Effects of type of dietary fat and protein on gluconeogenesis in isolated hepatocytes from BHE/cdb rats

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The effect of feeding a whole-egg diet or one matched in protein and fat content on gluconeogenesis by isolated hepatocytes was studied. BHE/cdb male weanling rats were fed one of five diets, which differed in source of protein and/or fat: casein/lactalbumin with corn oil, casein/lactalbumin with menhaden oil + corn oil, egg white with corn oil, egg white with menhaden oil + corn oil, or whole egg. At 74 ± 14 days of age, the rats were killed and isolated hepatocytes were prepared. The cells were incubated with 10 mM lactate, 10 mM pyruvate, 10 mM ethanol and 10 mM lactate, 10 mM lactate and 5 mM NH_4Cl, or 10 mM glutamate. The hepatocytes from the rats fed menhaden oil differed little in glucose production from the hepatocytes isolated from rats fed corn oil, regardless of protein source. Glucose production from lactate, pyruvate, lactate + ethanol, and lactate + ammonium chloride was significantly lower in hepatocytes from rats fed whole egg, compared with cells from rats fed the other diets. These data suggest that certain components of whole egg have effects on the control of gluconeogenesis. (J. Nutr. Biochem. 5:227–231, 1994.)

Keywords: gluconeogenesis; BHE/cdb rats; dietary fat; dietary protein

Introduction

We have reported that isolated hepatocytes from BHE/cdb rats, an animal model useful for the study of non-insulin-dependent diabetes mellitus (NIDDM), synthesize significantly more glucose from an assortment of substrates than do cells from Wistar rats.[1] Because an alteration in the regulation of gluconeogenesis is a complicating characteristic of NIDDM, this phenomenon has been extensively investigated in other animal models[2–4] as well as in human subjects.[5,6] Previously, we have shown that the composition of the diet can influence gluconeogenesis by hepatocytes isolated from 48-hour starved BHE/cdb rats.[7–10] Rats fed a 65% sucrose diet synthesized more glucose from a variety of substrates than rats fed a 65% starch diet.[7,8] Rats fed a 64% sucrose-6% coconut oil diet synthesized more glucose than rats fed a 64% sucrose-6% corn oil diet.[9,10] Studies of whole body glucose turnover likewise revealed that nonstarved rats fed a 6% coconut oil diet converted more alanine into glucose than rats fed a 6% corn oil diet.[11] Dietary fat also influenced Cori cycle activity: rats fed coconut oil had a greater rate of glucose recycling than rats fed corn oil. Lastly, these diet differences in glucose homeostasis were reversed when the fat source was menhaden oil. Rats fed menhaden oil converted less alanine to glucose and recycled less glucose than rats fed corn oil.[12] In all of these diets, purified ingredients were used.

Recently, in the course of studying longevity as affected by feeding dehydrated whole egg as the protein and fat source in a purified diet, we noticed that normally glucose-intolerant BHE/cdb rats were not as intolerant as expected.[13] Because glucose intolerance is generally characterized by an impaired glucose uptake as well as by an increased rate of gluconeogenesis,[4–6] we wondered whether BHE/cdb rats had a decreased gluconeogenic activity when fed this diet. Hence, the present paper reports the results of our studies of glucose synthesis by hepatocytes isolated from BHE/cdb rats fed this whole-egg diet or diets matched in protein and fat content where the protein was either dehydrated egg white or a 1:1 casein:lactalbumin, and the fat was either corn oil or 1% corn oil plus 17.5% menhaden oil. We found that feeding whole egg resulted in a decline in glucose synthesis.

Supported by Georgia Agricultural Experiment Station project #911 and the S.E. Egg Board.
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Received July 6, 1993; accepted December 8, 1993.

Methods and materials

Animals, diets, and experimental design

Weanling male BHE/cdb rats (University of Georgia Colony) were randomly assigned to one of five diet groups *(Table 1)*. The diets were analyzed for their fatty acid content using gas chromatography. The fatty acid content is shown in *Table 2*. Rats were housed in individual cages under controlled conditions of temperature (20 ± 1° C), light (0600 to 01800), and humidity (45 to 50%). Rats were fed ad libitum, and water was always available. Body weights and food intakes were determined weekly. At 74 ± 14 days of age, the animals were starved 46 ± 2 hours before use. The rats were cared for in accordance with the standards for humane care set forth by United States Department of Agriculture, NAS, and the Association of Laboratory Animal Caretakers. When used for the preparation of cells, the animals were weighed and

Table 1 Diet ingredients (g/kg diet)

Diets:	C-A	M-A	M-C	C-C	W-E
Ingredients					
Lactalbumin	—	—	115	115	—
Casein	—	—	117	117	—
Dehydrated whole egg	—	—	—	—	450
Dehydrated egg white	265	265	—	—	—
Corn oil*	185	10	10	185	—
Menhaden oil†	—	175	175	—	—
AIN vitamin mix	15	15	15	15	15
AIN mineral mix	49	49	49	49	49
Cellulose	32	32	32	32	32
Sucrose‡	450	450	483	483	450
Tocopherol	4	4	4	4	4

Abbreviations used: C-A, corn oil-dehydrated egg white; M-A, menhaden oil-dehydrated egg white; M-C, menhaden oil-casein:lactalbumin; C-C, corn oil-casein:lactalbumin; W-E, whole egg.
*Gift from Best Foods Inc., Union, NJ USA.
†Gift from Zapata Haynie, Reedville, VA USA.
‡Gift from Savanah Foods, Savanah, GA USA.

Table 2 Diet analysis

	C-A	M-A	M-C	C-C	W-E
Protein, %*	22	17.5	20	20	20.5
Lipid, %†	17	17.5	17.5	16	19
Fatty acids, mole%‡					
>14:0	—	0.02	0.14	—	—
14:0	0.20	11.14	10.62	0.19	0.43
15:0	0.10	0.69	1.06	—	0.13
16:0	11.17	22.23	23.66	10.61	27.94
16:1	0.53	16.21	18.08	0.19	4.17
18:0	2.22	5.56	5.80	1.96	9.09
18:1	29.54	16.15	15.13	30.00	41.74
18:2	55.06	7.21	6.23	55.75	13.84
18:3	1.15	2.39	2.28	1.09	0.47
20:0	—	0.40	0.37	—	0.04
20:1	—	—	—	—	—
20:2	0.02	—	—	0.02	0.09
20:3	—	—	—	—	1.84
20:5	—	1.00	0.90	0.03	—
21:0	—	2.77	2.43	—	—
22:0	0.02	0.18	0.11	0.03	0.20
22:1	0.04	0.92	0.66	0.03	1.58
22:4	—	12.73	11.94	—	0.05
22:5	—	0.37	0.69	—	0.01
22:6	—	—	—	—	0.17
24:0	0.03	0.03	0.02	0.07	0.01

Abbreviations used: C-A, corn oil-dehydrated egg white; M-A, menhaden oil-dehydrated egg white; M-C, menhaden oil-casein:lactalbumin; C-C, corn oil-casein:lactalbumin; W-E, whole egg.
*% protein determined by Kjeldahl.
†%lipid determined after lipid extraction.
‡Gas chromatography, courtesy of Dr. Ronald Etheridge, University of Georgia, Athens, GA USA.

anesthetized with a solution of sodium pentobarbital (0.1 cc/100 g body weight).

Preparation of cells

Isolated hepatocytes were prepared by the enzymatic perfusion methods of Cornell and Krebs[14,15] and Berry and Friend[16] as modified by Seglan[17] and Failla and Cousins.[18] The perfusion media was a calcium-free Krebs-Ringer-bicarbonate-HEPES (KRB-HEPES) solution (pH 7.4), containing 2.5% bovine serum albumin fraction V. After the perfusion with collagenase, cells were preincubated and gently shaken for 30 minutes in a siliconized Erlenmeyer flask at 37° C, while simultaneously being gassed with 95% O_2/5% CO_2. Viability was checked by trypan blue exclusion. Cells from these preparations consistently exceeded 90% viability.

Incubation

Cells, suspended in Krebs-Henseleit buffer, were incubated in 25 mL Erlenmeyer flasks. The cells were incubated without the added substrates or with the addition of 10 mM lactate, 10 mM pyruvate, 10 mM ethanol + 10 mM lactate, or 5 mM NH_4Cl + 10 mM lactate. As an additional test of cell viability, cells were incubated with 10 mM glutamate. The amount of glucose produced in these incubation samples was not significantly different from that of cells incubated without added substrates. This is to be expected in a quality preparation, as viable cells do not readily synthesize glucose from glutamate because glutamate does not traverse the intact plasma membrane. After 60 min of incubation at 37° C, cells were killed with the addition of 0.2 mL of 60% perchloric acid. Before incubation, a sample of the hepatocytes was treated with 60% perchloric acid to provide values for the initial concentration of glucose. After precipitation of protein with perchloric acid, the cells were neutralized with 1 M K_2CO_3.

Determination of glucose

The glucose content of the cells was determined by glucose oxidase (Sigma kit 510, Sigma Chemical Co., St. Louis, MO USA). Glucose production rates are reported as micromoles per gram wet weight per hour using the 3.7 conversion factor of Cornell[14] and Krebs.[15]

Statistics

A one-way analysis of variance (ANOVA), followed by an unpaired *t* test, was used to determine significantly different means (SAS, SAS Institute, Cary, NC USA). An unpaired *t* test was also employed to establish differences within groups between rates of glucose production from lactate and other substrates.

Results

Although the rats did not differ in their food consumption, their weight gain differed. Those rats fed the control diet (corn oil-casein:lactalbumin) gained weight faster than those fed the menhaden oil-egg albumin *(Figure 1)*. Rats fed the other diets were between these two. Prior to being starved, the final weights of the rats were 325 ± 49 g, 287 ± 39 g, 260 ± 20 g, 275 ± 25 g, and 307 ± 36 g for the control diet, menhaden oil-casein diet, menhaden oil egg white diet, corn oil-egg white diet, and the whole egg diet, respectively. The rats lost an average of 22 g during the starvation period.

As shown in *Table 3,* glucose concentrations in the initial samples (0 time) or samples void of substrate (60 minutes), did not differ between diet groups. As has been previously

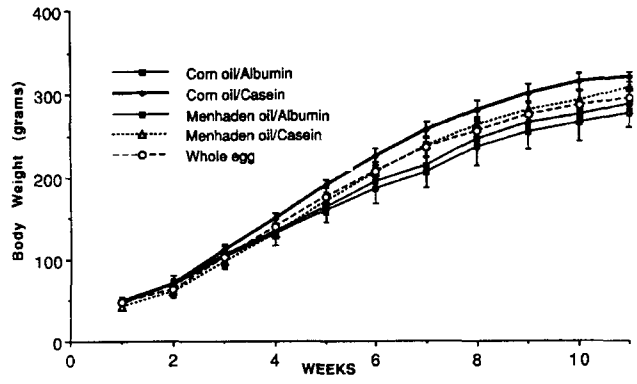

Figure 1 Growth curve. Each point on figure represents mean ± SEM, n = 5. Weekly body weight data were analyzed by ANOVA, and no significant differences were found at $P < 0.05$.

reported,[1,7-10] the addition of lactate resulted in an increase in glucose synthesis. However, cells from rats fed the whole-egg diet produced significantly less glucose from lactate, pyruvate, lactate + ethanol, and lactate + ammonia, when compared with cells from rats fed menhaden oil or corn oil. No significant differences in glucose production by hepatocytes from any substrate were found between corn oil- and menhaden oil-fed rats.

Intergroup comparisons between the synthesis of glucose from different substrates to that of lactate were made. These comparisons revealed that, in cells from each diet group, glucose synthesis from lactate + ethanol was significantly lower than that of lactate. As expected, glucose concentrations at time 0 and after 60 minutes of incubation were significantly lower than concentrations in lactate-incubated samples for each diet group. In hepatocytes from each of the diet groups, differences in the production of glucose from lactate plus ammonium chloride or pyruvate and lactate were not statistically significant.

Discussion

The purpose of the present work was to test the hypothesis that the improved glucose tolerance of BHE/cdb rats fed a whole-egg diet[13] was due to a diet effect on hepatic gluconeogenesis. Glucose synthesis is greater in BHE/cdb rats than in normal rats, and diet can affect this process.[1,7-10] Our hypothesis was correct in part. We found that when pyruvate or lactate plus ethanol or lactate plus NH_4Cl was provided as substrate, glucose production was considerably less in cells from the whole egg–fed group than in cells from the rats fed the other diets. Glucose production was also lower in these cells when only lactate was provided, but the variability of the results was such that ANOVA could not identify the differences in these values as being statistically significant. Unpaired *t* tests do show that when cells from rats fed the whole egg diet were compared with cells from rats fed either the corn oil-casein diet or the menhaden oil-casein diet, glucose production from lactate was significantly different. The question that arises is why diet should have these effects on cells harvested from rats that had been without food for 48 hours. The diet effect obviously could not have

Table 3 Effects of diet on glucose production by isolated hepatocytes

Incubation medium	corn oil control	corn oil albumin	menhaden oil control	menhaden oil albumin	whole egg	ANOVA
0 time	$28.9 \pm 5.4^{a,*}$	$19.3 \pm 5.0^{a,*}$	$20.8 \pm 3.6^{a,*}$	$21.6 \pm 5.2^{a,*}$	$16.3 \pm 3.1^{a,*}$	N.S.
60 minutes	$43.4 \pm 10.1^{a,*}$	$32.7 \pm 3.9^{a,*}$	$26.8 \pm 2.3^{a,*}$	$23.3 \pm 6.3^{a,*}$	$19.7 \pm 4.7^{a,*}$	N.S.
10 mM Lactate	$95.3 \pm 10.8^{a,b}$	82.4 ± 5.0^{a}	103.9 ± 19.9^{b}	82.9 ± 9.7^{a}	59.8 ± 6.6^{c}	0.05
10 mM Pyruvate	73.9 ± 9.0^{a}	84.8 ± 10.8^{a}	$63.7 \pm 10.0^{a,b}$	65.9 ± 6.2^{a}	44.1 ± 4.1^{b}	0.05
10 mM Lactate +10 mM ethanol	$52.3 \pm 10.7^{a,*}$	$44.9 \pm 3.2^{a,*}$	$72.6 \pm 12.9^{a,*}$	$54.2 \pm 3.9^{a,*}$	$26.34 \pm 1.9^{b,*}$	0.05
10 mM Lactate +5 mM ammonium chloride	87.9 ± 9.3^{a}	96.9 ± 10.9^{a}	79.4 ± 12.6^{a}	73.0 ± 9.7^{a}	53.5 ± 4.35^{b}	0.05

Data are expressed as micromoles of glucose per hour per gram of cells, wet weight.
Mean ± SEM (n = 6). Means in the same row having different letter superscript[a,b] are significantly different at the *P* value indicated.
Values for 0 time samples are micromoles of glucose per gram of cells, wet weight.
*Significantly differing from lactate ($P < 0.05$).

been a short-term one. One might argue that the difference in glucose production might have been due to diet-induced differences in hepatic glycogen stores. Indeed, the zero- and 60-minute basal values were much higher than previously reported[1,7-10] for BHE/cdb rats. Previously we found that BHE/cdb rats had approximately 2 μmoles glycogen/g tissue. This is equivalent to between 20 and 36 μmoles of glucose. At zero time ~3 μmoles glucose/g cells and at 60 minutes ~9 μmoles glucose/g cells were found. These values were 40 to 50% higher than those found in cells from a control strain. These values were obtained from cells incubated without added substrates and thus represent glycogenolysis. In the present work, diet affected the difference between the 0 and 60 min glucose values in these cells. The cells from the control animals produced 14.5 μmoles of glucose, those from the corn oil-albumin–fed rats produced 13.4 μmoles of glucose, and 6, 1.7, 3.4 μmoles of glucose were produced by the cells from the menhaden oil groups and the whole-egg group, respectively. This does not represent a lot of glycogenolysis, but nonetheless should be acknowledged. One mole of glycogen is assumed to release between 10 to 18 moles of glucose. One can figure back (given the difference between the zero- and 60-minute figure) the probable amount of glycogen involved and when one does so, it is very small indeed. That glycogen was available after 48 hours of starvation was surprising. However, in other work we reported measurable amounts of glycogen in starved rats of this strain.[1] Despite these diet effects on glycogen, when one examines glucose production from the added substrates, glucose production was affected. This suggests that some control aspect of the process was affected by components of the whole-egg diet. The diets differed in their lipid form. In all but the whole egg diet, pure oils were used.

It has been demonstrated that the composition of the dietary fat can produce specific modifications in the fatty acid profile of the plasma,[19,20] mitochondrial,[21,22] and nuclear membranes.[23,24] Further, it has been shown that the diabetic state is associated with a reduced activity of insulin-sensitive hepatic Δ^6- and Δ^9- desaturases and subsequent alterations in membrane composition.[25] As a compounded consequence of these dietary and hormonal influences, the physical and chemical properties of membrane phospholipids could be affected. This would result in an alteration in the processes that are reliant on membrane function.[10,20,26] The control of gluconeogenesis rests in part on the activity of its enzymes (and their allosteric regulators) and, in part, on the functionality of the mitochondrial membranes. Embedded in these membranes are components of the malate-aspartate shuttle, the acyl carnitine transport system, and the adenine nucleotide synthesis and transport system. Each of these systems have control properties vis à vis gluconeogenesis. For example, gluconeogenesis is increased when the malate aspartate shuttle is more active.[27] Likewise, gluconeogenesis is increased when respiration is less tightly coupled to ATP synthesis.[28] Inhibition of gluconeogenesis was found when palmitoylcarnitine oxidation was inhibited,[28] when pyruvate carboxylase was inhibited,[29] or when pyruvate kinase was activated.[28] Most of these processes can be affected by the composition of the lipid milieu (the mitochondrial membrane) in which they exist. Could the whole-egg diet have affected the composition of these membranes such that the activity of gluconeogenesis was affected? It is possible, but very difficult to explain. All the diets contained the same amount of fat and protein. We analyzed the fatty acid composition of the diet *(Table 2)* and found only minor differences. We also analyzed the dietary mineral content and found that the whole-egg diet contained twice as much phosphorous as the other diets, but that all other mineral components were equivalent. This phosphorous likely was contained by the phospholipids that comprise a large portion of the lipid in whole egg. It does not seem likely that the egg phospholipids would be the dietary component that down regulated gluconeogenesis. There may be other complex lipids or unique actions of protein-lipid combinations that can affect glucose synthesis in rats fed whole egg. We don't know what these might be. Detailed studies of the components of egg will be needed to discover why gluconeogenesis was decreased in BHE/cdb rats fed this whole-egg diet.

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