Nutrient–gene interactions determine mitochondrial function: effect of dietary fat

MOON-JEONG CHANG KIM[1] AND CAROLYN D. BERDANIER[2]
Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602–3622, USA

ABSTRACT **The effect on mitochondrial respiration of feeding hydrogenated coconut oil, corn oil, or menhaden oil (MO) to diabetes-prone BHE/cdb rats and normal Sprague Dawley (SD) rats was studied. Both fat source and strain affected the temperature dependence of succinate-supported respiration. The transition temperature was greater in BHE/cdb rats than in the SD rats. The efficiency of ATP synthesis as reflected by the ADP:O ratio was decreased in the BHE/cdb rats compared to SD rats, with the exception of the comparison made at 37°C with the MO-fed rats; at this temperature, the ADP:O ratios were similar. The diet and strain differences suggest a dietary lipid–gene interaction with respect to the mobility of subunit 6 of the F_1F_0ATPase. This subunit has two errors in its gene: one that affects the proton channel and another that likely affects its mobility within the inner mitochondrial membrane.—Kim, M.-J. C., Berdanier, C. D. Nutrient–gene interactions determine mitochondrial function: effect of dietary fat. *FASEB J.* 12, 243–248 (1998)**

Key Words: dietary fat saturation · Arrhenius plot · mitochondrial respiration · BHE/cdb rats

IN AN EARLIER COMMUNICATION we reported that rats of the diabetes-prone BHE/cdb strain have a mitochondrial genomic mutation in the region that encodes subunit 6 (also called subunit a) of F_0ATPase (1). The mutation changes the amino acid sequence of that portion of the subunit that forms the proton channel, whereby an asparagine is substituted for aspartate. We had previously reported a reduction in ATP synthesis efficiency (2) as well as a reduction in sensitivity to oligomycin (3) and an increase in sensitivity to changes in calcium ion concentration (4).

The F_0 portion of F_1F_0ATPase is embedded in the inner mitochondrial membrane. Cross and Duncan (5) 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). Because the F_0 portion is embedded in the inner mitochondrial membrane, its degree of movement is likely affected by the fluidity of that membrane. We reported previously that hepatic mitochondria from BHE/cdb rats fed hydrogenated coconut oil (HCO)[3] appeared less well coupled when respiration was supported by pyruvate or succinate (6, 7), but were coupled normally when the respiration was supported by fatty acid substrates 6). In none of these studies had we compared mitochondria from BHE/cdb rats with those from Sprague-Dawley (SD) rats fed the same diet; hence, the present work. In this work we asked the question of whether the genetic differences in the F_0ATPase subunit 6 would determine a difference in response to diet-induced differences in membrane lipid. To answer this question, we examined the dependence on temperature of succinate-supported oxygen consumption by isolated mitochondria from SD and BHE/cdb rats fed a 6% HCO, corn oil (CO), or menhaden oil (MO) diet. We found striking differences due to diet among the strains, which suggested that the asparagine for aspartate at position 101 and the leucine for serine substitutions at position 129 affected the function of subunit 6 within the membrane when the fatty acid composition of that membrane became more saturated.

METHODS

Groups of six (SD and BHE/cdb) male 28-day-old rats (57–67 g body weight) were fed for 4 wk either a 6% HCO, a 6% CO, or a 6% MO diet.[4] A total of 36 rats were used.

The rats were housed individually in hanging wire mesh cages in a room controlled for temperature (20±1°C), humidity (45–50%), and light (lights on 0600–1800). Food and

[1] Present address: Department of Home Economics, Kookmin University, Sungbak-ku, Seoul, 136–702, Korea.

[2] Correspondence: Department of Foods and Nutrition, Dawson Hall, University of Georgia, Athens, Georgia 30602–3622, USA.

[3] Abbreviations: ADP, adenosine diphosphate; CO, corn oil; HCO, hydrogenated coconut oil; MO, menhaden oil; RC, respiratory control; SD, Sprague Dawley; T_3, triodothyronine; T_4, thyroxine.

[4] Composition of the diet in percent by weight: sucrose, 64; casein, 10; lactalbumin, 10; AIN vitamin mix 1: AIN mineral mix, 4; fiber (Alphacel), 4; corn, menhaden oil, or hydrogenated coconut oil, 6. All ingredients except the corn oil and menhaden oil were purchased from ICN Nutritional Biochemicals, Cleveland, Ohio. The corn oil was a gift from Best Foods, Union, N.J. Menhaden oil was a gift of Zapata Haynie, Reidville, Va.

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water were always available. Food intake and body weight were determined weekly. The animals were cared for in accordance with the guidelines set forth in the NRC Guide for the Care and Use of Laboratory Animals (publication #85–23).

Rats were killed by decapitation at ~8 weeks of age. The liver was quickly excised, chilled, weighed, and used for the preparation of isolated mitochondria by differential centrifugation. The procedures of Johnson and Lardy (8) were followed. High-quality mitochondrial preparations were assured as described previously (9). The temperature dependence of succinate-supported respiration was determined at 3°C intervals from 4°C to 37°C in the presence and absence of 0.16 mM adenosine diphosphate (ADP). The methods used to prepare the Arrhenius plot and to calculate the transition temperature, as well as the activation energies, were the same as those used earlier (9, 10). Briefly, this consisted of measuring state 3 succinate-supported oxygen consumption at 3°C temperature intervals and using these values to calculate the activation energies and transition temperatures according to equation 1, as derived by Arrhenius:

$$\frac{d\ ln\ k}{dT} = \frac{Ea}{RT^2} \quad (1)$$

where k is the rate constant, R is the gas constant ($8.312 J(mol^{-1} \cdot k^{-1})$), Ea, the activation energy, and T the temperature in degrees Kelvin. Integration of equation 1 and conversion to base 10 logarithms gives equation 2:

$$\log\left[\frac{k_2}{k_1}\right] = \frac{Ea}{2.303R}\left(\frac{T_2 - T_1}{T_1 T_2}\right) \quad (2)$$

from which it can be seen that the value of Ea can be obtained from the slope of the straight line obtained when the logarithm of k is plotted against the reciprocal of the absolute temperature.

Details of the incubation media are shown as footnotes to **Table 1**. Using the definitions and assumptions of Lehninger (11) and Chance and Williams (12), respiratory rates (nmol oxygen consumed/mg protein × min^{-1}) were calculated after the addition of 0.16 mmol/l ADP (state 3) and after all the added ADP was phosphorylated to ATP (state 4, so-called resting state). The respiratory control (RC) ratio was calculated as the oxygen consumption rate for state 3 vs. that for state 4. The ADP:O ratio was calculated as the amount of added ADP converted to ATP to the amount of oxygen used during state 3 respiration. Where there was a failure to return to the resting state after ADP addition, the respiration was regarded as uncontrolled and no RC or ADP:0 ratios were calculated.

Mitochondria age after being isolated (13) and their quality may also deteriorate. To verify their consistency, respiration was determined at 25°C at the beginning, middle, and end of the temperature transition measurements. Any preparation that failed to retain its original quality was discarded and the values for that animal eliminated.

Because we suspected that diet might affect thyroid status, we measured serum levels of triodothyronine (T_3) and thyroxine (T_4) (Cambridge Medical Diagnostics Billerica, Mass.) in the neck blood collected at decapitation. We also measured triglyceride levels in the serum (Sigma kit #339, Sigma Chemical Co., St. Louis, Mo.).

Statistically significant means were identified by using SAS (Statistical Analysis Systems, Raleigh, N.C.) programs to analyze this 2 × 3 design. Where appropriate, pairs of means were compared by using a Student's *t* test. Regression analysis was used to determine changes in the slopes of the Arrhenius plots, followed by goodness of fit using Chi square. The r_2 and the residual sum of squares were calculated for all possible

TABLE 1. *Dietary fat effects on the dependence on temperature of succinate-supported respiration by mitochondria[1] from BHE/cdb and SD rats*

	HCO[2]		CO		MO	
	BHE/cdb	SD	BHE/cdb	SD	BHE/cdb	SD
Tt, °C	23.88 ± 1.10[3x]	20.15 ± 1.52[ay]	23.01 ± 1.11[x]	21.15 ± 1.40[xy]	23.45 ± 1.57[x]	18.43 ± 1.71[ay]
Ea (upper) kcal/mol	9.55 ± 0.60[x]	9.58 ± 1.01[x]	9.81 ± 0.55[x]	9.78 ± 0.33[x]	8.49 ± 1.07[x]	10.13 ± 0.79[ay]
Ea (lower) kcal/mol	17.48 ± 1.23[x]	19.34 ± 1.20[x]	18.13 ± 1.03[x]	18.05 ± 1.73[x]	19.09 ± 0.95[x]	20.06 ± 2.36[x]
37°C						
State 3[2]	102 ± 12	106 ± 7	129 ± 14	107 ± 8	115 ± 8	114 ± 6
State 4[2]	40 ± 2	42 ± 3	42 ± 3	33 ± 2[a]	38 ± 3	36 ± 3
RC[2]	2.6 ± 0.4	2.6 ± 0.2	3.1 ± 0.2	3.3 ± 0.2	3.1 ± 0.3	3.2 ± 0.2
ADP:O[2]	1.4 ± 0.02	1.7 ± 0.01[a]	1.5 ± 0.01	1.6 ± 0.02[a]	1.6 ± 0.01	1.6 ± 0.02
25°C						
State 3[2]	55 ± 4	60 ± 2	71 ± 8	60 ± 4	65 ± 5	64 ± 3
State 4[2]	22 ± 5	21 ± 1	19 ± 3	18 ± 2	19 ± 1	21 ± 1
RC[2]	2.6 ± 0.1	2.8 ± 0.1	4.2 ± 0.4	3.4 ± 0.3[a]	3.5 ± 0.4	3.1 ± 2.7
ADP:O[2]	1.4 ± 0.01	1.5 ± 0.01[a]	1.3 ± 0.01	1.5 ± 0.01[a]	1.3 ± 0.01	1.5 ± 0.01[a]
7°C						
State 3[2]	8.4 ± 0.5	8.7 ± 0.7	10.8 ± 2.1	8.9 ± 0.9	8.7 ± 1.3	8.7 ± 0.7
State 4[2]	—	—	—	—	—	—
RC[2]	—	—	—	—	—	—
ADP:O[2]	—	—	—	—	—	—

[1] 2.2 mg/ml mitochondrial protein were suspended in a medium containing 75 mmol/l glycine, 10 mmol/l phosphate buffer (pH 7.4), 75 mmol KCl, 5 mmol/l Mg SO_4, 10 mmol succinate, 30 μmol/l rotenone in a 1.8 ml chamber. State 3 respiration was produced by the addition of 0.16 mmol/l ADP. [2] Abbreviations: HCO, hydrogenated coconut oil; CO, corn oil; MO, menhaden oil; T_t, transition temperature; Ea, activation energy; state 3 and 4 respiration is expressed as nmoles O_2 consumed/mg protein·min^{-1}; RC, respiratory control ratio = state 3/state 4; ADP:O, mmoles ADP used/mmol oxygen consumed. [3] Mean ± SEM, N = 6; [a] Strain difference is significant ($P<0.05$) by Student's *t* test; xy, unlike letter superscripts indicate significantly different means ($P<0.05$) by ANOVA.

TABLE 2. *Dietary fat effects on food intake, body weight gain, hepatic weight, and serum measures of BHE/cdb and Sprague-Dawley rats*

	HCO		CO		MO	
	BHE/cdb	SD	BHE/cdb	SD	BHE/cdb	SD
DFI, g/100 g BW	7.7 ± 0.2[1x]	7.2 ± 0.4[x]	7.1 ± 0.7[x]	6.6 ± 0.2[x]	6.6 ± 0.9[x]	7.0 ± 0.4[x]
Weight gain, g	190 ± 11[x]	204 ± 14[x]	177 ± 16[x]	230 ± 8[ax]	172 ± 14[x]	226 ± 18[ax]
Liver weight, g	10.4 ± 0.8[xa]	11.6 ± 0.6[xa]	9.3 ± 1.1[b]	12.6 ± 0.7[a]	8.6 ± 1.0[b]	12.4 ± 0.6[a]
Triglycerides, mg/dl	123 ± 19[x]	88 ± 7[ay]	113 ± 29[x]	95 ± 15[xy]	64 ± 9[z]	37 ± 5[au]
T_4, μg/dl	9.5 ± 0.9[x]	8.2 ± 2.9[x]	7.1 ± 1.0[x]	5.2 ± 0.3[ay]	5.6 ± 0.5[y]	5.3 ± 0.3[y]
T_3, ng/dl	133 ± 10[x]	121 ± 17[x]	98 ± 8[y]	116 ± 8[xy]	95 ± 12[y]	116 ± 8[xy]
T_4:T_3 × 100	7.4 ± 1.1[x]	6.4 ± 1.3[x]	7.2 ± 0.9[x]	4.6 ± 0.3[ay]	6.6 ± 1.1[x]	4.6 ± 0.3[ay]

	ANOVA						
	DFI	Weight gain	Liver weight	TG	T_4	T_3	T_4:T_3
Diet	NS	NS	NS	0.01	0.01	0.01	NS
Strain	NS	NS	0.05	0.05	0.01	0.01	0.05
Interaction	NS	NS	NS	0.05	0.01	0.01	0.05

[a] Strain difference is significant ($P < 0.05$) by Student's *t* test; xyzu, unlike superscripts indicate significantly different means ($P < 0.05$) by ANOVA. Abbreviations: HCO, hydrogenated coconut oil; CO, corn oil; MO, menhaden oil; DFI, daily food intake; T_4, thyroxine; T_3, triiodothyronine; ANOVA, analysis of variance. Mean ± SEM, N = 6.

combinations of points fitted to two straight lines from the lower to upper temperature extremes. A change in slope was considered to occur at the first minimum for the sum of the residual sum of squares of the two straight lines. The calculations were continued, using all the points from the calculated point of a change in slope to the upper temperature extreme. The statistical significance of the differences in slope (m) and the intercept (b) for the straight lines, defined by mx + b = y, was tested and was significant at $P < 0.05$ based on the F distribution with n-2 df and the t distribution with n-2 df, where n = the total number of points on the two straight lines about the point at which the slope is changed.

RESULTS AND DISCUSSION

There were no diet or strain differences in daily food intake (**Table 2**), but body weight revealed both strain and diet differences. Those SD rats fed the HCO diet did not gain as much weight as SD rats fed the CO and MO diets. This is a typical response to an essential fatty acid-deficient diet (6, 7, 14–21). Although we have previously reported a similar decline in food efficiency in HCO-fed BHE/cdb rats compared to MO- or CO-fed rats, we did not observe this in the present work. Compared to previous work, the feeding period was 1 wk longer and the rats were 1 wk older when started on the HCO diet. Obviously, they were not depleted of their store of essential fatty acids and were not in the deficient state, which might have affected their response. Note that the SD rats were heavier than the BHE/cdb rats fed either the CO or MO diet. Strain but not diet differences were observed: liver weights of the SD rats were heavier than those of the BHE/cdb rats fed either CO or MO diets. No strain difference in liver weight was observed in rats fed the HCO diet.

Both strain and diet differences were observed in serum triglyceride levels. BHE/cdb rats had higher triglyceride levels than SD rats when fed the HCO diet. These levels fell when the rats of both strains consumed the MO diet, yet were unchanged when the CO diet was provided. There was a significant strain difference in the triglyceride levels of rats fed the MO diet, with BHE/cdb rats having the higher value. Thyroxine and T_3 levels were influenced by diet and strain. The SD rats had lower T_4 values than BHE/cdb rats when fed the CO diet, and rats of both strains fed the MO diet had lower T_4 values than when the HCO diet was consumed. Declines in T_3 levels were observed in the BHE/cdb rats when those fed the HCO diet were compared with those fed either the MO or CO diet. No such decline was observed in the SD rats. Calculation of the ratios of T_4 to T_3 suggest that deiodination was impaired in the BHE/cdb rats. Whereas the ratio fell in the SD rats fed CO or MO compared to HCO, this fall did not occur in the BHE/cdb rats.

Succinate-supported respiration at 37, 25, and 7°C is shown in Table 1, along with the calculated transition temperatures and the upper and lower activation energies. Significant strain differences in state 4 respiration were observed in rats fed the CO diet at 37°C and in the RC of these same rats when respiration was assessed at 25°C. This suggests there might have been an increased leak rate for these mitochondria. Alternatively, a relatively high state 4 oxygen use by BHE/cdb mitochondria suggests that some sort of compensatory reaction might be occurring. The adenylate kinase reaction could be more active and could provide supplementary ADP, which in turn could stimulate oxygen consumption. When this reaction is suppressed by using a 8:1 mixture of

AMP:ADP, one can observe a 30% reduction in state 4 oxygen use (C. E. Mathews and C. D. Berdanier, unpublished observations). Diet was without effect on these measurements except when ATP synthesis efficiency was evaluated as the ADP:O ratio. This ratio was influenced by strain in rats fed either the HCO or CO diet at either 37 or 25°C and in rats fed the MO diet at 25°C. In all these comparisons, mitochondria from BHE/cdb rats were significantly less efficient in capturing the energy of the proton gradient in the high-energy bond of ATP than were mitochondria from SD rats. The exception was in those rats fed the MO diet. The ADP:0 ratio was higher in the BHE/cdb rats fed this diet compared with those fed the HCO or CO diet when the mitochondria were evaluated at body temperature, 37°C. At the lower temperature, the strain difference was apparent. At 7°C, the mitochondria from both strains were so slowed by the cold that valid measurement of state 4 respiration was not possible, and thus the RC and ADP:0 ratio could not be calculated.

With respect to the temperature dependence of mitochondrial respiration, examination of the Arrhenius plots (**Fig. 1**) reveals a discontinuity in the lines influenced by the type of dietary fat and the rat strain. Note the significant strain difference in the break point in the rats fed the HCO diet and the lack of such a difference in rats fed the CO diet. In the MO groups, the lines are almost identical, yet there was a significant difference in transition temperature (Table 1) as well as a strain difference in the upper activation energy (Ea upper). In groups of rats fed the CO diet, the lines are parallel, with break points at nearly the same temperature. These Arrhenius plots are typical of membrane-bound systems, where one expects discontinuous behavior due to a phase change in the surrounding lipid. At the low temperatures, phospholipids in the membrane exist as gels containing crystalline hydrocarbon chain regions. As temperature increases, molecular movement of these hydrocarbon chains gradually increases until, at a transition temperature, a sharp increase in that absorption occurs and the mobility of the chain abruptly increases. This gives rise to the liquid crystalline state. The mobility of the hydrocarbon chain is determined by its degree of unsaturation. Hence, highly unsaturated fatty acids are more mobile than are very saturated fatty acids. In studies of mitochondria isolated from temperature-sensitive plants and animals, electron spin resonance techniques, which use spin-labeled compounds as well as differential scanning calorimetry, have confirmed that this discontinuity in Arrhenius plots is due to a phase change in the mitochondrial membrane lipid (10). This paper shows that this phase change can be influenced by saturation of the dietary fat and the genetic background of the consumer. The question that now arises is how could these factors influence

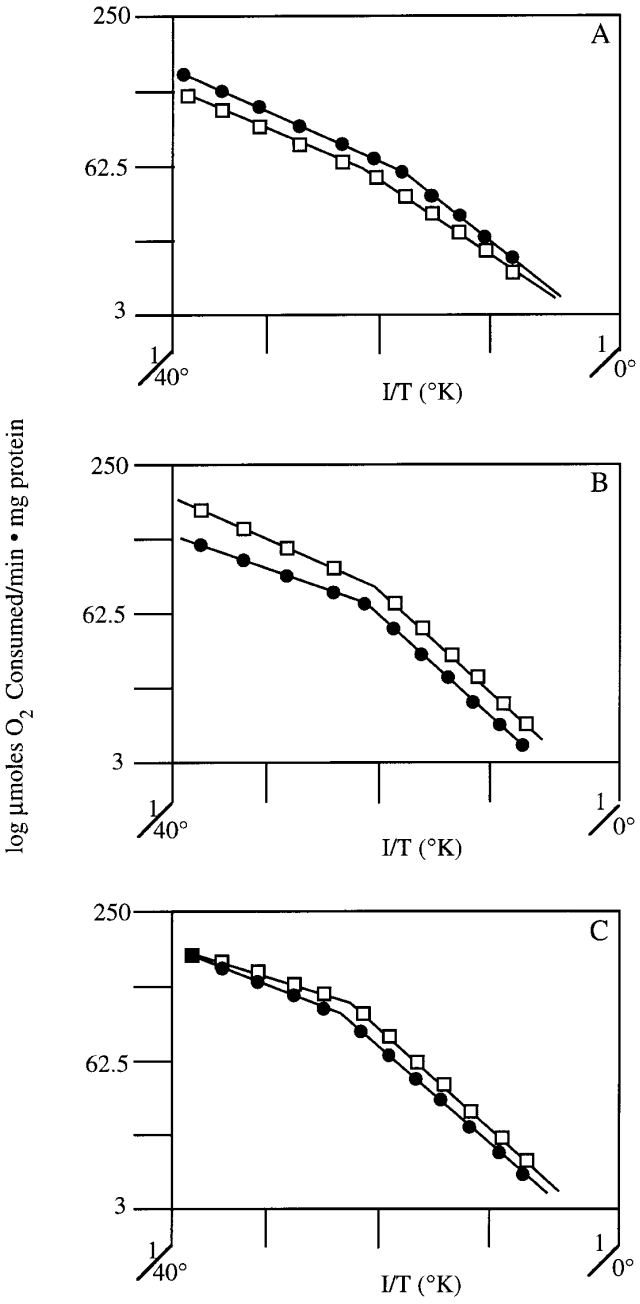

Figure 1. Arrhenius plot of the log of the oxidation rate for succinate-supported respiration vs. the reciprocal of the absolute temperature. Each point represents the mean of six observations using hepatic mitochondria isolated from BHE/cdb and Sprague Dawley (SD) rats fed a 6% hydrogenated coconut oil (*A*), 6% corn oil (*B*), or a 6% menhaden oil (*C*) diet. BHE/cdb (□); SD (●). The transition temperature and the upper and lower activation energies are given in Table 1.

the phase change in succinate-supported respiration?

One possibility is that BHE/cdb rats may not have a normal thyroid hormone status. Thyroid hormone affects both membrane fatty acid saturation (22–25) and mitochondrial respiration (26–38). Disturbances in thyroxine deiodination to triiodothyronine in the BHE/cdb rats could explain some of the present re-

sults. Table 1 shows a strain difference in T_4:T_3 ratios in rats fed the CO and MO diets. Deiodination of thyroxine to the active hormone apparently proceeded at a less active rate in the BHE/cdb rats than in the SD rats. We have previously reported on the strain difference in 5′ deiodinase activity (39). However, we have also reported that the induction of hyperthyroidism through daily injections of either T_4 or T_3 was without effect on respiration (6), yet increased mitochondrial membrane fatty acid unsaturation (22). These results suggest that abnormalities in thyroid hormone status cannot explain the present strain and dietary effects on phase transition.

Recently, considerable discussion has developed on the mechanism of action of the F_0ATPase. Pedersen (40) as well as Cross and Duncan (5) have suggested that the subunits of ATPase rotate within the mitochondrial membrane. This rotation could be hindered if the membrane loses fluidity as a function of its fatty acid saturation-to-unsaturation ratio and/or if the subunit secondary, tertiary, and quaternary structure has been modified genetically. In the present case, base substitutions in the mitochondrial gene for subunit 6 (subunit a) have been found (1). These substitutions result in amino acid substitutions at positions 101 and 129, which in turn could be assumed to affect the subunit's secondary structure. Molecular modeling of this protein using the predictions of Garnier, Osguthorp, and Robson (41) suggests that the substitution at position 129 would result in the formation of a pleated sheet rather than an amino acid loop. This change in protein conformation would probably confer a degree of rigidity to this portion of the molecule (42–46). Since molecular mobility is essential to ATPase function (5, 40, 44, 45), a small change in protein structure potentiated by the relative rigidity of the surrounding lipid could explain the strain difference in transition temperature as well as the decrease in ATP synthesis efficiency observed in the BHE/cdb rats fed the HCO diet. Further studies are needed to determine whether this explanation is plausible. FJ

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