# Effect of Dietary Theophylline on Pyruvate Metabolism and Respiratory Activities in Liver Mitochondria[1] (36698)

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Theophylline is known to influence carbohydrate (1), and lipid metabolism (2, 3) as well as the levels of calcium (4, 5) and phosphorous (5). Studies on the mode of action of theophylline have shown it to competitively inhibit the enzyme cyclic 3′, 5′-nucleotide phosphodiesterase (6–8) which results in the accumulation of cyclic AMP. The physiological roles of cyclic AMP have been studied extensively and been shown to affect many cellular processes including gluconeogenesis, ketogenesis, insulin release, and cellular permeability (9, 10, 11). In addition, cyclic AMP activates pigeon heart pyruvate decarboxylase (12) and pyruvate carboxylase in intact rat liver mitochondria (13).

In the present study we investigated the effect of dietary theophylline on pyruvate metabolism and respiratory activities of rat liver mitochondria.

*Methods.* Male Sprague-Dawley rats, weighing approximately 135 g, were fed *ad libitum* either a synthetic test diet or the test diet plus theophylline.[2] In Expt 1, 0.5% theophylline was added and the animals fed for 16 days. In initial studies animals were not able to tolerate well the 0.5% theophylline, therefore the dosage was cut to 0.2% and the time extended to 28 days for Expt 2. The isolation of intact liver mitochondria, incubation, processing of samples, analysis for pyruvate, citrate and $^{14}CO_2$ incorporation, and respiratory activities were performed according to previously cited methods (14–16). Neutralized supernatants and whole blood were analyzed for acetoacetate and β-hydroxybutyrate according to the method of Williamson, Mellanby and Krebs (17). Glycogen was hydrolyzed to glucose according to the method of Pfleiderer (18). Glucose was then determined by the glucose oxidase method (Calbiochem).

*Results and Discussion.* As shown in Table I, the addition of 0.5% theophylline to the diet decreased both the final body weight and adipose tissue weight. This is in agreement with the findings of Brodie, Krishna and Hynie (3) who reported that theophylline stimulates lipolysis in rat epididymal fat pads. It has also been shown that cyclic AMP, which accumulates in the presence of theophylline, stimulates the adipose tissue lipase (9). Results in Table II show the concentrations of blood ketone bodies and glucose and liver glycogen for control and theophylline fed rats. A significant decrease in β-hydroxybutyrate levels, total ketone bodies, and the β-hydroxybutyrate to acetoacetate ratios was

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[2] Diet composition: vitamin-free casein, 18%; sucrose, 67%; corn oil, 10%; salt mixture 4%. Salt mixture (No. 2 USP XIII) contained: calcium biphosphate, 13.58%; calcium lactate·5 $H_2O$, 2.7%; potassium phosphate (dibasic), 23.98%; sodium diphosphate·2 $H_2O$, 8.72%; sodium chloride, 4.35%. Vitamin mixture contained in grams per 100 pounds of diet: vitamin A (200,000 units/g), 4.5; vitamin D (400,000 units/g), 0.25; α-tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; manadione, 2.25; *p*-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0; calcium pantothenate, 3.0; in milligrams per 100 pounds of diet: biotin, 20; folic acid, 90; vitamin B-12, 1.35. Diet was obtained from Nutritional Biochemicals. All animals were supplemented with 60 μg of thiamine hydrochloride administered orally three times a week. This dosage was found to be adequate for maintaining normal growth when rats were fed *ad libitum*.

TABLE I. Effect of Dietary Theophylline[a] on Epididymal Fat Pad Weights.[b]

| Rat treatment | Body wt (g) | Adipose tissue wt (g/100 g) |
|---|---|---|
| None | 191 ± 5 | 0.631 ± 0.066 |
| Theophylline | 116 ± 7 | 0.101 ± 0.037 |
| $p$ | <.001 | <.001 |

[a] Fed 0.5% theophylline for 16 days. Animals were fasted overnight.

[b] Results expressed as mean ± SEM for four animals.

observed. The significance of these observations will be discussed in more detail in a later section on the liver ketone body concentrations. The significant increase in liver glycogen observed in the theophylline fed rats seems to be opposition to reports on the effect of cyclic AMP. Sutherland and Robison (9) have discussed the role of cyclic AMP in glucose production and have noted its suppression of glycogen synthetase as well as overall stimulation of gluconeogenesis. Exton and Park (10) have studied the effects of cyclic AMP on perfused liver and have noted an increase in glucose and a decrease in glycogen levels with cyclic AMP treatment. The reason for these differences are not readily apparent.

In the theophylline fed rats there was also a significant decrease in pyruvate utilization and radioactive bicarbonate incorporation (Table III). There was no change, however, in the concentration of citrate or the ratio of pyruvate used to radioactive bicarbonate incorporated.

Cyclic AMP stimulates the multi-step conversion of pyruvate to phosphoenolpyruvate (11, 19–22). Although the exact method of control is not known, Haynes has shown that cyclic AMP stimulates pyruvate carboxylation in intact mitochondria (13) and that glucagon which is mediated by cyclic AMP can increase the rate of pyruvate transfer into the mitochondria. In addition, cyclic AMP stimulates lipolysis which in turn may deplete the fat stores. Since fatty acids activate pyruvate carboxylase and stimulate gluconeogenesis (14, 15, 21, 24–26), cyclic AMP may be indirectly involved in pyruvate utilization through its lipolytic action.

The evidence of lipolysis shown by decreased body and adipose tissue weight as well as decreased blood ketone body levels and the decrease in pyruvate utilization suggest that theophylline may reduce pyruvate metabolism through cyclic AMP stimulation of lipolysis rather than through the direct action on gluconeogenic enzymes as noted by Haynes (13). It is probable that this is a direct result of the pharmacologically high dose (0.5%) of theophylline fed.

Analysis of the supernatant for ketone bodies revealed a significant decrease in β-hydroxybutyrate, total ketone bodies, and the β-hydroxybutyrate to acetoacetate ratio. This is similar to the findings reported for blood in Table II. Williamson *et al.* (27) have noted that the β-hydroxybutyrate to acetoacetate ratio may be used to measure the NADH to $NAD^+$ ratio in mitochondria. Since β-hydroxybutyrate dehydrogenase is absent from the blood, a decrease in the

TABLE II. Effect of Dietary Theophylline[a] on Blood Ketone Bodies and Glucose Levels and Liver Glycogen.[b]

| Parameters examined | Control | Theophylline |
|---|---|---|
| Acetoacetate (μmoles/ml) | 0.058 ± 0.002 | 0.068 ± 0.010 |
| β-Hydroxybutyrate (μmoles/ml) | 0.569 ± 0.054 | 0.068 ± 0.019[c] |
| Total ketone (μmoles/ml) | 0.628 ± 0.053 | 0.136 ± 0.020[c] |
| Ratio β-hydroxybutyrate to acetoacetate | 9.87 ± 1.12 | 1.08 ± 0.40[c] |
| Blood glucose (mg/100 ml) | 66 ± 3 | 89 ± 10 |
| Liver glycogen (mg/g wet wt) | 0.199 ± 0.012 | 0.288 ± 0.022[c] |

[a] Fed 0.5% theophylline for 16 days. The animals were fasted overnight.

[b] Results expressed as mean ± SEM for four animals.

[c] $p < .05$ with respect to control.

TABLE III. Effect of Dietary Theophylline[a] on Pyruvate Metabolism in Rat Liver Mitochondria.[b]

| Metabolite changes | Experimental | | | |
|---|---|---|---|---|
| | Control | | Treated | |
| | Without OC[c] Group A | With OC Group B | Without OC Group C | With OC Group D |
| | ($\mu$moles/2.4 mg N/8 min) | | | |
| Pyruvate used | 20.3 ± 0.2[d] | 15.7 ± 0.6[e] | 16.8 ± 0.7[d] | 13.4 ± 0.8[e] |
| $^{14}CO_2$ incorporated | 8.5 ± 1.2[d] | 9.7 ± 0.3 | 6.4 ± 0.5 | 9.8 ± 0.3 |
| Citrate | 3.2 ± 0.2 | 2.2 ± 0.1 | 3.1 ± 0.1 | 2.5 ± 0.2 |
| Ratio pyruvate used/ $^{14}CO_2$ incorporated | 2.5 ± 0.3 | 1.6 ± 0.1 | 2.7 ± 0.1 | 1.5 ± 0.02 |
| $\beta$-Hydroxybutyrate | 0.258 ± 0.021[d] | 1.592 ± 0.093[e] | 0.094 ± 0.012[d] | 1.154 ± 0.117[e] |
| Acetoacetate | 0.034 ± 0.004 | 0.079 ± 0.004 | 0.038 ± 0.003 | 0.070 ± 0.007 |
| Total ketones | 0.293 ± 0.018[d] | 1.672 ± 0.096[e] | 0.133 ± 0.013[d] | 1.225 ± 0.124[e] |
| Ratio $\beta$-Hydroxybutyrate/ acetoacetate | 8.0 ± 1.3[d] | 20.1 ± 0.8[e] | 2.5 ± 0.3[d] | 16.7 ± 0.8[e] |

[a] Fed 0.5% theophylline for 16 days. Animals were fasted overnight.
[b] Results are expressed as mean ± SEM for four animals.
[c] OC = L-Octanoylcarnitine.
[d] $p < .05$ for Group A compared to Group C.
[e] $p < .05$ for Group B compared to Group D.

ketone body ratio implies a decrease in the NADH to $NAD^+$ ratio and a low concentration of reducing equivalents. These authors also noted an increase in the NADH to $NAD^+$ ratio accompanied gluconeogenesis from pyruvate in perfused liver. The decrease noted in this study confirms the observed decrease in pyruvate metabolism. The low levels of ketone bodies also suggest a depletion of storage lipids with theophylline feeding.

Fatty acids are known to inhibit oxidation of pyruvate in mitochondria from liver (25, 26, 28, 29). In the presence of L-octanoylcarnitine, the utilization of pyruvate, formation of citrate, and the ratio of pyruvate utilization to radioactive bicarbonate incorporation were significantly decreased in mitochondria from both control and theophylline fed rats. In addition, the octanoylcarnitine significantly increased the amount of radioactive bicarbonate incorporated in the theophylline fed but not in the control rats. In fact, there was no significant difference between bicarbonate incorporation in the control and the theophylline fed rats in the presence of octanoylcarnitine. Thus, it is probable that decrease in pyruvate carboxylation as suggested earlier is due to a decreased availability of fatty acids, which provide reducing equivalent, acetyl CoA and ATP.

As would be expected, the additions of the fatty acid significantly increased ketone body formation. Since the concentration of $\beta$-hydroxybutyrate increased to a greater extent than did the acetoacetate, there was a corresponding increase in the ketone body ratio. Moreover, the ratio was increased only 2.5-fold in the control but over 6-fold in the theophylline fed rats. Heimberg, Weinstein and Kohout (30) have also reported that cyclic AMP stimulates fatty acid oxidation and ketogenesis. The ketogenic effect may be quite substantial in the presence of high levels of exogenous free fatty acids, although small and inconsistent in the absence of these substrates.

Table IV summarizes the respiratory activities of liver mitochondria from control and theophylline fed rats. In state 3 where all required components are present and the respiratory chain itself is the rate limiting factor, theophylline feeding caused no change in oxygen utilization. The respiratory rate in state 4 where ADP is lacking and the respiratory control ratio (state 3 to state 4) were

TABLE IV. Effect of Dietary Theophylline[a] on Liver Mitochondrial Respiratory Activities.

| Rat treatment | Substrate (m*M*) | Parameters studied[b] | | | |
|---|---|---|---|---|---|
| | | Respiratory rate ($\mu$moles $O_2$/min/g protein) | | | |
| | | State 3 | State 4 | RC | ADP/O |
| Control | Succinate (7.5) | 77.8 ± 2.5 | 14.90 ± 0.44 | 5.25 ± 0.25 | 1.72 ± 0.03 |
| Theophylline | Succinate (7.5) | 84.2 ± 1.8 | 12.80 ± 0.31 | 6.58 ± 0.22 | 1.80 ± 0.02 |
| *p*[c] | | NS | <.005 | <.025 | <.001 |
| Control | α-Ketoglutarate (7.5) | 31.7 ± 1.2 | 6.96 ± 0.48 | 4.94 ± 0.72 | 2.52 ± 0.05 |
| Theophylline | α-Ketoglutarate (7.5) | 32.4 ± 1.3 | 5.70 ± 0.21 | 5.61 ± 0.17 | 2.70 ± 0.04 |
| *p* | | NS | NS | NS | <.025 |
| Control | Pyruvate (7.5) | 22.1 ± 1.7 | 6.24 ± 0.48 | 3.54 ± 0.07 | 2.65 ± 0.08 |
| Theophylline | Pyruvate (7.5) | 19.4 ± 2.3 | 5.29 ± 0.39 | 3.73 ± 0.42 | 2.76 ± 0.06 |
| *p* | | NS | NS | NS | NS |

[a] Fed 0.2% theophylline. Animals were not fasted.

[b] Results are expressed as mean ± SEM for six animals.

[c] Probability of significant difference between means of control and of experimental values by Student's *t* test. NS indicates "not significant" ($p > 0.05$).

significantly increased only when succinate was used as the substrate. The ADP/O ratios which represent the efficiency of oxidative phosphorylation were slightly but statistically significantly increased when either succinate or α-ketoglutarate was used as a substrate. The reason for this increase in the ADP/O ratio remains obscure.

*Summary.* Diets containing theophylline were fed *ad libitum* to rats. Theophylline caused a significant decrease in body weight and adipose tissue weight. A significant decrease in the blood and liver β-hydroxybutyrate level and the β-hydroxybutyrate to acetoacetate ratio was noted for rats fed theophylline. Theophylline feeding also elevated the blood glucose level and the concentration of liver glycogen.

Rat liver mitochondria from control and theophylline fed rats were incubated in the presence of ATP, $Mg^+$, pyruvate and radioactive bicarbonate. Theophylline significantly decreased pyruvate utilization and radioactive bicarbonate incorporation. Addition of L-octanoylcarnitine decreased pyruvate utilization and increased the total ketone bodies as well as their ratio in both groups.

Respiratory activities for control and theophylline fed rats were determined using succinate, α-ketoglutarate, and pyruvate as substrates. The efficiency of oxidative phosphorylation was significantly increased in the theophylline fed rats when succinate or α-ketoglutarate was used as the substrate.

It is concluded that pharmacologically large dosages of dietary theophylline deplete storage lipids through activation of lipases. This in turn decreases pyruvate carboxylation in mitochondria.

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