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Effect of Dietary Fat on Hepatic Mitochondrial F_1F_0ATPase Characteristics in NIDDM-prone Rat

Sook-Bae B. Kim† and Chang-Im Kim*

Department of Food Science and Human Nutrition, Chonbuk National University, Chonju 561-756, Korea
**Department of Foods and Nutrition, Hyejeon College, Chungnam 320-702, Korea*

Abstract

The present work was designed to determine whether change in fluidity of the mitochondrial membrane affects mitochondrial F_1F_0ATPase characteristics in NIDDM-prone BHE/Cdb rat. Isolated mitochondria from BHE/Cdb rat fed a 6% coconut oil or corn oil were functionally tested by an analysis of its respiration and the coupling of this process to ATP synthesis in presence of oligomycin, a specific inhibitor of oxidative phosphorylation (OXPHOS), that binds to the F_1F_0ATPase. Mitochondria from rats fed coconut oil were more responsive to the inhibitory action of oligomycin with respect to state 3 respiration, respiratory control (RC) ratio and ADP:P (P/O) ratio than were mitochondria from rats fed corn oil. In state 3 respiration, mitochondria from rats fed coconut oil consumed less oxygen than did mitochondria from rats fed corn oil. RC ratio was lower in the mitochondria from rats fed coconut oil than was mitochondria from rats fed corn oil. In P/O ratio, the mitochondria from rats fed coconut oil had a lower P/O ratio than did mitochondria from rats fed corn oil. The data showed that the change in fluidity of the mitochondrial membrane by dietary fat affected mitochondrial F_1F_0ATPase characteristics. The present study on diet differences in F_1F_0ATPase characteristics provides considerable insight into the role diets play in the control of mitochondrial function, especially OXPHOS in NIDDM with mitochondrial defects.

Key words: dietary fat, oligomycin, oxidative phosphorylation, BHE/Cdb rat, NIDDM, F_1F_0ATPase

INTRODUCTION

The BHE/Cdb rat, a substrain of the heterogeneous BHE[1)] rat strain, was developed specially for the study of non-insulin dependent mellitus (NIDDM) in the absence of obesity (1-4). The BHE/Cdb rat mimics human NIDDM. It develops moderate hyperglycemia and impaired glucose tolerance as it ages as well as a number of diabetic complications. Feeding purified diets hastens the appearance of glucose intolerance. Using a diet that approximates the composition of the human diet, glucose intolerance has been observed as early as 100 days of age. Prior to the development of glucose intolerance, various hepatic abnormalities in metabolic control have been observed. Among these are: 200% increase in *de novo* fatty acid and cholesterol synthesis (2), 40% increase in gluconeogenesis (3), and 20% reduction in ATP synthesis efficiency (4). The reasons for this loss in ATP synthesis efficiency have been sought because it was believed to be related to the above described features of the BHE/Cdb rat and because the results in this rodent might help us understand NIDDM in the human with a similar mitochondrial defect.

†Corresponding author. E-mail: sbkim@moak.chonbuk.ac.kr
Phone: 82-63-270-3823, Fax: 82-63-270-3854

1) The initial BHE were taken from a US Department of Agriculture subunit, the Bureau of Home Economics. Animals of the parent strain are no longer available except as breeding pairs from the Animal Resource Center, NIH, Bethesda, MD. Animals for the BHE/Cdb substrain can be obtained from Dr. Berdanier, Univ. of Georgia, Athens, GA.

The observations that hepatic mitochondria in BHE/Cdb rats may be dysfunctional were reported (5). Mitochondria isolated from young growing BHE/Cdb rats, fed a stock diet, respired at a slower rate than did mitochondria from rats of the Wistar strain. McCusker et al. also found slower mitochondrial respiratory rates, and subsequently reported that as these rats aged, there were further decreases in their respiration (6). Efforts to clarify the dysfunctional character of mitochondria in the BHE/Cdb rats have revealed the existence of genetic differences between these and normal rats.

Recent studies of the BHE/Cdb rat have shown that the hepatic mitochondrial DNA has a base substitution at position 523 in the area that codes for subunit 6 of F_1F_0ATPase (7). At this position the BHE/Cdb codon is "GAC" while the Sprague Dawley codon is "AAC". The amino acid substitution (aspartic acid substituted for asparagine) at this critical location in the F_0 should have an effect on F_1F_0ATPase characteristics and mitochondrial function. The previous study (8) showed that the BHE/Cdb genotype with an abnormal subunit 6 contributed to aberrant mitochondrial F_1F_0ATPase characteristics. The responsiveness of hepatic mitochondria isolated from NIDDM-prone BHE/Cdb and normal Sprague Dawley (SD) rats to oligomycin was performed. Oligomycin blocked proton conductance primarily through its binding to the F_1F_0ATPase. Mitochondria from BHE/Cdb rats were more sensitive to oligomycin inhibition than were those from SD rats. These inherent differences in the functional attributes of the mitochondrial F_1F_0ATPase suggested that the defect of F_1F_0ATPase accounts for the defective mitochondrial function,

OXPHOS, which in turn precedes the development of NIDDM.

Although there is a strong genetic element, NIDDM has a multi-factorial etiology in which environmental factors like diet are important modifiers. It should be noted that the mitochondrial F_1F_0ATPase characteristics are managed by diet. In mammals the mitochondria have a dual bilayer membrane structure that surrounds OXPHOS system. Cross and Duncan (9) have suggested that the various subunits of ATPase rotate or move within the confines of the matrix (the F_1 portion) and the inner mitochondrial membrane (the F_0 portion). The membranes of the mitochondrion contain phospholipids that are specific to the particular membrane and may affect its function of the various proteins embedded in it. This is probably of importance to the F_0 which must have some degree of flexibility within its lipid environment for F_1F_0ATPase characteristics. The change in the lipid composition of the mitochondrial membrane could alter the properties of F_1F_0ATPase. It is thus proposed that change in fluidity of the mitochondrial membrane affects mitochondrial F_1F_0ATPase characteristics. In the present study, isolated mitochondria from BHE/Cdb rats fed a 6% coconut oil or corn oil were functionally tested by an analysis of its respiration and the coupling of this process to ATP synthesis in the presence of oligomycin.

MATERIALS AND METHODS

Animal and diets

Two groups of three week old BHE/Cdb rats (12/group) were housed individually in hanging, wire mesh cages in a room in which room temperature (21±1°C), humidity (45~50%), and lights (lights on, 06:00~18:00) were controlled. The rats were fed a diet containing 64% sucrose, 6% corn oil or coconut oil, 10% lactalbumin, 10% casein, 5% fiber, 1% AIN-93 vitamin mix, 4% AIN-93 mineral mix for 150 days of age. The diet ingredients were purchased from ICN Nutritional Biochemicals (Cleveland, OH). Rats were weighed and food intakes were determined every week.

Preparation of mitochondria

The rats were killed by decapitation and the livers were quickly excised, chilled in Tris-buffered (pH 7.2) 0.25 M sucrose, and weighed. Mitochondria were prepared by the procedure of Johnson and Lardy (10). The liver was homogenized in cold, Tris-buffered 0.25 M sucrose and the mitochondria were isolated from the homogenate by differential centrifugation, washed and resuspended three times. After the final wash, the mitochondria were resuspended in the buffer. The mitochondrial protein content was determined by the biuret method using bovine serum albumin as standard (Sigma kit # 541-2, Sigma Co., St. Louis, MO).

Determination of oligomycin sensitivity in OXPHOS

Oxygen consumption was determined with an oxygen electrode (Model 5331, Yellow Springs Instrument Co., Yellow-Springs, OH), 2.5 mL chamber and oxygen meter (UGA Instrument Design Group, Athens, GA). The reaction chamber was fitted with a magnetic stirrer and temperature was controlled at 25°C. Respiration buffer (11) (75 mM glycine, 10 mM phosphate buffer, pH 7.4, 75 mM KCl, 5 mM $MgSO_4$, 10 mM Tris-HCl, pH 7.2) was preequilibrated with air by shaking in a water bath at 25°C and introduced into the chamber by syringe. All subsequent additions to the chamber were made with Hamilton syringes passed through the capillary on top. The following reagents were stored frozen (-80°C) in small aliquots: 25 mM ADP and 0.65 M succinate (pH 7.2). In a typical experiment, freshly isolated mitochondria (2.5 mg of mitochondrial protein) were added to the chamber containing respiration buffer and 5 mM succinate. After 2 min, 375 nmol of ADP was added to stimulate state 3 respiration. Usually two additions of ADP were made before addition of uncoupler (50 μM DNP, 2,4-dinitrophenol). In order to determine the oligomycin sensitivity in OXPHOS, oligomycin (Sigma Co., St. Louis, MO) was added 0.04 μg oligomycin per mg of mitochondrial protein before addition of ADP. State 3 respiration (in the presence of ADP) and state 4 respiration (after the added ADP had been converted to ATP) rate were calculated according to Chance and Williams (12) using assumptions from Reynafarje et al. (13). The RC ratio and P/O ratio were calculated according to Estabrook (14).

Statistical analysis

2×2 repeated measures design was utilized. Diet (corn oil vs. coconut oil) was between-subject factor, treatment (with oligomycin vs. without oligomycin) was within-subject factor. Multiple t-tests were conducted. Paired comparison was performed for the within factor, and independent samples comparison was done for the between factor. The results are expressed as mean values with their standard errors. SPSSWIN 8 was used for all statistical analyses.

RESULTS AND DISCUSSION

The food intake, body weight, liver weight and relative liver size (RLS) are presented in Table 1. There were no diet differences in daily food intake, liver weight and RLS. But body weight revealed diet differences. The rats fed coconut oil gained less weight than the rats fed corn oil. This is a typical response to an essential fatty acid-deficient diet.

Succinate-supported mitochondrial respirations are presented in Fig. 1~4. In the absence of oligomycin, the state 3 respi-

Table 1. Effects of corn oil or coconut oil diets on food intake, body weight and liver weight in NIDDM-prone BHE/Cdb rats

Diet	Food intake (g/100g body wt/day)	Body weight (g)	Liver weight (g)	RLS[1)] (%)
Corn oil	6.9±0.7[2)]	495.6±16.5[a]	19.5±1.3	4.0±0.3
Coconut oil	7.2±1.1	470.1±13.1[b]	18.9±1.0	4.1±0.3

[1)]RLS, relative liver size. (liver weight/body weight)×100

[2)]Values are means±SE for 12 rats. Values within a column with different superscript letters were significantly different ($p<0.05$)

ratory rate, the state 4 rate, the RC ratio and P/O ratio were similar to those previously reported (15) and were evidence of well prepared mitochondria. Feeding hydrogenated coconut oil resulted in a decrease in state 3 oxygen consumption which affected the RC ratio and P/O ratio. State 3 respiration was lower in the mitochondria from rats fed coconut oil (HCO) than in the mitochondria from rats fed corn oil (CO) (68.06 ±3.14 vs. 74.99±3.93 *ng* atoms O/min/mg of mitochondrial protein) (Fig. 1). There were no significant differences in state 4 respiration between mitochondria from HCO and those from CO (18.82±0.94 vs. 17.64±0.83 *ng* atoms O/min/mg of mitochondrial protein) (Fig. 2). The calculated RC ratio was lower for the mitochondria from HCO than was mitochondria from CO (3.61±0.19 vs. 4.25±0.21) (Fig. 3). With respect to P/O ratio, mitochondria from HCO had lower P/O ratio than did mitochondria from CO (1.80±0.09 vs. 2.21±0.01) (Fig. 4).

In the presence of oligomycin, the extent of inhibition was very different between mitochondria from HCO and mitochondria from CO. Mitochondria from HCO were respiring far slower than mitochondria from CO. Feeding coconut oil released oligomycin-inhibited state 3 respiration, RC ratio and P/O ratio. Mitochondria from HCO were more responsive to the inhibitory action of oligomycin with respect to state 3 respiration, RC ratio and P/O ratio than were mitochondria from CO. In state 3 respiration, mitochondria from HCO consumed less oxygen than did mitochondria from CO. Mitochondria from HCO was 27.48±2.35 *ng* atoms O/min/mg of mitochondrial protein (59.6% inhibition); mitochondria from CO was 44.76±2.15 *ng* atoms O/min/mg of mitochondrial protein (40.3% inhibition) (Fig. 1). There were no significant differences in state 4 respiration between mitochondria from HCO and those from CO. Mitochondria from HCO was 13.22±0.63 *ng* atoms O/min/mg of mitochondrial protein (27.4% inhibition); mitochondria from CO was 12.65±0.66 *ng* atoms O/min/mg of mitochondrial protein (28.3% inhibition) (Fig. 2). In RC ratio, the mitochondria from HCO had a lower RC than did those from CO. Mitochondria from HCO was 2.22± 0.13 (38.5% inhibition); mitochondria from CO was 3.01±0.15

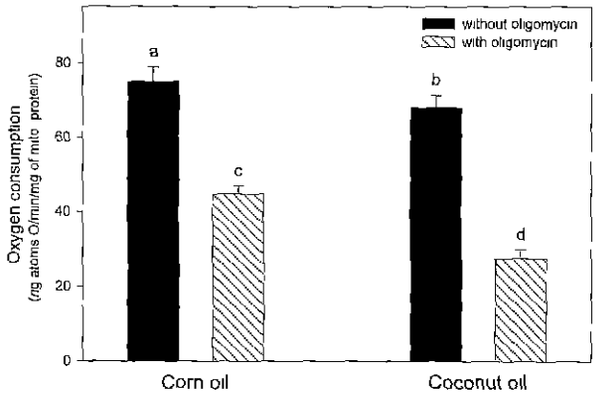

Fig. 1. State 3 respiration of hepatic mitochondria from BHE/Cdb rats fed 6% corn oil or coconut oil with and without oligomycin (0.04 μg/mg of mito. protein). Each bar with different letters is significantly different ($p<0.05$). n=6

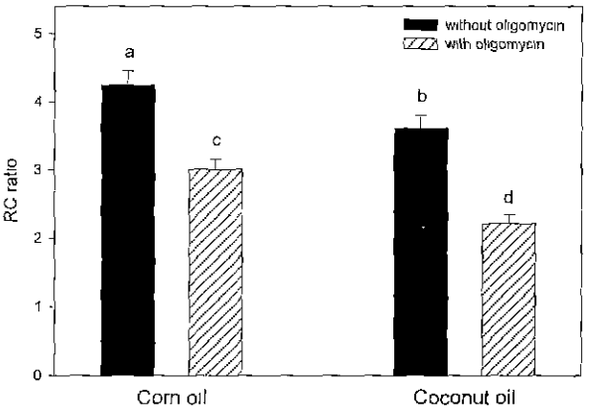

Fig. 3. RC ratio of hepatic mitochondria from BHE/Cdb rats fed 6% corn oil or coconut oil with and without oligomycin (0.04 μg/mg of mito. protein). Each bar with different letters is significantly different ($p<0.05$). n=6

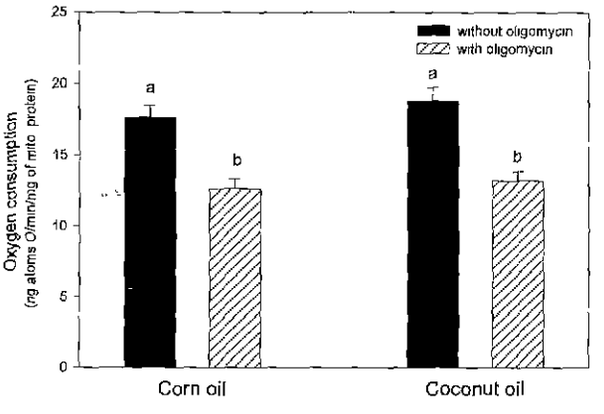

Fig. 2. State 4 respiration of hepatic mitochondria from BHE/Cdb rats fed 6% corn oil or coconut oil with and without oligomycin (0.04 μg/mg of mito. protein). Each bar with different letters is significantly different ($p<0.05$). n=6

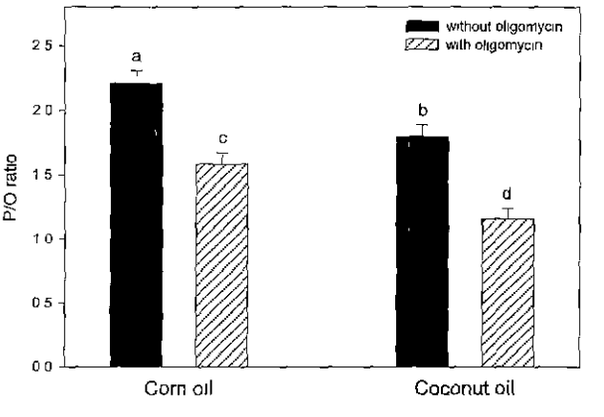

Fig. 4. P/O ratio of hepatic mitochondria from BHE/Cdb rats fed 6% corn oil or coconut oil with and without oligomycin (0.04 μg/mg of mito. protein). Each bar with different letters is significantly different ($p<0.05$). n=6

(29.4% inhibition) (Fig. 3). P/O ratio was lower in the mitochondria from HCO than was those from CO. Mitochondria from HCO was 1.16±0.08 (35.5% inhibition); mitochondria from CO was 1.58±0.09 (28.5% inhibition) (Fig. 4). In all these comparisons, the data showed that mitochondria from HCO reduced mitochondrial respiratory function and enhanced sensitivity to oligomycin compared to mitochondria from CO.

How does dietary fat relate to the mitochondrial F_1F_0ATPase characteristics? Perhaps there may be specific fatty acids associated with the HCO diet that have the effect on the membrane fluidity. The previous study (16) has shown the diet affects membrane fluidity. The influence of dietary fat components on the composition of hepatic mitochondria lipids was demonstrated. The response to dietary fat composition was magnified in the fatty acid composition of hepatic mitochondrial phospholipid. The rats fed coconut oil had more saturated phospholipid fatty acids than rats fed corn oil. Mitochondria from HCO contained more 16:1, 18:1 and less 18:2, 20:4 fatty acids in the phospholipids than mitochondria from CO. The unsaturation index and the ratio of the concentrations of unsaturated fatty acids to concentrations of saturated fatty acids (UFA/SFA) were lower in mitochondria from HCO than in mitochondria from CO. It is well known that the membranes of the mitochondrion contain phospholipids that are specific to the particular membrane and may affect its function of the various proteins embedded in it. Pedersen (17) as well as Cross and Duncan (9) have suggested that the subunits of F_1F_0ATPase rotate within the mitochondrial membrane. Any change in mitochondrial membrane fluidity should have effects on its characteristics.

Change in membrane fluidity may affect the mitochondrial F_1F_0ATPase characteristics in proton conductance. The previous study (16) showed perturbation of the membrane lipid through the feeding of hydrogenated coconut oil affected proton conductance. Change in membrane fluidity at proton channel resulted in a change in proton conductance. In this study, as expected, the differences in RC ratio, a criterion useful in evaluating coupling, and the P/O ratio showed that the rats fed coconut oil were more uncoupled and less efficient with respect to mitochondrial ATP synthesis than were the rats fed corn oil. It is postulated that coconut oil induces proton leak of F_1F_0ATPase by acting on the mitochondrial membrane through their lipophilic properties and protonporic activities.

In conclusion, rats fed coconut oil change proton conductance in F_0 portion of F_1F_0ATPase by change in proton permeability: it releases respiratory control and increases in oligomycin response of mitochondrial F_1F_0ATPase. The present study on diet differences in F_1F_0ATPase characteristics provides considerable insight into the role diets play in the control of mitochondrial function, especially OXPHOS in NIDDM with mitochondrial defects.

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