Influence of Menhaden Oil on Mitochondrial Respiration in BHE Rats (42974)

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Abstract. **The effects of corn or menhaden oil and thyroxine treatment on hepatic mitochondrial respiration was studied. BHE rats were fed a 64% sucrose, 6% corn, or menhaden oil diet until they were 60–70 days of age. Succinate-supported mitochondrial respiration was studied at 3°C intervals from 4 to 40°C. Upper and lower activation energies and transition temperatures were determined through the calculation of Arrhenius plot. Menhaden oil plus daily thyroxine injection resulted in higher and lower activation energies than the other treatments. This combined treatment also resulted in lower state 3 and higher state 4 respiration rates and tighter coupling of respiration to ATP synthesis. These effects were thought to be due to the effect this treatment combination had on membrane fluidity.** [P.S.E.B.M. 1989, Vol 192]

It is well documented that different dietary fats can influence the fatty acid composition of both the depot fat and the lipid portion of the cellular membranes. Feeding highly saturated fat such as hydrogenated coconut oil results in highly saturated depot lipids and membrane phospholipids with a higher than normal percentage of saturated fatty acids. Several studies have shown that mitochondrial membranes and mitochondrial function can be altered by the inclusion of saturated fat in the diet (1–4). Yamaoka *et al.* (5) recently reported that feeding a 20% sardine oil diet which was rich in omega 3 unsaturated fatty acids resulted in omega 3 fatty acid elevations in the phospholipids of heart and liver. The isolated heart mitochondria of these rats consumed less oxygen during state 3 succinate or glutamate respiration than heart mitochondria from rats fed corn oil. State 4 succinate-supported respiration was not affected; however, state 4 glutamate plus malate-supported respiration was also lower in the sardine oil-fed rats compared with the corn oil-fed rats. The respiratory control ratios and the ADP:O ratios were not affected, yet the activities of both the F_1F_0ATPase and cytochrome *c* oxidase were. The ATPase activity was higher and the cytochrome *c* oxidase activity was lower in the sardine oil-fed rats than in the corn oil-fed rats. Hepatic mitochondrial activity was not studied. In an earlier study, we reported that feeding hydrogenated coconut oil to BHE rats resulted in uncontrolled state 3 pyruvate supported respiration while the succinate-supported respiration was controlled (4). The mitochondria from the coconut oil-fed group had higher state 3 and state 4 respirations and a lower respiratory control ratio than mitochondria from corn oil-fed BHE rats.

In view of the results of Yamaoka *et al.* (5), we wondered if the coconut oil effect could be reversed by feeding menhaden oil. Since thyroxine (T_4) can also affect membrane phospholipid fatty acid saturation (2, 6–8) and mitochondrial respiration (8, 9), we wondered whether the combination of T_4 and menhaden oil feeding would be additive in their effects on mitochondrial respiration. To answer these questions, we fed BHE weanling rats a diet containing 6% corn oil or menhaden oil. For 7 days prior to measuring hepatic respiration, these rats were injected daily with either T_4 or vehicle. We found that compared with corn oil feeding, menhaden oil feeding lowered hepatic state 3 respiration and this reduction was lowered further with T_4 treatment.

Materials and Methods

Male weanling BHE rats (~50 g), 10/group, were fed a diet containing 10% casein, 10% lactalbumin, 64% sucrose, 1% AIN Vitamin Mix, 5% AIN Mineral Mix, 4% fiber (Alfacel) and 6% corn (Gift of Best Foods, Union, NJ), or menhaden oil (Gift of Zapata Haynie, Reedville, VA). Except for the oils, the diet ingredients were purchased from ICN Nutritional Biochemicals,

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Cleveland, OH. The fatty acid profiles of these oils are given in Table I. The rats were housed individually in hanging wire mesh cages in a room regulated for temperature (21 ± 1°C), humidity (45–50%), and light (lights on 0600–1800). Rats were weighed and food intakes were determined weekly. Care was taken to minimize autooxidation of the diet. The diet contained 400 mg of vitamin E/kg. The diet was mixed and stored frozen under a layer of nitrogen and fresh diet was offered daily just before the dark period. During the fourth week, the rats were injected with either T_4 (10 μg of T_4/100 g body wt) or 0.005 *M* NaOH, the vehicle for the T_4. The timing of the T_4 treatment was such that each T_4-treated rat was injected with T_4 for the 7 days preceding use. Thus, at 23 days of age they were started on the dietary treatments, at 53 days of age they were started on the T_4 treatment, and at 60 days of age they were killed. The rats were killed by decapitation, blood collected from the neck wound, and the livers quickly excised, chilled, and weighed. The sera were harvested after centrifugation (0–4°C, 500*g*) and used for the determination of T_4 (10), triiodothyronine (T_3) (11), insulin (12), glucose (13), and triglycerides (14). Mitochondria were prepared according to the procedures of Johnson and Lardy as previously described (4, 15). Oxygen consumption was determined at 3°C intervals from 4° to 37°C in the presence and absence of 0.16 *M* ADP as described previously (15). 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 (15). 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 using the equation 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 (1.987 cal/(mol.k) or 8.312 J/(mol.k), Ea the activation energy, and T the temperature in degrees Kelvin. Integration of Equation 1 and conversion to base 10 logarithms gives

$$\log\left(\frac{k_2}{k^1}\right) = \frac{Ea}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (2)$$

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.

The means were compared using an analysis of variance for this 2 × 2 experiment (16). Where appropriate, two means were compared for significant difference ($P < 0.05$) using the Student's t test (17).

Table I. Fatty Acid Composition of Corn and Menhaden Oil

Fatty acid	% of Total fatty acids	
	Corn oil	Menhaden oil
14:0	Trace	9
16:0	12.5	15
18:0	Trace	4
16:1	—	13
18:1	33	14
18:2	51	2
18:3	2	1
18:4	—	7
20:4	—	2
22:1	—	3
20:5	—	20
22:6	—	11

Results

In these rats, the T_4 and diet treatments had little effect on food intake, body weight gain, or relative liver size (data not shown). Analysis of variance of these data did not reveal any significant treatment effects. Table II shows the serum parameters in these rats. Rats fed

Table II. Serum Parameters in BHE Rats Fed Corn or Menhaden Oil and Treated with T_4 or Placebo

Diet	T_4[a]	T_4 (μg/dl)	T_3 (ng/dl)	Insulin (microunits/dl)	Glucose (mg/dl)	Triglycerides (mg/dl)
Corn oil	−	7 ± 1[b]	98 ± 8	40 ± 9	140 ± 10	113 ± 29
Corn oil	+	17 ± 2[c]	307 ± 28[c]	55 ± 14	157 ± 16	124 ± 24
Menhaden oil	−	6 ± 1	95 ± 13	57 ± 12	139 ± 5	64 ± 9[d]
Menhaden oil	+	7 ± 1[d]	188 ± 11[c,d]	57 ± 11	143 ± 10	55 ± 7[d]
			Analysis of variance[e]			
Diet		0.01	0.01	NS	NS	0.01
Thyroxine		0.01	0.01	NS	NS	NS
Interaction		0.05	0.01	NS	NS	NS

[a] T_4 was administered ip, 10 μg/100 g body wt/day.
[b] Mean ± SE, 10 rats/group.
[c] Within diet groups, the T_4 effect was significant ($P < 0.05$).
[d] Within T_4-treated groups, the diet effect was significant ($P < 0.05$).
[e] Significant treatment effects were noted as $P < 0.05$ or $P < 0.01$ or NS, nonsignificant.

corn oil and treated with T_4 had higher serum T_3 and T_4 levels than their nontreated controls. Those rats fed menhaden oil and treated with T_4 had lower T_4 and T_3 levels than T_4-treated corn oil-fed rats. Analysis of variance of these data indicated that both diet and T_4 treatments affected these values. Serum insulin and glucose levels were not affected by either dietary or T_4 treatments. Serum triglycerides were lower in both menhaden oil-fed groups than in the corn oil-fed groups. This is consistent with reports in the literature (18–20) on blood lipid-lowering effect of the omega 3-rich fish oils. Probably this was due to the effect of these oils on hepatic triglyceride release (21).

Mitochondria from rats fed menhaden oil did not differ from mitochondria from their corn oil-fed cohorts with respect to transition temperature (T_t), upper activation energy (Ea), or lower activation energy (Ea) (Table III). Transition temperature is the temperature at which the membrane lipid passes from a fluid lamellar phase to a liquid crystalline phase. This transition requires energy. Thus, the parameters of fluidity, T_t and Ea, indicate membrane fluidity. Within the menhaden oil groups, T_4 treatment resulted in mitochondria having a higher upper activation energy and a lessened lower activation energy. Thus, mitochondria from these rats had a broader transition temperature range than mitochondria from any of the other groups and the treatments of menhaden oil and T_4 were additive. A broader transition temperature range indicates that the variety of mitochondrial phospholipid fatty acids in the membrane were changing their physical state between lamellar and reverse hexagonal liquid-crystalline phases. This transition may have importance in the function of the lipid bilayer as the medium in which the proteins of the membrane function. Some of these proteins function by changing their shape. In a very fluid medium, this function is facilitated. If the medium is more crystalline, the function of the imbedded proteins is not facilitated. Thus, membrane fluidity has a role in the regulation of pathways such as respiration, anion/cation flux, and metabolic flux, which are membrane associated.

Analysis of variance of the data presented in Table IV shows that dietary fat type affected the state 3 succinate-supported respiration when the mitochondria were studied at 25° and 37°C. At 7°C there was no measurable state 4 respiration and very little state 3 respiration. At this temperature there were no diet or

Table III. Transition Temperatures and Upper and Lower Activation Energies for Succinate-Supported Respiration by Isolated Hepatic Mitochondria from BHE Rats Fed Menhaden or Corn Oil and Treated with T_4 or Placebo

Diet	T_4[a]	T_t (°C)	Upper Ea (kcal/mol)	Lower Ea (kcal/mol)
Corn oil	−	23.01 ± 1.11[b]	9.81 ± 0.55	18.13 ± 1.03
Corn oil	+	21.22 ± 1.11	8.64 ± 0.66	17.40 ± 1.78
Menhaden oil	−	23.45 ± 1.57	8.49 ± 1.07	19.09 ± 0.95
Menhaden oil	+	21.88 ± 1.62	9.75 ± 0.65	16.14 ± 0.88[c]

[a] T_4 was administered ip, 10 μg/100 g body wt/day.
[b] Mean ± SE, six rats/group.
[c] The effect of T_4 treatment was significant ($P < 0.05$) in rats fed the same diet.

Table IV. Effect of Dietary Fat and T_4 Treatment on the Succinate-Supported Oxygen Consumption by Isolated Hepatic Mitochondria at Three Temperatures

Diet	T_4	nmol O_2 consumed/mg protein/min									
		37°C				25°C				7°C	
		+ADP	−ADP	RC	ADP:O	+ADP	−ADP	RC	ADP:O	+ADP	−ADP
Corn oil	−	129 ± 14[a]	42 ± 3	3.1 ± 0.2	1.5 ± 0.1	71 ± 8	19 ± 3	4.2 ± 0.4	1.3 ± 0.1	11 ± 2	—
Corn oil	+	133 ± 15	39 ± 4	3.5 ± 0.1	1.5 ± 0.1	79 ± 8	27 ± 2[b]	3.4 ± 0.3	1.4 ± 0.2	11 ± 1	—
Menhaden oil	−	115 ± 8	38 ± 3	3.1 ± 0.3	1.6 ± 0.1	65 ± 5	19 ± 1	3.5 ± 0.4	1.3 ± 0.1	8.7 ± 1.3	—
Menhaden oil	+	100 ± 4[b,c]	46 ± 4	2.3 ± 0.2[b,c]	1.8 ± 0.1[c]	55 ± 2[b,c]	25 ± 2[b]	2.2 ± 0.1[b]	1.3 ± 0.2	9.5 ± 0.6	—
		Analysis of variance									
Diet		0.05	NS	NS	NS	0.05	NS	0.05	NS	NS	—
Thyroxine		NS	NS	NS	NS	NS	0.05	0.05	NS	NS	—
Interaction		NS	NS	0.05	NS	NS	NS	0.05	NS	NS	—

[a] Mean ± SE, $n = 6$.
[b] Within dietary treatment, the T_4 effect was significant ($P < 0.05$).
[c] Within T_4 treatment, the dietary effect was significant ($P < 0.05$).

T_4 treatment effects. At 25° and 37°C, mitochondria from both T_4-treated and untreated rats fed menhaden oil had lower respiratory rates than mitochondria from their corn oil-fed cohorts. This is consistent with the report of Yamaoka *et al.* (5) who used heart mitochondria from rats fed corn oil or sardine oil. Within the menhaden oil groups, T_4 had a significant effect on state 3 respiration. T_4 treatment and menhaden oil were additive with respect to their effects on state 3 respiration. This is consistent with our observations on membrane fluidity (Table III). Mitochondria from these rats consumed less oxygen in state 3 and more oxygen in state 4 with the result that the respiratory control (RC) ratio was significantly lower in this group than in the other three groups. At 25°C, analysis of variance revealed that both treatments and an interaction of these treatments affected the RC. Also, at 25°C but not at 37°C, T_4 was shown to have an effect on state 4 respiration. By using the simple *t* test for the group means, we found that most of the difference found significant upon analysis of variance could be attributed to the combined treatments of menhaden oil and T_4.

Discussion

Several reports have shown that the type of dietary fat can affect mitochondrial activity of the heart and liver (3–5). There are also numerous reports (e.g., 7–9) in the literature which indicate that T_4 treatment can affect these same parameters. This report is unique because it shows not only that fat type and T_4 can affect mitochondrial metabolism but that these two treatments were additive. The question which arises is how this additive effect occurred.

We reported (Table II) that fat type affected the T_3 levels in T_4-treated rats. Corn oil-fed rats had significantly more T_3 than did menhaden oil-fed rats when both groups were injected daily with the same dose of T_4. This suggests that the 5′-deiodinase reaction was more active in the corn oil-fed rats. Alternatively, it could also suggest that more rapid degradation of the T_3 might have occurred in the menhaden oil-fed rats. The latter suggestion is supported by the observation that the T_4-injected corn oil-fed rats had twice as much serum T_4 as did the T_4-injected menhaden oil-fed rats. Although BHE rats have been reported to be less active in the conversion of T_4 to T_3 (22), it is clear from the present work that exogenous T_4 can "push" the reaction forward such that T_3 levels can rise.

When T_3 levels rise a number of responses are elicited. Among them are a rise in the unsaturation of the membrane phospholipid fatty acids (2, 6–8). This results in a more fluid membrane. If the membrane is the plasma membrane, the cell will compensate for this rise in fatty acid unsaturation by increasing the amount of cholesterol, thereby keeping the membrane fluidity within very narrow limits. The mitochondrial membrane is not as closely controlled. It contains very little cholesterol to serve as a counterbalance to rising unsaturation in the phospholipid fatty acids. Thus, when rats are fed an unsaturated fat, the cell must accommodate these unsaturated fatty acids by down-regulating the desaturase enzymes and incorporating newly synthesized saturated fatty acids into the mitochondrial membrane.

In menhaden oil-fed rats, triglyceride output is decreased (21). In the present work, the diet consisted of 64% sucrose, which should have stimulated desaturase activity (via its effect on insulin) and 6% menhaden oil or corn oil. Since triglyceride output was presumed to be decreased in the menhaden oil-fed rats and since the fatty acids of the mitochondrial membrane would undergo rapid exchange with those in the cytosol, one could assume that more unsaturated fatty acids from the cytosol would be found in the membrane, making it more fluid. When T_4 treatment was superimposed on the dietary treatment, then fluidity was certain to rise and processes such as respiration, which depends, in part, on the ease with which its components can move about in the lipid milieu, would be affected. Hence, we saw a rise in the upper activation energy and a decrease in the lower activation energy and, in effect, a broader transition state.

One might ask how this could affect respiration. Note in Table IV that succinate-supported respiration was decreased in state 3 and increased in state 4 in the T_4-treated menhaden oil-fed rats. This resulted in a decrease in RC but also an increase, at 37°C, in the ADP:O ratio. This latter ratio means that more ADP was converted to ATP per unit of oxygen consumed than by the mitochondria from other groups. The effect of the diet was to provide more long chain unsaturated fatty acids and the effect of T_4 was probably to increase the unsaturation via the activity of the desaturase enzymes. Collectively then, these two treatments resulted in a milieu which favored tight coupling of respiration to ATP synthesis because it favored a more fluid lipid milieu.

There could be another explanation of the additive effects of T_4 and menhaden oil. Horrum *et al.* (23) suggested that T_4 stimulates state 4 respiration by creating a disequilibrium of redox states on or near cytochrome *b*. They found that thyroxine injection resulted in a more reduced redox state for cytochrome *c* in contrast to the relative oxidized state for cytochrome *b*. Thus, there is an increased content of cytochrome *b* and an unchanged content of cytochrome c_1. The result of these T_4-induced changes in the redox states of the cytochromes is an increase in respiration capacity and looser coupling. This latter explanation of the diet-T_4 additive effect seems more likely and we will need to determine whether such occurs.

Last, it should be pointed out that BHE rats are

not normal rats with respect to their responses to T_4 treatment. Normal rats when treated with T_4 increase their mitochondrial respiration, ATP synthesis, and shuttle activity (24–26) as well as mitochondrial ATPase activity (9) and cytosolic gluconeogenic activity (27, 28). As shown in the corn oil-fed rats in Table IV, T_4 by itself had little effect on state 3 respiration at 37°C and 25°C and influenced only slightly the state 4 respiration at 25°C. Earlier, we reported that T_4 treatment of BHE rats resulted in an increase in hepatic mitochondrial shuttle and ATPase activities (29) and stimulated glucose synthesis from lactate (30). These observations are consistent with those in the literature for normal rats. However, T_4 inhibited glucose synthesis from alanine (30). This is not consistent with the literature on T_4. In addition, T_4 counteracted the effect of feeding hydrogenated coconut oil on membrane phospholipid fatty acid saturation (6) but did not counteract the loss in respiratory control seen in rats fed hydrogenated coconut oil (31). These observations suggest that the inherent error in the lipemic BHE rat may reside in that portion of the mitochondrial metabolism involved in respiratory control. That portion may be induced in normal rats by T_4 but not in these rats. Hence, T_4 did not have the large expected stimulatory effect on oxygen consumption and respiratory control. Further work is needed to elucidate the reasons why BHE rats are abnormal in this respect.

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