 10 m*M* | 12.7 ± 0.9 (12) | 7.23 ± 0.27 (10)[c] | 12.1 ± 0.2 (12) | 11.4 ± 0.7 (9) |
| Pyruvate, 10 m*M* | 12.3 ± 0.9 (14) | 9.32 ± 0.61 (10)[c,d] | 21.9 ± 0.6 (14)[d] | 22.5 ± 1.8 (10)[d] |
| Lactate, 10 m*M* + pyruvate, 1 m*M* | 12.3 ± 1.0 (14) | 7.09 ± 0.33 (10)[c] | 12.1 ± 0.2 (14) | 13.2 ± 0.9 (10) |
| Lactate, 10 m*M* + $NH_4Cl$, 5 m*M* | 7.32 ± 0.9 (14)[d] | 4.13 ± 0.31 (10)[c,d] | 19.3 ± 0.8 (14)[d] | 24.0 ± 2.0 (10)[d] |
| Lactate, 10 m*M* + oleate, 1 m*M* | 57.6 ± 2.1 (12)[d] | 33.8 ± 1.1 (9)[c,d] | 5.45 ± 0.2 (12)[d] | 4.13 ± 0.24 (10)[d] |
| Lactate, 10 m*M* + lysine, 2 m*M* | 14.2 ± 1.3 (14) | 7.99 ± 0.25 (10)[c] | 14.0 ± 0.4 (14) | 13.1 ± 0.9 (10) |
| Lactate, 10 m*M* + ethanol, 10 m*M* | 26.2 ± 0.6 (13)[d] | 12.9 ± 0.5 (10)[c,d] | 4.30 ± 0.2 (14)[d] | 2.81 ± 0.12 (10)[d] |
| Alanine, 10 m*M* | 6.61 ± 0.54 (10)[d] | 4.18 ± 0.22 (10)[c,d] | 16.3 ± 0.6 (12)[d] | 20.4 ± 0.8 (10)[d] |
| Glutamate, 10 m*M* | 11.0 ± 0.3 (14) | 6.31 ± 0.26 (10)[c] | 22.6 ± 0.3 (14)[d] | 30.9 ± 0.9 (10)[c,d] |
| Glycerol, 10 m*M* | 5.04 ± 1.25 (14)[d] | 2.36 ± 0.24 (10)[c,d] | 18.7 ± 0.1 (14)[d] | 20.4 ± 1.2 (10)[d] |
| Dihydroxyacetone, 10 m*M* | 4.44 ± 0.16 (14)[d] | 2.39 ± 0.19 (10)[c,d] | 18.7 ± 0.2 (14)[d] | 26.5 ± 1.3 (10)[c,d] |

[a] See Table I, footnote *a*.
[b] Mean ± SEM, number of observations in parentheses.
[c] Effect of strain is significant ($P < 0.05$).
[d] Significantly different from lactate ($P < 0.05$).

to the incubation media. In general, when βHB production was high, AcAc production was low and vice versa.

When the ATP contents of the isolated hepatocytes were determined, no strain differences were observed. The ATP content of these cells was similar to that reported by Krebs *et al.* (32) for isolated hepatocytes and to that reported earlier in freeze-clamped tissue (2). Of all the substrates used, only glycerol resulted in a significant fall (0.88 ± 0.03 and 0.81 ± 0.09 from 2.10 ± 0.11 and 2.12 ± 0.11 BHE and Wistar, respectively) in ATP content. Most likely, this was due to the need for ATP by the glycerol phosphorylation reaction catalyzed by glycerol kinase.

Last, there were no significant strain differences in either PEPCK activity or glycogen content (Table III). While the mean glycogen level of the BHE livers was twice that of the Wistar livers, the variability of the strain (glycogen levels ranged from 1.4 to 16.1 mg/g) ruled out statistical significance. Had our group size been larger, statistical significance would probably have been achieved.

**Discussion.** Compared to the numerous reports on the lipid metabolism of the BHE strain of rat ((5, 42), reviews), the production of glucose has received little attention. Aside from the measurements of glucose 6-phosphatase (38), no measurements of gluconeogenic enzymes have been reported despite the many suggestions that rats of this strain, as they age, become hyperglycemic. Diabetic animals generally have higher gluconeogenic rates than normal animals (15, 31, 44, 45). The BHE rats used in the current work were young rats (50–60 days of age) and were not hyperglycemic; yet, they had greater gluconeogenic capacities than their normal Wistar controls. The question which arises is why these hepatocytes are able to produce this "extra" glucose. Furthermore, what is the relevance of this increased gluconeogenic capacity to the development of hyperglycemia in middle age? With respect to the reasons for an increased gluconeogenic capacity, it may be the result of the animals' decreased ability to utilize stored carcass lipid for energy when starved. This would result in a compensatory increase in the utilization of carcass protein for fuel thus necessitating an increase in its ability to convert amino acids into glucose. Indirect evidence for this hypothesis was provided by an earlier report (46) which compared the carcass composition of BHE and Wistar rats before and after 24 hr without food. Whereas the Wistar rats lost carcass lipid with starvation, the BHE rats lost comparatively less carcass lipid and more carcass protein and water. Further work is needed in this area.

With respect to the relevance of the increased gluconeogenic capacity to the age-related development of hyperglycemia, the present data would suggest that this characteristic might preceed the typical hyperglycemia associated with the diabetic state. In animal models where diabetes is chemically or surgically induced, gluconeogenesis is stimulated and when insulin is given to control blood glucose levels, gluconeogenesis is inhibited. This suggests, then, that the gluconeogenic contribution to the diabetic hyperglycemic state may be more significant to this disorder than the resistance of cells to insulin-stimulated glucose uptake. In this situation there would be a continual supply of glucose to the circulation by the hepatocyte and not only would blood glucose rise because of the peripheral insulin

TABLE III. GLYCOGEN LEVEL AND PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) ACTIVITY IN 40-HR STARVED BHE AND WISTAR RATS

| Strain | Liver weight | Relative liver size[a] | Glycogen (mg/g liver) | PEPCK (units/100 g body wt) |
|---|---|---|---|---|
| Wistar | 6.7 ± 0.1[b] | 3.6 ± 0.1 | 2.9 ± 0.3 | 3.89 ± 0.26 |
| BHE | 5.8 ± 0.8 | 3.9 ± 0.3 | 7.1 ± 2.9 | 4.06 ± 0.27 |

[a] Relative liver size = liver weight/body weight × 100.
[b] Mean ± SEM, five rats.

resistance but also because of the resistance of the hepatocyte to the down regulation of its gluconeogenic activity by insulin. In turn, this elevation in blood glucose would stimulate insulin release by the pancreas such that an inappropriate serum insulin level might be present in the starved individual. Hyperinsulinism is a feature of the maturity onset diabetic and of the 50-day-old BHE rat (47).

Perhaps it is now appropriate to broach the subject of the control of gluconeogenesis in the BHE rat compared to the Wistar rat under the above conditions. *In vitro*, gluconeogenesis has been shown to be stimulated by glucagon. BHE rats, however, have been shown to be hypoglucagonemic when starved for 24 hr (29, 30). Perhaps the measurements of glucagon in these early studies were inadequate and maybe glucagon levels were actually higher than were measured. An accelerated gluconeogenic capacity is inconsistent with abnormally low glucagon levels if, *in vivo*, glucagon is responsible for an accelerated glucose production in the 40-hr starved rat. The fact that there were no strain differences in PEPCK activity (Table III) was surprising since one would anticipate raised enzyme activity consistent with the *in vitro* measurements of an increased glucose production. However, the enzyme activity is measured *in vitro* under conditions which may not exist *in vivo*. Hence, the discordance reported may be understandable. However, one must now ask the question of whether other rate controlling factors in the gluconeogenic sequence are operative in these BHE rats. It has been suggested that the activity of the malate–aspartate shuttle controls gluconeogenesis (22, 23, 39) and when this shuttle is less active, glucose production is decreased. BHE rats have been shown to have a less active shuttle activity when ADP is limited but not when ADP is supplied (2). Since the ATP content of the cells was not different between the strains, one must assume that under the circumstances of this study (i.e., starvation for 40 hr), ADP was not limiting within the hepatocytes from the BHE rats. That the shuttle was probably quite active can be seen from the production of glucose when either $NH_4Cl$ or lysine was added to the lactate-containing media. These compounds interact with $\alpha$-ketoglutarate to form, intracellularly, glutamate, an important rate-limiting component of the malate–aspartate shuttle. If glutamate becomes limited, the shuttle activity becomes less active and gluconeogenesis slows down. In any event, starvation (which lowers phosphorylation state) increases the gluconeogenic capacity and this is probably due to both an increase in ADP and glutamate levels which in turn increase malate–aspartate shuttle activity. Whether one can further increase the gluconeogenic capacity in the BHE rats through a stimulation of the malate–aspartate shuttle or whether the gluconeogenic pathway is already operating at its maximum remains to be determined. Likewise, whether the suppression of gluconeogenesis can be achieved and whether the long-term effects of such inhibition would include a normalization of glucose homeostasis remains to be investigated.

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