# A Comparison of Magnesium Deficiency, Cold Acclimation and Thyroxine Administration on Mitochondrial Fatty Acid Composition[1] (36567)

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It has been reported by some that liver mitochondria of rats exposed to prolonged cold show a reduction in oxidative phosphorylation efficiency and exhibit more rapid swelling (1–3). Similarly it has been found that either thyroxine administration (4, 5) or dietary magnesium (Mg) deficiency (6–9) in animals at colony room temperature (24°) causes the same mitochondrial changes. In studies on the effects of dietary deficiencies in essential fatty acids, it has been shown (10, 11) that liver mitochondria from deficient animals have less unsaturated fatty acids, a more rapid rate of swelling, and decreased P/O ratios.

In view of the similar end-effects of cold acclimation, thyroxine administration and magnesium deficiency on mitochondrial uncoupling and resistance to swelling, we have performed experiments to see whether or not mitochondria from animals subjected to these three experimental situations show similar changes in patterns of distribution of unsaturated fatty acids.

*Materials and Methods.* Male Sprague-Dawley (Wisconsin) rats with an average body weight of 140 g were given glass-distilled water and fed *ad libitum* either a control or an Mg-deficient diet for 4 to 5 weeks. The composition of the control diet is shown in Table I. The Mg-deficient diet was prepared with the same composition as the control diet except that magnesium sulfate was replaced by dextrose. The control and the deficient diet by actual analysis contained 61 mg and 4 mg, respectively, of Mg/100 g diet. Each diet was fed to two separate groups of rats (10 to 15 rats/group) which were maintained at 23 ± 2° (warm acclimated) or at 5 ± 2° (cold acclimated) for the 4 to 5 week experimental period. An additional 31 rats were warm acclimated; and of these, 15 were fed the control diet, and 16 rats were fed the Mg-deficient diet. All 31 rats, after 4 weeks, were made hyperthyroid by daily injections of 2 mg/100 g body weight of L-thyroxine (sodium salt) (5) administered intraperitoneally for 8 days prior to sacrifice. All animals were housed individually in wire bottom cages, and light was cycled automatically every 12 hr starting at 6 a.m.

The rats were killed by decapitation, and serum and liver samples were obtained for analysis. Serum magnesium was determined by atomic absorption spectrometry (12), and liver mitochondria were isolated by the method of Hogeboom and Schneider (13) as described by Hoch and Lipmann (14). The mitochondrial pellet was suspended in 1 ml of 0.25 *M* sucrose solution and transferred to glass homogenizing tubes containing 10 ml of 2.5 *N* KOH in methanol (85%) solution. After homogenization, the total fatty acids (FA) were extracted by the method of Lasker and Theilacker (15) as modified by Williams and Platner (16). The fatty acids were methylated with boron trifluoride reagent as described by Metcalfe and Schmitz (17). The FA methyl esters were weighted and their compositions were analyzed by gas–liquid chromatography as previously described (18). In these studies the sum of the areas under the gas chromatographic peaks representing the following fatty acids were

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TABLE I. Composition of the Control Diet.[a]

| Ingredients | Percent |
|---|---|
| Dextrose | 60.8 |
| Soya assay protein[b] | 25.0 |
| Mineral salts[c] | 6.0 |
| Vitamins[d] | 2.0 |
| Corn oil (Mazola) | 6.0 |
| DL-Methionine | 0.2 |

[a] Our analysis showed the percentage composition of FA in the diet was as follows: palmitic, 12.2; stearic, 1.9; oleic, 25.2; linoleic, 59.2; and linolenic, 1.5.

[b] Water and alcohol extracted, obtained from GBI, Chagrin Falls, OH.

[c] Percentage composition: calcium carbonate, 16.60; calcium phosphate (dibasic), 47.60; copper sulfate, 0.017; ferric citrate, 0.333; magnesium sulfate, 5.00; manganese sulfate, 0.416; potassium chloride, 11.60; potassium iodide, 0.017; sodium chloride, 6.60; sodium phosphate (dibasic), 11.6; and zinc carbonate, 0.217.

[d] Mixture obtained from Nutritional Biochemical Corporation, Cleveland, OH. One kilogram of the mixture contained the following: vitamin A concentrate (200,000 units/g), 4.5; vitamin D concentrate (400,000 units/g), 0.25; alpha tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; *p*-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; thiamine hydrochloride, 1.0; calcium pantothenate, 3.0; biotin, 0.020; folic acid, 0.090; vitamin $B_{12}$, 0.00135.

equated to 100%: myristic (14:0), myristoleic (14:1), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), arachidonic (20:4) and docosahexaenoic (22.6). The percentages of the total unsaturated fatty acids (TUFA) were calculated by subtracting the total saturated fatty acids (TSFA) from 100. The diets were analyzed for FA composition using the same procedure as described for the mitochondria. All data were anlayzed for statistically significant differences between treatment means by using the Kruskal-Wallis H test (19) and the Mann-Whitney U test (20).

*Results.* In animals on a control diet, prolonged cold exposure caused a significant rise in serum magnesium (Table II), but in deficient animals it had no significant effect in preventing the lowering of serum magnesium. On the other hand, thyroxine had no effects on serum magnesium levels in animals on control or magnesium-deficient diets. The percentage compositions of fatty acids of the liver mitochondria lipids under the different experimental conditions are presented in Table III. As a result of Mg deficiency, the relative concentration of myristoleic acid increased in liver mitochondria of rats kept at 23° and decreased in those kept at 5°. The relative concentration of myristic acid decreased and that of docosahexaenoic acid increased in the latter group. In mitochondria of thyroxine-treated, Mg-deficient animals, the percentage of linoleic acid was decreased compared to the control group.

Comparing cold-acclimated rats on the control diet with the warm-acclimated control group (Table III), it is apparent that cold acclimation alone produced significant decreases in the percentages of palmitic and arachidonic acids and significant increases in the percentages of myristoleic, stearic, and linoleic acids. Similarly there was a significant decrease in the total unsaturated fatty acids in mitochondria of the thyroxine-treated animals on a control diet when compared with those of warm-acclimated controls. The effect of thyroxine in decreasing the total unsaturated fatty acids was more pronounced than the effects of cold (Table III). The significant reduction in the total unsaturation of liver mitochondria lipids was due in part to a significant increase in the proportion of stearic acid and significant de-

TABLE II. Rat Serum Magnesium Concentration (mg/100 ml).

| Treatment | Control diet | Mg-deficient diet | $p$[a] |
|---|---|---|---|
| Warm control | 1.96 ± 0.07[b] | 1.00 ± 0.05 | <.001 |
| Cold acclimated | 2.42 ± 0.08[c] | 1.14 ± 0.09 | <.001 |
| Thyroxine treated | 1.70 ± 0.03 | 0.99 ± 0.05 | <.001 |

[a] Probability level of significance for rats fed Mg-deficient diet, compared to control diet.

[b] Mean ± SEM.

[c] Significant ($p < .01$) when compared with the warm controls.

TABLE III. Percentage Composition of Fatty Acids from Liver Mitochondrial Lipids of Rats After Magnesium Deficiency, Cold Acclimation, and Thyroxine Administration.

| | Warm acclimated | | | Cold acclimated | | | | Thyroxine-treated warm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fatty acid | 1 Control | 2 Mg-deficient | (1 vs 2) $p$[a] | 3 Control | (1 vs 3) | 4 Mg-deficient | (3 vs 4) $p$ | 5 Control | (1 vs 5) | 6 Mg-deficient | (5 vs 6) $p$ |
| 14:0 | 0.9 ± 0.2[b] | 1.3 ± 0.3 | NS | 0.9 ± 0.1 | NS | 0.4 ± 0.1 | ≤.005 | 1.0 ± 0.2 | NS | 1.3 ± 0.1 | NS |
| 14:1 | 0.5 ± 0.1 | 1.7 ± 0.6 | NS | 1.2 ± 0.2 | ≤.001 | 0.2 ± 0.05 | ≤.001 | 0.7 ± 0.2 | NS | 0.6 ± 0.2 | NS |
| 16:0 | 22.5 ± 0.4 | 21.3 ± 0.8 | NS | 19.2 ± 0.8 | ≤.001 | 20.1 ± 0.3 | NS | 21.1 ± 0.5 | NS | 20.5 ± 0.7 | NS |
| 16:1 | 1.1 ± 0.3 | 1.6 ± 0.4 | NS | 0.5 ± 0.1 | NS | 0.7 ± 0.1 | NS | 1.7 ± 0.2 | NS | 1.4 ± 0.2 | NS |
| 18:0 | 15.2 ± 0.6 | 14.2 ± 0.7 | NS | 20.6 ± 0.5 | ≤.001 | 18.6 ± 1.1 | NS | 27.4 ± 0.9 | ≤.001 | 27.8 ± 0.6 | NS |
| 18:1 | 11.7 ± 1.5 | 11.8 ± 1.6 | NS | 9.7 ± 0.4 | NS | 10.9 ± 0.4 | NS | 11.9 ± 0.4 | NS | 12.3 ± 0.3 | NS |
| 18:2 | 18.8 ± 0.5 | 21.1 ± 0.7 | NS | 21.1 ± 0.4 | ≤.01 | 20.6 ± 0.5 | NS | 15.6 ± 0.5 | ≤.001 | 13.8 ± 0.3 | ≤.005 |
| 20:4 | 26.6 ± 1.1 | 25.1 ± 1.3 | NS | 24.5 ± 0.6 | ≤.05 | 25.4 ± 0.7 | NS | 17.7 ± 1.0 | ≤.001 | 19.1 ± 0.9 | NS |
| 22:6 | 2.9 ± 0.6 | 1.9 ± 0.4 | NS | 2.3 ± 0.2 | NS | 3.2 ± 0.2 | ≤.005 | 3.1 ± 0.8 | NS | 3.2 ± 0.5 | NS |
| TUFA[c] | 61.6 ± 0.6 | 63.2 ± 0.7 | NS | 59.3 ± 0.8 | ≤.02 | 61.0 ± 1.2 | NS | 50.7 ± 1.2 | ≤.001 | 50.4 ± 1.1 | NS |
| | (10)[d] | (14) | | (14) | | (15) | | (15) | | (16) | |

[a] Probability.
[b] Mean ± SEM.
[c] Total unsaturated fatty acids.
[d] Values in parentheses indicate number of animals in the group.

creases in the proportion of linoleic and arachidonic acids.

The combination of Mg deficiency with cold exposure or thyroxine treatment in general did not change the fatty acid alterations induced by cold or thyroxine treatment alone. However, there was a slight but significant reduction in the relative percentage of linoleic acid due to thyroxine treatment in the Mg-deficient animals. This reduction in linoleic acid was so small that it could be attributed to the effect of thyroxine alone, since Mg-deficiency alone produced no change in the relative percentage of linoleic acid.

*Discussion.* More than 90% of the total lipids in mitochondria are phospholipids, and these characteristically show a high degree of unsaturation (2). Although the morphological alterations in the mitochondria from Mg-deficient and thyroxine-treated rats have been reported to be similar (7), and both treatments produce uncoupling of oxidative phosphorylation (10), we did not find similar changes in the fatty acid composition of mitochondrial lipids in this study. Mg deficiency alone apparently caused no significant changes in the relative amounts of major fatty acid components such as palmitic, stearic, linoleic, or arachidonic acid. However, it did affect the relative amounts of myristic, myristoleic, and docosahexaenoc acid which together accounts for only about 4 to 5% of the total fatty acids present. On the other hand, thyroxine treatment alone produced marked changes in the percentages of stearic, linoleic, and arachidonic acids which constituted more than 60% of the total fatty acids in the mitochondria. The sharp decline of 18:2 may have been due to the fact that it is a very active substrate in $\beta$-oxidation; and according to Björntorp (21) is oxidized at the fastest rate of the long chain fatty acids. It would, therefore, appear that the decrease in the efficiency of oxidative phosphorylation in the mitochondria from Mg-deficient rats, as reported by others (10) is more likely due to a change in some other biochemical mechanism, rather than to an alteration in the fatty acid distribution.

Mitochondria isolated from the livers of rats chronically exposed to cold also have been reported (1, 8, 9) to show swelling and decreased phosphorylative efficiency. Previous experiments in this laboratory showed that the proportion of stearic acid was increased significantly in the liver mitochondria of cold-acclimated rats (18). In this study we have confirmed this finding. The increase in stearic acid was associated with a decrease in palmitic acid. This might indicate that in the cold-acclimated rat there was an increased conversion of palmitic to stearic acid possibly as a result of the increased activity of the chain elongation system of fatty acid synthesis in the mitochondria. Also, an increase in the percentage of linoleic acid with a decrease of arachidonic acid might suggest that in a cold-acclimated rat there is a depression in the conversion of linoleic to arachidonic acid in the liver microsomes which is reflected in the fatty acid distribution of the mitochondrial lipid.

The results in the present study showed that the liver mitochondria of thyroxine-treated and cold-acclimated rats undergo similar changes in fatty acid composition. In both there was a significant increase in the proportion of stearic acid with a decrease in the total unsaturated fatty acids, but the thyroxine effects were more pronounced and produced a greater increase in stearate and a more striking decrease in arachidonate. Thus, if we relate the alterations in fatty acid composition of the mitochondrial lipid to the structural and functional change of mitochondria from cold-acclimated animals and from thyroxine-treated animals, the increase in the proportion of stearic acid with the resultant decrease in total unsaturation might contribute to the swelling and fragility of the mitochondrial membrane and uncoupling of oxidative phosphorylation. Although Mg deficiency has been reported by others (10) to produce swelling and fragility of the mitochondrial membrane and uncoupling of oxidative phosphorylation, this does not appear to be the result of changes of fatty acids in the structural lipids of mitochondria. Thus uncoupling and swelling of mitochondria of magnesium-deficient animals is not associated with a change in total unsaturated fatty

acids. This lack of effect of magnesium deficiency was also evident with cold or thyroxine since it failed to substantially alter the fatty acid composition of mitochondria when in combination with these treatments, suggesting that antagonism between magnesium and thyroxine does not exist at this level.

*Summary.* A study was made to compare the effects of dietary magnesium deficiency, cold acclimation, and thyroxine administration on liver mitochondrial fatty acid composition. Groups of singly caged rats were fed synthetic diets containing either a control level (61 mg %) or a low level (4 mg %) of magnesium, and of these some were kept in the cold (5 ± 2°) and others at normal colony room temperature (23 ± 2°). A selected number of these rats were made hyperthyroid by ip injection of 2 mg/100 g body weight of L-thyroxine sodium daily for 8 days before sacrifice. The fatty acid composition of the mitochondrial lipids was determined by gas chromatography. Cold acclimation alone significantly decreased the percentages of palmitic and arachidonic acids and increased the percentages of myristoleic, stearic, and linoleic acids. Throxine treatment increased the percentage of stearic acid and decreased linoleic and arachidonic acids. Magnesium deficiency, on the other hand, had little effect on the major components of the mitochondrial fatty acids and no effect on the total unsaturated fatty acids. It is concluded that mitochondrial swelling and uncoupling produced by magnesium deficiency does not seem to be related to changes in the total unsaturation of mitochondrial fatty acids.

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