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Pyruvate metabolism in BHE/cdb rats is altered by the type of dietary fat

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The effect of feeding corn oil (CO), hydrogenated coconut oil (HCO), or menhaden oil (MO) on hepatic pyruvate metabolism of diabetes-prone BHE/cdb rats was studied. Pyruvate-related metabolites in frozen clamped livers as well as pyruvate related enzyme activities were determined after 7 weeks of feeding. Small differences in feed efficiency were noted, as were small differences in metabolites and several key reactions. The difference in metabolism was greatest between the MO rats and the other two groups. The CO and HCO rats were similar in many respects. Lactate levels were increased in CO and HCO rats compared to MO rats. Membrane-bound lactate dehydrogenase (LDH) activities were different in the three groups of rats. Rats fed MO had lower lactate levels, but their LDH activity was greatly increased. Pyruvate levels were similar in all groups, but pyruvate carboxylase and pyruvate kinase were less active in the MO-fed rats than in the other diet groups. These findings suggest that a diet fat gene interaction has occurred that accommodates the mutation reported previously in the mitochondrial genome for F_0ATPase such that the type of diet fat can potentiate or suppress the genetically determined metabolic features that characterize the BHE/cdb rat. (J. Nutr. Biochem. 8:275–278, 1997)

Keywords: pyruvate metabolism; BHE/cdb rats; menhaden oil; corn oil; hydrogenated coconut oil

Introduction

Previously, we have reported that the type of dietary fat can have large effects on a variety of metabolic features in the BHE/cdb rat. Menhaden oil (MO) compared to beef tallow or corn oil (CO) shortens the life spans of these rats while delaying the onset of impaired glucose tolerance.[1] This dietary treatment also improves insulin binding to the hepatic and adipose cell membrane and increases intracellular glucose oxidation and use.[2,3] In contrast, feeding hydrogenated coconut oil (HCO) impairs glucose use,[4] while also having marked effects on oxidative phosphorylation.[5,6] Mitochondrial respiration supported by pyruvate is poorly coupled to ATP synthesis[5,6] and gluconeogenesis is markedly increased[7,8] when BHE/cdb rats are fed this fat compared to corn oil. Studies of whole body glucose turnover[4] in nonfasted rats also show a similar diet fat effect on glucose synthesis. Feeding menhaden oil reverses these metabolic differences; however, long-term menhaden oil feeding does not optimize life span.[1]

The BHE/cdb rat has a point mutation in the mitochondrial genome in the region of subunits 6,8 of the oligomycin sensitive portion of the F_1F_0ATPase.[10] This mutation explains the inefficiency reported previously in the coupling of respiration to ATP synthesis.[11] When diet fat is manipulated as described above, the diet effect either potentiates this genetic feature or rescues it.[5,6,9] We hypothesized that this potentiation or rescue could be observed at the level of pyruvate. Hence, the present paper reports the results of several feeding trials designed to assess the use or production of pyruvate. To this end we measured levels of pyruvate related metabolites in frozen-clamped liver and the activities of enzymes which either use or produce pyruvate.

Methods and materials

Three groups of male weanling rats were fed one of three diets from weaning until used 5 to 7 weeks later. The diets consisted of sucrose (64%) 1:1 lactalbumin: casein (20%), fiber (alphacel) (4%), AIN mineral mix (5%), AIN vitamin mix (1%), and either corn oil (CO) (Mazola corn oil, gift of Best Foods, Union, NJ USA), hydrogenated coconut oil (HCO), or menhaden oil (MO) (6%) (gift of Zapata Haynie, Reedsville, VA USA). Except as

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noted, these ingredients were purchased from Nutritional Biochemical (Cleveland, OH USA). Food intake was calculated on a daily basis corrected for the change in body weight because of growth. Thus, the values are reported as grams food consumed per day per 100g body weight. Body weight and food intakes were determined each week. Food and water were always available. Special care was taken to ensure that these fats did not autooxidize. Fresh diet was provided daily just before the dark phase of the lighting cycle, the fats were kept frozen and under a layer of nitrogen, and the menhaden oil was mixed into the dry ingredients just before the diet was provided to the animals. Periodic assessment of the peroxides in the diet assured us that our precautions minimized diet peroxidation.

The animals were housed individually in hanging wire mesh cages in a room controlled for temperature (21° ± 1°C), humidity (40 to 50%), and light (lights on, 0600 to 1800). Animal care followed the recommendations of the American Association for Laboratory Animal Care as outlined in the National Institutes of Health (NIH) Publication 88-23, 1985, *NIH Guide for the Care and Use of Laboratory Animals.*

Freeze clamp studies

Nonstarved animals were killed by a double guillotine which allowed for the rapid excision of the liver. The liver was then clamped between two copper plates that had been precooled in liquid nitrogen. The clamped tissue was finely ground in liquid nitrogen and stored at −80°C until used for the metabolite assays. The mean time between killing and clamping was 4.5 seconds. Rapid freeze clamping is essential to the preservation of phosphorylated intermediates as pointed out by Faupel et al.[12] Perchloric extracts were prepared, and the various glycolytic and citric acid cycle intermediates were determined using the procedures outlined by Lowry and Passonneau.[13] Oxaloacetate levels in the mitochondria and in the cytosol were calculated[14] as were redox and phosphorylation states using the equations of Veech and Krebs[15] and those of Williamson.[14]

Enzyme studies

Nonstarved rats were killed by decapitation and the liver quickly excised, chilled, and weighed. The activities of the various pyruvate-related enzymes in this liver were determined using established methods. Pyruvate carboxylase was determined using the methods of Berndt et al.[16] as outlined in Bergmeyer.[17] Pyruvate kinase was assessed by the methods of Sillero et al.[18] Phosphoenolpyruvate carboxykinase (PEPCK) was determined using the methods of Opie and Newsholme.[19] Lactate dehydrogenase was determined using the methods of Kline et al.[20] The activity of this enzyme was determined in the cell supernatant and in the mitochondrial fraction. Alanine amino transferase activity was assessed using a Sigma kit (No. 59, Sigma Chemical, St. Louis, MO USA). Malic enzyme determination followed the procedures of Freedland.[21] Total pyruvate dehydrogenase activity was measured using the methods of Wieland et al.[22]

Statistical analysis

The means of each of the diet groups for each of the measurements were compared using a one-way analysis of variance (ANOVA). This analysis was followed by a Bartlett's test to identify significantly different means (Statistical Analysis System, SAS Institute, Cary, NC USA).

Table 1 Initial weight, final weight, and food intake of BHE/cdb rats fed a 6% CO, HCO, or MO diet

	CO	HCO	MO
Initial wt (g)[1]	59 ± 4[2]	56 ± 4	56 ± 4
Final wt (g)[1]	279 ± 9[a]	246 ± 8[b]	271 ± 8[a]
Liver wt (g)[3]	12.8 ± 0.8	11.5 ± 0.7	13.1 ± 0.7
Daily food intake (g/100 g b.w.)[1]	9.4 ± 0.3[a]	10.1 ± 0.2[b]	8.8 ± 0.2[c]

[1]n = 20/group.
[2]Mean ± SEM; means having unlike letter superscripts ([a, b, c]) are significantly different ($P < 0.05$) as determined by a one-way ANOVA followed by a Bartlett's test.
[3]n = 10/group.
Abbreviations: CO, corn oil; HCO, hydrogenated coconut oil; MO, menhaden oil.

Results

The rats were used after 7 weeks (70 days of age). Shown in *Table 1* are the initial and final body weights, food intakes, and liver weights of rats fed the different diets. The rats fed the HCO diet gained less weight, but ate more food than rats fed the other diets. This means that food efficiency was affected. This has been reported previously by many investigators,[23–27] as well as ourselves[4–9] and is a characteristic of the rat response to a diet deficient in essential fatty acids. Because the duration of the study was short, the tissues were probably not devoid of essential fatty acids.[5] Note that the liver weights are from half of the animals. The freeze clamping procedure does not permit one to weigh the liver at harvest. The metabolites measured in these frozen liver powders are shown in *Table 2*. A one way ANOVA followed by a Bartlett's test identified few significantly

Table 2 Pyruvate related metabolites in frozen clamped hepatic tissue from BHE/cdb rats fed a 6% corn oil (CO), hydrogenated coconut oil (HCO), or menhaden oil (MO) diet

	CO[1]	HCO	MO
		n moles/g	
Lactate	2031 ± 238[2a]	2555 ± 396[a]	1543 ± 138[b]
Malate	833 ± 75[a]	816 ± 82[a]	671 ± 33[b]
Alanine	1697 ± 200	1514 ± 199	1682 ± 236
Glutamate	2543 ± 106	2368 ± 179	2446 ± 160
Pyruvate	58 ± 10	65 ± 15	58 ± 6
PEP	20 ± 4[a]	32 ± 14[ab]	37 ± 4[b]
Oxalacetate (calc.)	6 ± 1	6 ± 2	8 ± 1
ATP	2198 ± 89[ab]	1847 ± 123[a]	2339 ± 135[b]
ADP	1459 ± 88	1443 ± 67	1416 ± 72
AMP	490 ± 46	523 ± 34	513 ± 48
Pi	6633 ± 263[a]	6822 ± 321[a]	7485 ± 295[b]
ATP:ADP.HPO_4	405 ± 42[a]	344 ± 41[b]	331 ± 30[b]
NAD^+:$NADH^+_{LDH}$	270 ± 52[a]	267 ± 81[a]	397 ± 68[b]

[1]Abbreviations used: CO, corn oil; HCO, hydrogenated coconut oil; MO, menhaden oil; PEP, phosphoenolpyruvate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; Pi, inorganic phosphate.
[2]Mean ± SEM, n = 10. Means having unlike letter superscripts ([a, b, c]) are significantly different ($P < 0.05$) as determined by a one-way ANOVA.

Table 3 Pyruvate related enzyme activity in hepatic tissue from BHE/cdb rats fed a 6% corn oil (CO), hydrogenated coconut oil (HCO), or menhaden oil (MO) diet

Enzyme	CO	HCO	MO
		Activity (units[1]/g tissue)	
Pyruvate kinase	90 ± 6	81 ± 21	47 ± 8
Pyruvate carboxylase	40 ± 3[a]	42.7 ± 2.8[a]	19.8 ± 6.5[b]
Malic enzyme	10.4 ± 0.9[a]	51 ± 6[b]	9.8 ± 0.7[a]
Alanine aminotransferase	2.4 ± 0.4	2.7 ± 0.3	1.6 ± 0.4
Phosphoenolpyruvate carboxykinase	5.2 ± 0.5	7.0 ± 1.3	5.5 ± 0.7
Lactate dehydrogenase (outer membrane)	0.555 ± 0.059[a]	0.284 ± 0.071[b]	1.794 ± 0.59[c]
Lactate dehydrogenase (cytosol)	0.898 ± 0.141	0.839 ± 0.196	0.646 ± 0.200
Pyruvate dehydrogenase	339 ± 95	359 ± 85	No Value

[1]Unit is defined as the amount of enzyme needed to catalyze one μ mole of substrate to product per minute.
[2]Mean ± SEM, n = 10. Means having unlike letter superscripts ([a, b, c]) are significantly different ($P < 0.05$) as determined by one-way ANOVA.

different means; however, by using a t test for two group comparisons, some differences were noted. Lactate levels were lowest in the MO-fed rats and highest in the HCO-fed rats. Malate levels in MO-fed rats were lower than those in CO- and HCO-fed rats. Phosphoenolpyruvate was lowest in the CO-fed rats, intermediate in the HCO-fed rats, and highest in the MO-fed rats. The difference between the ATP level of the HCO group and the MO group was significantly different. ATP levels were least in the HCO-fed rats, whereas those in the CO and MO rats were not different. ADP and AMP levels were not influenced by diet. Inorganic phosphate (Pi) was highest in the MO-fed rats and not different in the CO- and HCO-fed rats.

The activities of the various enzymes using or producing pyruvate are shown in *Table 3*. Because of a shortage of tissue, pyruvate dehydrogenase was not determined in the MO rats. The activity of this enzyme was similar in the CO and HCO rats. Diet significantly affected pyruvate carboxylase, malic enzyme, and membrane-bound lactate dehydrogenase. The activity of pyruvate carboxylase was lowest in MO rats. In contrast, MO rats and CO rats had similar malic enzyme activities, which were significantly exceeded by the activity of the enzyme in the HCO rats. The activity of the cytoplasmic lactate dehydrogenase was unaffected by diet, whereas that of the outer mitochondrial membrane was least in the HCO rats, greatest in the MO rats, and intermediate in the CO rats. Lastly, phosphoenolpyruvate carboxykinase and alanine amino transferase activity were not different among the diet groups.

Discussion

The present work was designed to elucidate further the ways in which the source of dietary fat could affect pyruvate metabolism and thus explain the responses of BHE/cdb rats to these diets with respect to glucose homeostasis. These rats mimic humans with NIDDM and it is known that diet choice in humans can affect the age of onset and severity of NIDDM. Thus, we wanted to examine one component (the fat component) of the diet very carefully. Our earlier work suggested that pyruvate metabolism is quite sensitive to dietary fat type. Outlined in *Figure 1* are the pathways pyruvate can take. Some of the pathways require ATP, whereas others are influenced by the phosphorylation state or the redox state. Examination of *Table 2* reveals that ATP levels are slightly less in the HCO-fed rats. This relates to our earlier report of a partial uncoupling of oxidative phosphorylation in rats fed this diet[5,6] and to our recent report of a point mutation in the mitochondrial genome in the region of the F_0ATPase 6,8.[10] This mutation results in an inefficiency in ATP synthesis that likely is potentiated by the HCO-induced, relatively more rigid, inner mitochondrial membrane. One would suspect that the animal defends itself from the consequences of this diet-gene interaction by developing or strengthening alternative pathways of metabolite use. Pryor et al.[27] has reported that an artificially induced oxidative phosphorylation inefficiency results in a decrease in gluconeogenesis. The inefficiency was induced through the use of a low dose of a mild uncoupler, DCMU. The report of Pryor et al. is in contrast with our earlier reports of a decrease in ATP synthesis efficiency[5,6] and an increase in gluconeogenesis in HCO fed rats.[7,8] We have

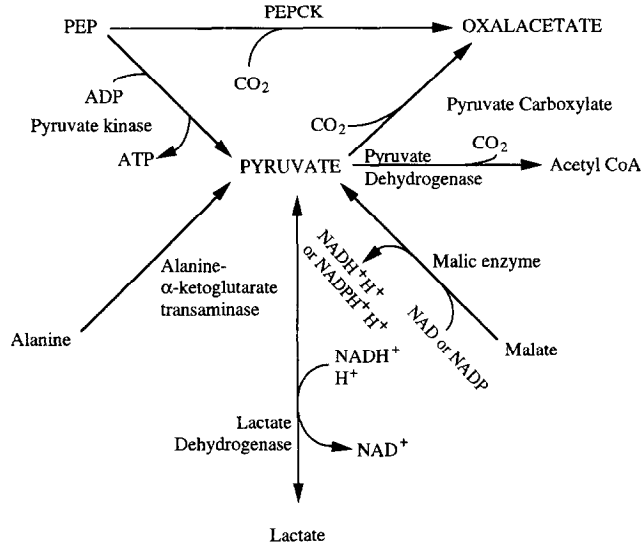

Figure 1 Overview of pyruvate metabolic pathways.

also reported that feeding MO increases ATP synthesis efficiency[9] and decreases gluconeogenesis. Using DCMU in normal rats increases fatty acid synthesis[28] so one might anticipate that lipogenesis would be greater in rats with ATP synthesis inefficiency. BHE/cdb rats are characterized by hyperlipogenesis, as well as hyperactive gluconeogenesis.[29–31] All of these processes feed off the central three carbon metabolite, pyruvate. As can be seen in *Tables 2* and *3*, diet fat affects the direction of pyruvate flow. Slightly less lactate and malate, plus a less active pyruvate kinase and pyruvate carboxylase and a more active membrane-bound lactate dehydrogenase, were found in the MO-fed rats when compared to the rats fed the other diets. This would explain the lack of a diet fat difference in pyruvate levels.

Examination of the HCO group shows a different picture, which would be consistent with our earlier reports. The HCO rat seems to defend itself against the untoward effects of the HCO diet, which may explain the slightly higher lactate levels with less active membrane-bound lactate dehydrogenase activity. In contrast, the rats fed the MO diet had lower lactate levels and higher membrane-bound lactate dehydrogenase. The cytoplasmic redox state, which is calculated from the tissue levels of lactate and pyruvate, is much higher in this group than in the other two groups; thus, suggesting that the source of the dietary fat affected the pyruvate crossroad of intermediary metabolism. In addition, the direction of metabolite flow seems to depend on both the diet and gene effects on ATP synthesis efficiency.

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