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# INVESTIGATIONS OF THE EFFECTS OF THEOPHYLLINE ADMINISTRATION ON CARNITINE ACETYLTRANSFERASE ACTIVITY OF RAT HEART

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The effects of oral theophylline administration (100 mg/kg b.w./day) on the activity of carnitine acetyltransferase (CAT) of rat heart for five-week interval treatment were studied. The result indicated that the body weights of placebo groups were not significantly changed as compared to control groups ($P < 0.1$), but theophylline treatment caused a significant decrease in the body weights of rat ($P < 0.01$) as compared to either control or placebo groups throughout the five-week interval treatments. Daily administration of theophylline to rats did not significantly affect heart weights as compared to either control or placebo groups ($P < 0.1$) for the five week-interval treatments. Our data indicated that the activity of CAT was not significantly changed in placebo groups as compared to control groups ($P < 0.1$), however, there was a significant increase in the activity of CAT in heart of theophylline-treated groups ($P < 0.01$) as compared to either control or placebo groups. The increase in the activity of CAT was noticed in the first three weeks of theophylline treatments followed by a gradual return toward normal activity by the fourth and fifth weeks of continued treatment. The observed changes in activity of CAT of heart might be due to theophylline-enhanced mobilization of lipid from adipose tissues which consequently stimulated increased L-carnitine transport into the heart tissues to form fatty acyl-carnitine groups for subsequent $\beta$-oxidation inside the heart mitochondria. Accumulations of acyl-carnitine groups in heart mitochondria may increase the catalytic action of CAT and possible mechanisms are discussed.

*Keywords:* Rat heart; L-carnitine; Carnitine acetyltransferase; Theophylline; Lipolysis; $\beta$-oxidation

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## INTRODUCTION

Carnitine acetyltransferase (acetyl-CoA: carnitine o-acetyltransferase, EC 2.3.1.7, CAT), catalyzes the reversible transfer of short-chain acyl groups (2–6 carbons in length) between coenzyme A (CoA) and L-carnitin[1,2] according to the reaction:

$$\text{Acyl-carnitine} + \text{CoA} \rightleftharpoons \text{Acyl-CoA} + \text{L-carnitine}. \quad (1)$$

The importance of CAT stems from its role in $\beta$-oxidation of fatty acids, in maintaining acetyl-CoA homeostasis and also in detoxification of certain poorly metabolized branched-chain acyl groups generated by amino acid metabolism.[3] CAT is widely distributed in animal tissues, and is present as a membrane-bound enzyme in mitochondria, microsomes and peroxisomes. The shuttling of short-chain acyl groups between CoA and L-carnitine by CAT gives this enzyme an important role in intermediary metabolism. It facilitates transfer of short-chain acyl groups across the inner mitochondrial membrane[4] which helps in maintaining acetyl-CoA/CoA homeostasis across cellular organelles, especially in the mitochondria.[5] By coupling CoA to branched-chain acyl groups generated from amino acid catabolism and transporting them out of mitochondria, CAT helps in detoxifying these poorly metabolisable secondary products.[3–6] Clinically, CAT is used in evaluating L-carnitine and its ester levels in body fluids and tissues of many physiological,[7] pathological,[8,9] and exogenous factors that promote lipolysis are known to have profound effects on the L-carnitine levels.[10,11] This has gained importance, as L-carnitine and its esters are increasingly being implicated in both congenital as well as inherited disorders.[12]

CAT has been purified and well characterized from human liver,[13] rat liver and heart,[14] mouse liver,[15] pigeon muscle,[16] yeast[17] and camel muscle.[18–20]

Theophylline (1,3-dimethylxanthine) is a frequently used drug in the treatment of acute and chronic obstructive lung disease, in modern therapeutics[21] and in the management of apnea of prematurity.[22] It is known for its narrow therapeutic range, interactions with other drugs and many side effects. Minor side effects tend to occur in some patients with plasma levels above 15 μg/mL which are especially frequent with levels above 25 μg/mL (therapeutic range 10–20 μg/mL).[23] Major toxic complications are cardiac arrhythmias, hypotension and seizures and are often difficult to control. These toxic events can be lethal or lead to permanent neurological damage despite optimal supportive treatment and extracorporeal drug removal.[24]

It is known to stimulate skeletal muscle,[25] central nerve system respiratory centers[26] and relax airway smooth muscle.[27] Theophylline causes an increased lipolysis in the adipose tissue and consequently enhances the levels of plasma free fatty acid.[27] Accumulation of cAMP levels following inhibition of phosphodiesterase,[28] and antagonism of adenosine receptors have been also reported due to theophylline treatment.[29] Recently, we investigated the effect of oral theophylline administrations on the L-carnitine levels in the skeletal muscle and the liver of rats. A significant rise in total carnitine and long-chain acyl-carnitine levels was observed in the skeletal muscle but not in the liver.[30] Moreover, we observed that theophylline feeding caused a significant increase in plasma levels of L-carnitine in rats.[31]

The objective of the present study was to determine the effect of daily administration of theophylline on the activity of carnitine acetyltransferase in rat heart. To the best of our knowledge, no data are available on the effect of theophylline treatments on carnitine-dependent enzymes in any species.

## MATERIALS AND METHODS

### Chemicals

L-Carnitine hydrochloride, tris-(hydroxymethyl)aminomethane (Tris), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid disodium salt (EDTA) and lithium salt of acetylcoenzyme A, were purchased from Sigma Chemical Company, St Louis, MO, USA. Theophylline was obtained from Fluka Chemie AG, Buchs, Switzerland. All other chemicals used were of analytical grade, and glass distilled water was used throughout.

### Animal Care

A total of 150 male Wistar rats weighing between 200–265 g were obtained from the Breeding Laboratory, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. They were housed separately and fed water and rodent chow *ad libitum*. They were subjected to a one-week acclimatization period in an air-conditioned room maintained at 21°C with a relative humidity of 60%. A recurring cycle of 12 h each of light and darkness periods was maintained throughout the experimental period. At

the beginning of the experiment, the animals were randomly divided into three groups, each comprising of 50 rats in properly suspended metabolic cages with a stainless steel wire-mesh floor.



### Dosing Method

Group 1 (control), 2 (placebo) and 3 (theophylline-treated) consisted of control without dosing, placebo receiving saline solution and treated receiving theophylline solution, respectively. An aqueous solution of theophylline (100 mg/kg b.w.) prepared freshly on the day of treatment was dosed orally every day to each rat in the treated group via a gastric lavage technique using a curved needle intubator. Placebo group rats received the equivalent amount of saline solution in the same volume. The solution was introduced directly into the stomach and failure to regurgitate ensured that the entire dose remained inside. At intervals of 0, 1, 2, 3, 4 and 5 weeks, 10 rats from each group were killed under ether anesthesia, their hearts removed quickly, weighed and frozen in liquid nitrogen. They were stored in a frozen condition at −70°C until further processed.

### Sample Preparation

Hearts were homogenized (10% w/v) in ice-cold 50 mM potassium phosphate buffer pH 7.5 containing 0.5 mM EDTA and 0.1% Triton X-100 using a Potter-Elvehjem Teflon-glass homogenizer, at 4°C.

### Enzyme Assay

CAT activity was measured in the direction of acetyl-CoA formation. The colorimetric procedure described previously by Alhomida *et al.*[18–20] with a slight modification was used in the current study. The assay is based on measuring the initial rate of total CoA formation, by the DTNB reaction from acetyl-CoA, by heart homogenate separately with and without L-carnitine. Cuvette A contained 100 mM Tris HCl buffer, pH 7.8, 1.25 mM EDTA, 0.1 mM DTNB, 0.15 mM acetyl-CoA and 1.25 mM L-carnitine in a final volume of 1.0 mL to measure the total CoA formed from acetyl-CoA by CAT plus all other competing reactions that produce CoA such as acyl-CoA hydrolase, as well as any other reactions that produce reduced thiol groups. Cuvette B contained the identical substances in cuvette A except that 1.25 mM L-carnitine solution was omitted to measure the total CoA formed by the competing reactions, the hydrolase assay, minus the

CAT activity. The CAT activity was calculated as follows: Total CoA formed by CAT = total CoA formed in cuvette A minus total CoA formed in cuvette B.

The reaction was initiated by the addition of 10 μL heart homogenate, mixing immediately and the rates were followed at 25°C by monitoring the change in absorbance at 412 nm using a LKB Ultrospec II recording spectrophotometer. The molar extinction coefficient ($\varepsilon$) of 13600 $M^{-1} cm^{-1}$ for 5′-thio-2-nitrobenzoate was used for the calculations. One unit of CAT activity is defined as the amount of enzyme catalyzing the release of 1 nmol CoA/min/mg NCP under the assay conditions.

To evaluate the possible interference of theophylline with the analytical determination of CAT a theophylline solution was added to 1 mL samples of heart homogenate, and the activity of CAT in the samples was determined and compared to values obtained before theophylline addition. No interference was observed for theophylline concentrations of 50, 100, and 150 mg/mL.

### Protein Determination

Non-collagen protein (NCP) was determined by the procedure described by Lilienthal *et al.*[32] 50 μL heart homogenate was added to 50 mM NaOH and incubated at room temperature for 18 h. After centrifugation, the protein was then estimated by the modified Lowry method using bovine serum albumin as protein standard.[33]

### Determination of Theophylline Levels

Theophylline was measured using a fluorescence polarization immunoassay method (Abbott TDx system, Abbott Laboratories, Wokingham, Berks, UK).

### Statistical Analysis

The data from each sample were run in duplicate and the CAT activity was expressed as means ± SD nmol/min/mg NCP for $n = 10$ rats per week. The Shapiro-Wilk test was applied to all variables to check for normal distribution. For normally distributed variables, the statistical significance of differences was determined by approximate parametric tests (Student's $t$, Snedecor's $F$, etc.). For non-normally distributed variables, non-parametric

tests were used (Wilcoxon's test, etc.). Means were considered significantly different if $P < 0.05$.[34]

## RESULTS

The effects of orally administrated theophylline (100 mg/kg b.w./day) on the activity of heart CAT were evaluated in adult male rats for five-week interval treatments. Figure 1 shows the means ($\pm$ SD) of body weight of control, placebo and theophylline-treated rats for five-week interval treatments. No significant difference in body weight was observed in placebo groups as compared to control groups ($P < 0.1$). However, the theophylline-treated groups showed a significant reduction in body weight as compared to either control or placebo groups ($P < 0.01$).

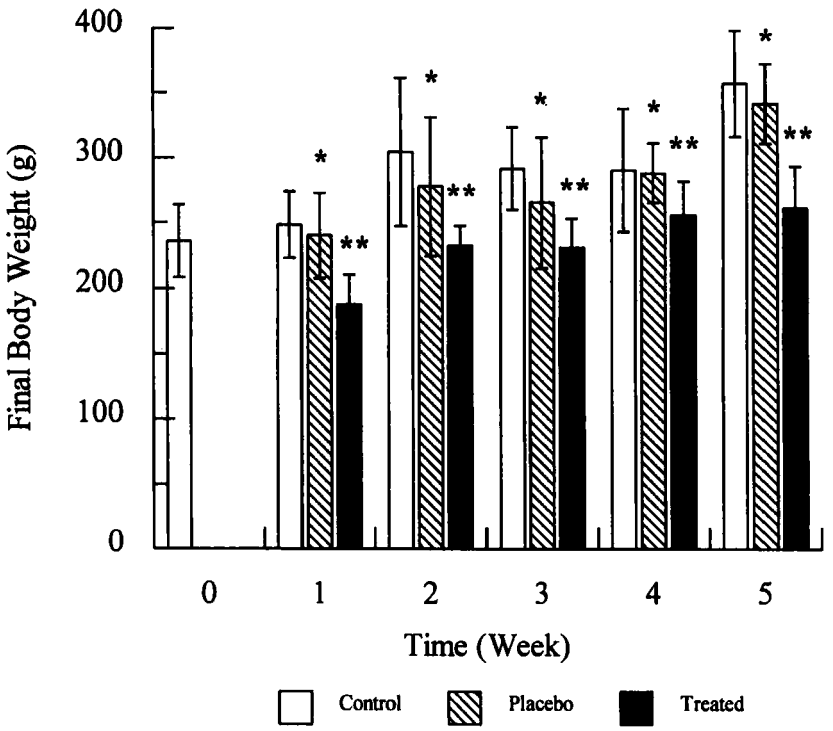


FIGURE 1 Final body weight of control, placebo and theophylline-treated rats for five-week interval treatments. Data are expressed as means $\pm$ SD, for $n = 10$ rats. *Values are not significantly different as compared to control groups, $P < 0.1$. **Values are significantly low as compared to either control or placebo groups, $P < 0.01$.

Figure 2 shows the means (±SD) of heart weight of control, placebo and theophylline-treated rats for five-week interval treatments. There was no significant difference in absolute heart weight in theophylline-treated groups as compared to either control or placebo groups ($P < 0.1$).

The means (±SD) of carnitine acetyltransferase activity of control, placebo and theophylline-treated heart of rats for five-week interval treatments are given in Table I. Our result indicated that the activity of CAT was not significantly different in placebo groups as compared to control groups ($P < 0.1$) for all five-week interval studies, however, theophylline administration caused a significant increase in the activity of CAT (>13%) as compared to either control or placebo groups ($P < 0.01$). The increase observed was sustained for the first, second and third weeks ($P < 0.01$, respectively) with a gradual fall back towards control activity of CAT. The fourth week CAT activity was less than that of the third week ($P < 0.1$). At

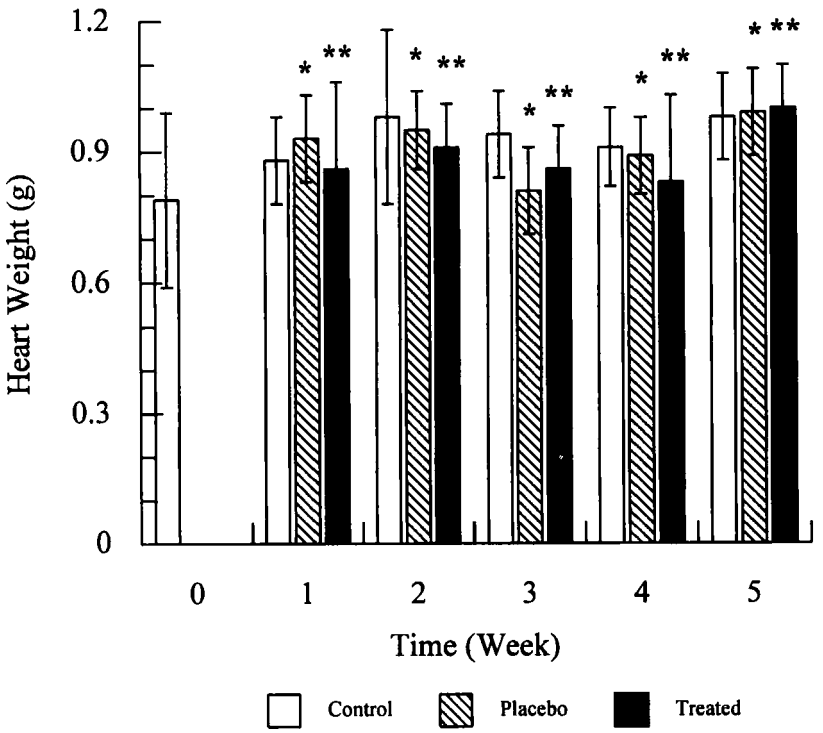


FIGURE 2 Heart weight of control, placebo and theophylline-treated rats for five-week interval treatments. Data are expressed as means ± SD, for $n = 10$ rats. *Values are not significantly different as compared to control groups, $P < 0.1$. **Values are not significantly different as compared to either control or placebo groups, $P < 0.1$.

TABLE I Carnitine acetyltransferase (CAT) activity in rat heart in control, placebo and theophylline-treated groups for five-week interval treatments

| *Time* (*Week*) | *CAT Activity* (nmol/min/mg *NCP*) | | |
|---|---|---|---|
| | *Control* | *Placebo* | *Treated* |
| 0 | 66.8 ± 11.3 | 65.8 ± 9.8 * | 75.2 ± 11.3 ‡ |
| 1 | 63.5 ± 8.5 | 55.9 ± 11.2 * | 73.3 ± 8.8 ‡ |
| 2 | 58.9 ± 10.6 | 62.1 ± 13.2 * | 78.6 ± 6.9 ‡ |
| 3 | 62.2 ± 12.2 | 67.3 ± 15.2 * | 76.8 ± 4.3 ‡ |
| 4 | 63.6 ± 10.7 | 57.8 ± 13.7 * | 65.9 ± 4.0 † |
| 5 | 66.2 ± 12.4 | 61.8 ± 14.6 * | 62.3 ± 4.6 † |

Data are expressed as means ± SD, $n = 10$ rats/week.
* Values are not significantly different as compared to control groups, $P < 1.0$.
† Values are not significantly different as compared to either control or placebo groups, $P < 0.1$.
‡ Values are significantly high as compared to either control or placebo groups, $P < 0.01$.

the fifth week, even though theophylline treatments were continued, no changes in the activity of CAT were noticed ($P < 0.1$) (Table I).

## DISCUSSION

A novel finding was that daily theophylline feeding induced changes in the activity of carnitine acetyltransferase in the heart of adult male rats. The efficacy of theophylline as a metabolic-inducing agent by increasing plasma levels of free fatty acids has been known for several years.[27] Since CAT facilitates transfer of short-chain acyl groups across the inner mitochondrial membrane[4] which helps in maintaining acetyl-CoA/CoA homeostasis across cellular organelles, especially in the mitochondria.[5] In this study we attempted to investigate the effect of theophylline feeding on the activity of CAT in rat heart.

In the present study, a dose of 100 mg/kg b.w./day was administered orally which resulted in a mean ± SD plasma theophylline concentration of 15.9 ± 0.7 μg/mL (initial study). This concentration is within the safe therapeutic range employed for humans (5–20 μg/ml).[23,35] At this dose, theophylline has been employed in bronchodilation in acute bouts of asthma and in the management and prevention of neonatal apnea.[36]

Our results indicated that body weight reductions were observed in theophylline-treated rats. However, the mean absolute heart weight was not significantly different from either control or placebo groups. These changes were, therefore, considered as related to treatment with theophylline. Recently we reported that theophylline treatment caused an increased food intake in theophylline-treated compared to either control or placebo

groups[30] which confirms the findings of Scammell and Fregly[37] who have reported that theophylline administration for two weeks increased food intakes in rats. The mechanism by which theophylline feeding caused these physiological changes, such as weight loss (i.e. mobilization of stored fat reserves),[38] a rise in basal metabolic rate[39] and increase in urinary excretion,[40] is unknown. It may involve an effect on hormone secretion e.g. thyroid and thyrotropin, thyroid stimulating hormone (TSH). In fact we recently reported that theophylline treatment caused a significant increase in plasma levels of L-carnitine in rats.[31] Moreover, we also observed that theophylline feeding caused a significant increase in urinary L-carnitine excretion in rats (submitted for publication). Indeed, Scammell and Fregly[37] observed that theophylline administration to rats caused an increase in both food consumption and faecal bulk. They suggested that theophylline administration may have a direct effect on the thyroid gland or an increase in the sensitivity of the gland to TSH.[37]

An interesting observation coming from this study is the finding of a marked elevation in the activity of CAT in rat heart after theophylline treatment. This result along with the above observations suggested that an elevation in the activity of CAT might be due to increased conversion of free carnitine to acyl-carnitine inside heart tissues. Two mechanisms could account for these changes. First, it is well known that CAT has an essential important role in maintaining CoA availability by converting accumulating acyl-CoA groups into the corresponding acyl-carnitine groups.[1-3] Indeed, we recently reported that theophylline treatment caused a striking increase in the skeletal muscle L-carnitine levels in rats, while no such changes in hepatic tissues were noticed.[30] Furthermore, we also observed that theophylline treatment caused a significant increase in total, free, short-chain acyl and long-chain acyl carnitine concentrations in heart (submitted for publication). The rise in the L-carnitine levels in the heart may demonstrate an increased flow of fatty acids into this tissue for subsequent transference from acyl-CoA groups to form the corresponding acyl-carnitine groups as catalyzed by CAT. Secondly, it could be that inversion of CAT might occur, causing a withdrawal of acyl-carnitine groups out of mitochondria. Either way, an increase in the activity of CAT has been suggested to protect cellular metabolism under conditions of abnormal acyl-CoA groups build up.[4] The efficacy of theophylline as a metabolic-inducing agent has been known for several years. For example, the effect of theophylline feeding on fat cells, phosphodiesterase inhibition, release of intracellular bound calcium, antagonism to the effects of endogenous adenosine, result in increasing cAMP accumulations and lipolysis in response to

lipolytic hormones.[27–29] Therefore, the increase in L-carnitine levels would help circumvent the enhanced fatty acids that are liberated due to increased lipolytic effects of theophylline.

Our data showed that there was a "tolerance" effect to theophylline feeding in adult male rats. Results showed that the activity of CAT gradually returned to normal activity by the fourth week despite the continuous theophylline treatment (Table I). This could be the result of "adaptation" in rats for long-term feeding. For example, such "adaptation" may include an increase in catabolism of theophylline in the tissues, particularly the liver, or an increase of theophylline excretion in kidneys. It should be noted that rats are probably more resistant to long-term theophylline feeding as suggested by Scammell and Fregly.[37]

In conclusion, the data showed a decrease in the body weight in theophylline-treated rats as compared to either control or placebo groups whereas there were no significant changes in both heart weight and the relative heart weight (g/100 g b.w.). Moreover, daily administrated theophylline to adult male rats also caused a striking increase in the activity of CAT in the rat heart as compared to either control or placebo groups. These changes in the activity of CAT may result from a mobilization of lipid from adipose tissues which consequently enhances an increased L-carnitine levels which is then transported into the heart tissues to form fatty acyl-carnitine groups for subsequent $\beta$-oxidation. These results are consistent with the hypothesis that theophylline feeding leads to increased fat lipolysis. It should be noted that we considered the possibility that theophylline treatment interfered with the analytical determination of the activity of CAT. However, estimation of the activity of CAT in heart homogenate, in the presence or the absence of theophylline, differed by less than 3%.

The results raise several questions that need further investigations at an intracellular level in tissues and/or key organs. For example, would supplementation of L-carnitine help in restoring normal activity to theophylline-treated rats? Does theophylline alter L-carnitine metabolism, L-carnitine-dependent enzymes or its distribution in plasma and/or tissues? These and other questions are currently being studied in our laboratory.

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