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Investigation of the Effect of Theophylline Administration on Total, Free, Short-Chain Acyl and Long-Chain Acyl Carnitine Distributions in Rat Renal Tissues

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This study is conducted to investigate the effect of oral theophylline administration on total (TC), free (FC), short- (SC), long-chain acyl (LC), acyl (AC) carnitine distributions as well as the ratio of acyl to free carnitine (AC/FC) in rat renal tissues. Theophylline was administrated at 100 mg kg^{-1} body weight day^{-1}, and effects were monitored after a treatment period that lasted between 1 week and 5 weeks. The results indicated that theophylline administration leads to significantly higher concentrations of TC, FC, SC, L and AC in renal tissues as compared to those of control and placebo groups ($P < 0{\cdot}001$). Moreover, the ratio of AC/FC was significantly increased ($P < 0{\cdot}001$) as compared to either control or placebo groups. These changes may result from theophylline-enhanced mobilization of lipids from adipose tissues, which consequently stimulates an increased carnitine transport into the renal tissues to form acylcarnitines for subsequent β-oxidation inside the renal mitochondria. © 1998 John Wiley & Sons, Ltd.

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KEY WORDS — acylcarnitine; carnitine; kidney; rat; lipolysis; theophylline

INTRODUCTION

Carnitine (L-3-hydroxy-4-*N*-trimethylaminobutyrate) is a quaternary amine that is synthesized from lysine and methionine in the liver, kidney and brain. It is an essential cofactor that plays an important role in fatty acid metabolism.[1] Since acyl-CoA ester cannot penetrate the inner mitochondrial membrane, they are trans-esterified to fatty acylcarnitine esters by carnitine acyltransferases, and translocated across the inner mitochondrial membrane by carnitine: acylcarnitine translocase in mitochondrial matrix where they undergo β-oxidation.[2] A second important function ascribed to carnitine is maintaining mitochondrial acetyl-CoA/CoASH homeostasis.[3] This function is of great significance since CoASH is an intermediate in several metabolic pathways. Another established function of carnitine is to detoxify certain poorly metabolized branched-chain acyl-CoAs that are generated from amino acid catabolism inside the mitochondria.[1,2]

Carnitine has widespread occurrence in nature and is believed to be present in all animals, many plants and microorganisms; its concentration, however, varies over a wide range between species and tissues.[1] Highest concentrations of carnitine are reported in tissues that require high amounts of energy such as skeletal and heart muscles.[3,4] Carnitine, when present, exists either in free form (FC) or esterified as acylcarnitine (AC). Many physiological and pathological conditions affect serum, urine and tissue carnitine distributions in both animals and humans including lifestyle, diabetes, renal and hepatic diseases, and some metabolic disorders.[5–9]

Theophylline (1,3-dimethylxanthine) is a drug frequently used in the treatment of acute and chronic obstructive lung disease, in modern therapeutics[10] and in the management of apnea of prematurity.[11] Minor toxic effects may occur in some patients at plasma levels above 15 μg ml^{-1},

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and are especially frequent with levels above 25 μg ml^{-1} (therapeutic range 10–20 μg ml^{-1}).[12] Major toxic complications are cardiac arrhythmias, hypotension and seizures, and are often difficult to control. These complications can be lethal or lead to permanent neurological damage despite optimal supportive treatment and extracorporeal drug removal.[13] Theophylline stimulates skeletal muscle,[14] central nerve system respiratory centres[15] and relaxes airway smooth muscle.[16] Theophylline causes an increased lipolysis in adipose tissue and consequently enhances the level of plasma free fatty acid.[16] Accumulation of cAMP levels following inhibition of phosphodiesterase,[17] and antagonism of adenosine receptors have also been reported due to theophylline treatment.[18] Recently, we reported that theophylline administration induced a significant increase in the level of total, free and long-chain acylcarnitine in the cardiac tissues and skeletal muscle but not in the liver of rats.[19,20]

The objective of the present study was to investigate the effect of daily administration of theophylline on total (TC), free (FC), short- (SC), long-chain acyl (LC) and acyl (AC) carnitine as well as the ratio of AC to FC in rat renal tissues.

MATERIALS AND METHODS

Chemicals

Carnitine hydrochloride, trs-(hydroxymethyl)-aminomethane (Tris), 5, 5′-dithiobis(2-nitrobenzoic acid), ethylenediaminetetraacetic acid disodium salt, lithium salt of acetylcoenzyme A and carnitine acetyltransferase (EC 2.3.1.7) were purchased from Sigma Chemical Company, MO, U.S.A. Theophylline was purchased from Fluka, AG, Chemische Febrik, Switzerland, Catalase (EC 1.11.1.6) was purchased from Winlab, Maidenhead, Berkshire, U.K. All other chemicals used were of analytical grade, and glass distilled water was used throughout.

Animal Care

A total of 150 adult male Wistar rats weighing 200–267 g (236 ± 31·5 g) were obtained from the College of Pharmacy, Breeding Laboratory, King Saud University, Riyadh, Saudi Arabia. Upon arrival, animals were individually identified, housed in clean and properly suspended metabolic cages, and given water and standard rodent chow *ad libitum*. The animal room was air-conditioned and maintained at 21 ± 1·5°C with a relative humidity of 60 ± 20 per cent. A recurring cycle of 12 h light (06.00 hours to 18.00 hours) and 12 h of darkness was put into effect. After 1-week acclimation period, animals were randomly assigned to one of three groups, each consisting of 50 rats. Group 1 was control without dosing, group 2 was placebo treated with saline solution, and group 3 was theophylline fed at 100 mg kg^{-1} body weight day^{-1} as described below.

Sample Collections

Rats were placed individually in metabolic cages for 24 h for urine collection and measurement of food intake. At the end of each week, 10 rats from each group were lightly anaesthetized with diethylether. Blood was immediately collected from EDTA-tubes using cardiac puncture. Plasma samples were obtained by centrifugation at 4°C. Rats were killed under ether anaesthesia, their kidneys were removed quickly, weighed and immediately placed in liquid nitrogen. All samples were kept frozen at −80°C until assay.

Dosing Method

Normal saline solution or theophylline solution was dosed orally via a gastric lavage technique. A curved-needle intubator (3″ length, 18 gauge, 2·25 mm ball diameter, Popper & Sons, Inc., New York, U.S.A.), was attached (via a Luer-lock) to a 3 ml plastic syringe. The intubator-attached syringe was filled with the desired solution. Dosing was accomplished by holding the unanaesthetized rat securely, and carefully inserting the cannula into its stomach. Then the solution was gently injected, the needle was carefully removed. Rats were unable to regurgitate, thus assuring that the entire dose remained in the stomach.

Carnitine Preparations

Carnitine extractions were prepared from 10 per cent kidney homogenate in ice-cold 1·2 M perchloric acid (PCA) using a stainless steel Omni-Mixer homogenizer (Omni International Inc., Gainesville, VA, U.S.A.) and the homogenate was centrifuged at 8000 *g* for 10 min. The pellet was washed twice by re-suspending in ice-cold 0·6 M PCA followed by centrifugation. All supernatants were pooled and used for estimation of both free

carnitine (FC) and total acid-soluble (TS) carnitine whereas the pellet was used for estimation of long-chain acylcarnitine (LC) as described below.

Determination of Carnitine Levels

Total acid-soluble (TS) and FC carnitine concentrations were estimated as described previously,[21] whereas total (TC), short- (SC), long-chain acyl (LC) and acyl (AC) carnitine levels were determined as described previously[19,20] with slight modification for use in the current study. Briefly, for FC estimation 1 ml of PCA extract was carefully neutralized with 1 M KOH. For estimation of TS, which is comprised of FC and SC, 1 ml of the PCA extract was hydrolysed with 1 M KOH and incubated for 30 mins at room temperature after which the extract was neutralized with ice-cold 1·2 M PCA, and following centrifugation the clear supernatant was used for TS. SC was calculated by subtraction of FC from TS. For LC estimation, the remaining PCA extract pellet was dissolved in 1 ml of 0·5 ml KOH and hydrolysed at 65°C for 1 h, then ice-cold 1·2 M PCA was added to bring the pH $< 2{\cdot}0$. After centrifugation, the supernatant was used for LC estimation, whereas, the pellet was used for non-collagen protein (NCP) determination as described below. The sum of SC and LC was referred to as AC while the sum of TS and LC was referred to as TC. Prior to the enzymic determination of carnitine, the free thiol groups of 0·8 ml of neutralized sample extracts were oxidized using 0·2 ml of hydrogen peroxide reagent (100 mM Tris pH 7·8, 1·25 mM EDTA pH 8 and 3 per cent hydrogen peroxide). The mixture was incubated for 10 min at room temperature and then excess peroxide was destroyed by adding 0·05 ml of catalase (5 U).

Carnitine Analysis

Carnitine was measured enzymically.[19,20] Full details about the optimization, linearity, precision and reproducibility of the method were described previously.[20] Generally, the method is based on the reaction of carnitine with acetyl-CoA specifically catalysed by carnitine acetyltransferase (CAT), forming CoASH which is detected by a reaction with DTNB. The assay mixture (1 ml) contained hydrogen peroxide-oxidized extract, 10 mM DTNB (pH 7·8) and 15 mM acetyl-CoA. The reaction was initiated by adding CAT (0·9 U). The increase in absorbance was measured 412 nm using an Ultrospec 2000 UV/Visible Spectrophotometer (Pharmacia Biotech Ltd, Science Park, Cambridge CB4 4FJ, U.K). Carnitine concentrations in the samples were calculated with reference to the corresponding absorbance obtained with standard carnitine solution. A new standard curve was obtained daily using serial dilutions of analytical grade carnitine solution (10–100 nmol ml^{-1}). Carnitine concentration in the samples was calculated by interpolating the absorbance from the standard curve and multiplying the concentration obtained by the dilution factor to correct for sample dilution by acid solution. To evaluate the possible interference of theophylline studied with the analytical determination of FC, SC, LC, AC and TC in renal samples, theophylline solutions were added to 1 ml renal extract, and the concentration of carnitine in the samples was then determined in duplicate. No interference of theophylline was observed at concentrations of 50, 100 and 150 mg.

Protein Determination

Non-collagen protein (NCP) was determined by a procedure described by Lilienthal *et al.*[22] The pellet obtained after LC extraction was dissolved in 50 mM NaOH and incubated at room temperature for 18 h. Protein was estimated by the modified Lowry method using bovine serum albumin as standard.[23]

Determination of Theophylline Levels

Theophylline was measured as described previously[24] using a fluorescence polarization immunoassay method (Abbott TDx system, Abbott Laboratories, Wokingham, Berks, U.K.) whereas the levels of creatinine were measured using a Beckman Synchron CX-5 Analyser, New York, U.S.A.

Statistical Analysis

The data from each sample were run in duplicate and expressed as means $\pm$ SD (nmol mg^{-1} non-collagen protein) for $n = 10$ rats per week. All statistical analyses were performed using Student's *t*-test. Means were considered significantly different if $P < 0{\cdot}05$.[25]

Table 1. Concentration of free (FC), acyl (AC) and total (TC) carnitine in control, placebo and theophylline-treated rat kidney.

	Control			Placebo			Treated		
Weeks of treatment	FC	AC	TC	FC	AC	TC	FC	AC	TC
				nmol mg^{-1} non-collagen protein					
0	2·22 ± 0·5	0·31 ± 0·2	2·61 ± 0·4						
1	2·61 ± 0·3	0·41 ± 0·1	3·01 ± 0·2	2·22 ± 0·1*	0·31 ± 0·4*	2·63 ± 0·2*	3·33 ± 0·7†	0·66 ± 0·4†	4·11 ± 0·4†
2	2·41 ± 0·1	0·43 ± 0·4	2·99 ± 0·4	2·61 ± 0·2*	0·42 ± 0·4*	3·45 ± 0·4*	3·12 ± 0·5†	0·78 ± 0·2†	3·89 ± 0·1†
3	2·72 ± 0·6	0·41 ± 0·2	3·14 ± 0·3	2·93 ± 0·4*	0·39 ± 0·1*	3·63 ± 0·2*	3·71 ± 0·1†	0·82 ± 0·4†	4·51 ± 0·3†
4	2·53 ± 0·5	0·32 ± 0·3	2·86 ± 0·2	2·72 ± 0·3*	0·41 ± 0·3*	3·37 ± 0·1*	4·45 ± 0·1†	0·88 ± 0·5†	5·31 ± 0·2†
5	3·34 ± 0·7	0·44 ± 0·2	3·81 ± 0·1	2·91 ± 0·4*	0·43 ± 0·4*	3·72 ± 0·6*	4·31 ± 0·4†	0·82 ± 0·3†	5·23 ± 0·1†

Data expressed as means ± SD, $n = 10$ rats.
* Values are not significantly different as compared to control groups, $P < 0·1$.
† Values are significantly higher than in either control or placebo groups, $P < 0·001$.

RESULTS

The effect of orally administrated theophylline on the status of renal carnitine distributions were evaluated in adult male rats. Table 1 shows means (±SD) of free (FC), acyl (AC) and total (TC) carnitine levels in renal tissues. Renal FC, AC and TC levels were not significantly different in placebo groups versus control groups ($P < 0·1$), however, a significant increase in FC, AC and TC levels was observed in theophylline-treated groups ($P < 0·001$).

Figure 1 shows the means (±SD) of short- (SC) (Panel A) and long-chain acyl (LC) carnitine (Panel B) levels in the kidney of control, placebo and theophylline-treated rats. No significant changes were noticed in SC or LC levels in placebo groups as compared to control groups ($P < 0·1$). However, SC or LC levels were significantly increased by more than 1·8- and 2-fold, respectively, in the theophylline treatment groups when compared to either control or placebo groups ($P < 0·001$).

Figure 2 shows ratios of acyl (AC) to free (FC) carnitine in the kidney of control, placebo and theophylline-treated rats. The results indicated that the ratio of AC to FC was not significantly different ($P < 0·1$) in placebo as compared to control groups, however, theophylline administration caused a significant 1·7-fold increase in AC to FC ratio ($P < 0·001$).

DISCUSSION

The main action of theophylline is to relax bronchospasm; it is used to prevent or alleviate asthma and acute obstructive airways disease. The efficacy of theophylline as a stimulating agent has also been known for several years. For example, theophylline feeding is reported to increase plasma levels of free fatty acids.[16] In this study, we investigated the effect of theophylline feeding on carnitine distributions in the kidney of adult male rats.

In our previous study we showed that daily theophylline feeding caused an increase in food intake, water consumption and urine volume.[19,20] The mechanism by which theophylline treatment caused these changes is unknown. Scammell and Fregly have observed that theophylline caused an increase in both total food consumption and fecal bulk.[26] They suggested that theophylline administration might have a direct effect on the thyroid gland or an increase in the sensitivity of the gland to thyrotropin.[26] Recently, we reported that daily theophylline treatment led to a significant loss of final body weight in theophylline-treated rats, but the actual weight of organs such as heart, kidneys, liver, etc, were not significantly different from either the control or placebo groups.[19,20] These physiological changes brought about by theophylline feeding may be due to mobilization of stored fat reserves,[27] a rise in basal metabolic rate[28] and/or an increase in urinary excretion.[29]

It should be noted that an oral dose of 100 mg kg^{-1} body weight day^{-1} resulted in plasma theophylline concentrations of 15·9 ± 0·7 μg ml^{-1} (mean ± SD). This concentration is within the safe therapeutic range (5–20 μg ml^{-1}) employed for humans,[30] and similar doses have been employed for bronchodilation and/or in the management and prevention of neonatal apnea.[31] Our present study demonstrated that daily theophylline feeding for several weeks caused a marked elevation of FC, AC, SC, LC and TC in the kidney of rats. The results further indicated that the ratio of AC/FC was significantly increased in theophylline-treated

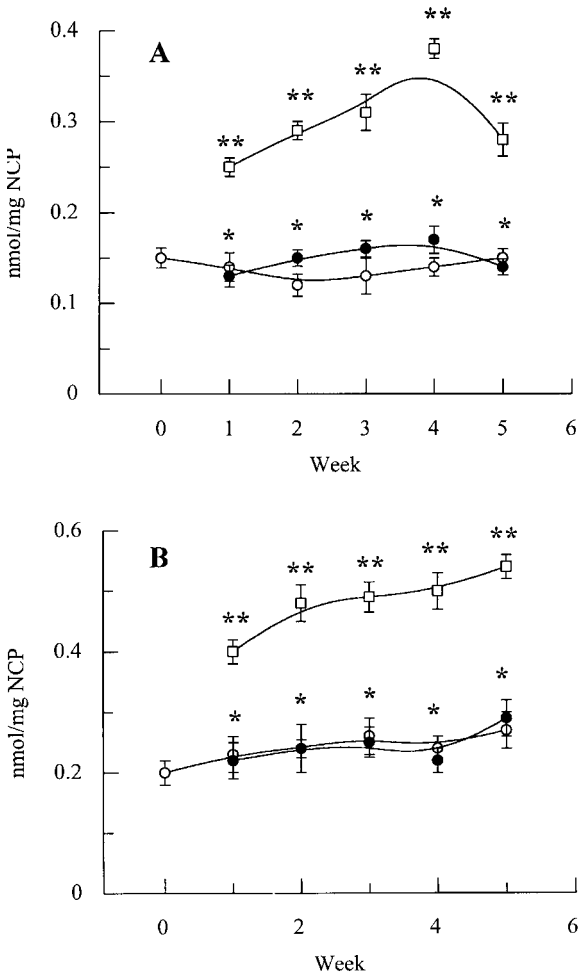

Figure 1. Panel A shows the concentrations of short-chain acyl (SC) carnitine in control (○–○), placebo (●–●) and theophylline-treated (□–□) rat renal tissues. Panel B shows the concentrations of long-chain acyl (LC) carnitine in control (○–○), placebo (●–●) and theophylline-treated (□–□) rat renal tissues. In both panels, data are expressed as means ± SD nmol mg^{-1} non-collagen protein for $n = 10$ rats. *Values are not significantly different from control groups, $P < 0{\cdot}1$. **Values are significantly higher than in either control or placebo groups, $P < 0{\cdot}001$.

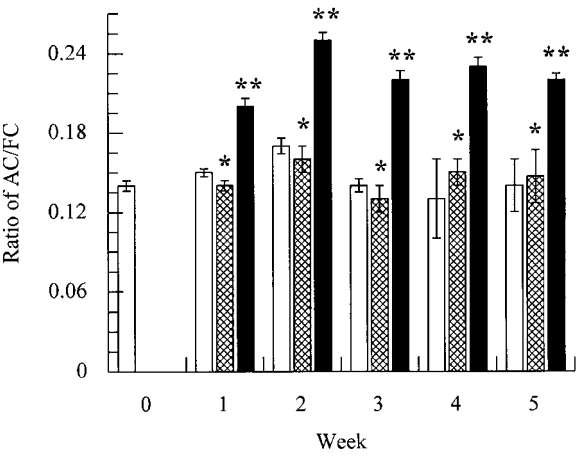

Figure 2. The ratio of acyl (AC) to free (FC) carnitine in control (□), placebo (▨) and theophylline-treated (■) rats. Data are expressed as means ± SD, for $n = 10$ rats. Other symbols are as reported in Figure 1.

rats as compared to either control or placebo groups. The possibility that the above results might be due to interference by theophylline in the analytical determination of carnitine levels was ruled out, carnitine levels determined in the presence of high concentration of theophylline differed marginally (<3 per cent) from those of control samples.[19,20]

The mechanisms by which theophylline feeding caused changes in carnitine level in renal tissues is unknown. However, there are many possible causes for elevations of carnitine levels in renal tissues: (i) carnitine is not degraded in mammalian systems and is eliminated in the urine as FC and AC,[1] (ii) renal clearance of carnitine is much less than the glomerular filtration rate, and the majority of the filtered carnitine is reabsorbed in the kidney through a specific, saturable transport system.[32,33] Thus theophylline treatment may alter after renal tubular reabsorption of carnitine and/or (iii) it is well known that theophylline has diuretic effects, raising the possibility that theophylline-induced diuresis and dehydration might come in part from interaction with antidiuretic hormone (ADH).[34] Indeed, we recently observed that theophylline administration caused an increase in urinary carnitine excretion in rats (submitted data for publication).

It has been suggested that the ratio of AC/FC may provide a useful marker of changes in carnitine metabolism.[10] This ratio can be used as a screening system to detect alterations in mitochondrial metabolism.[10] Our results showed that theophylline treatment caused significant increase in the ratios of AC/FC in renal tissues as compared to either control or placebo rats. The mechanisms by which theophylline administration caused changes in this ratio is unknown. However, there are many possible benefits for elevations of this ratio. For example, carnitine is essential for the movement of long-chain fatty acids across the inner mitochondrial membrane.[2] Higher carnitine concentrations have also been observed in a

number of stress conditions, where lipid metabolism is altered.[4–10] Increased carnitine concentrations would then help to compensate for the elevated fatty acid concentrations that are liberated due to increased lipolytic effects of the theophylline. An increase in the carnitine of the renal tissues could catalyse the flow of fatty acids into those tissues for subsequent metabolism. Theophylline treatment caused a striking increase in carnitine levels in the heart and skeletal muscle of rats, but not in hepatic tissues.[19,20]

Enhanced carnitine concentrations in renal tissues of the rats on theophylline treatment could be an accumulation of carnitine. The concentrations of carnitine in various tissues are higher than that in plasma.[35] An active Na^+-dependent transport system for carnitine uptake has been proposed[36] as well as a reversible diffusion transport system in exchange of intracellular deoxycarnitine (γ-butyrobetaine).[37] Theophylline may affect either of these transport systems so as to increase the flow of carnitine from the plasma into the cells. This is consistent with the observation that a significant depletion in plasma carnitine levels occurred in theophylline-treated rats.[24] An increase in the ratio during theophylline treatment could be due to mobilization of fatty acids with subsequent increases in long-chain fatty acyl carnitine esters in the tissues. The rise in AC concentrations in renal tissues further supports our contention that theophylline may increase transport of carnitine into tissues and/or may induce carnitine-dependent enzymes such as carnitine acetyltransferase[38–40] or carnitine palmitoyltransferase.[1,2] Recently, we observed that theophylline treatment significantly increased the activity of carnitine aceyltransferase in rat cardiac tissue compared to either control or placebo groups.[41]

In conclusion, our studies showed that theophylline treatment caused an increase in free, short-chain acyl, long-chain acyl and total carnitine in renal tissues as compared to either control or placebo groups. Moreover, daily theophylline administration to adult male rats also causes a significant increase in the ratio of acylated carnitine to free carnitine in renal tissue. These abnormalities in carnitine distribution may result from a mobilization of lipid from adipose tissue which subsequently results in increased carnitine transport into the tissues to augment β-oxidation inside the mitochondria. The results raised several points that need further investigation. For example, does theophylline alter carnitine metabolism and/or its distribution in urine, plasma and/or tissues, or induce carnitine-dependent enzymes?

Further work is being undertaken in our laboratory to investigate the mechanism of the effects of theophylline treatments in tissue uptake and metabolism of carnitine in short-and long-term treatments.

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REFERENCES

1. Bieber, L. L. (1988). Carnitine. *Ann. Rev. Biochem.*, **57**, 261–283.
2. Hoppel, C. (1992). The physiological role of L-carnitine. In: L-Carnitine and its Role in Medicine: from Functions to Therapy. (Ferrari, R., DiMauro, S. and Sherwood, G., eds.) Academic Press: California, pp. 5–19.
3. Lysiak, W., Toth, P. P., Swelter, C. H. and Bieber, L. L. (1986). Quantitation of the afflux of acylcarnitine from rat heart, brain and liver mitochondria. *J. Biol. Chem.*, **261**, 13698–13703.
4. Alhomida, A. S., Duhaiman, A. S., Al-Jafari, A. A. and Junaid, M. A. (1995). Determination of L-carnitine, acylcarnitine and total carnitine levels in plasma and tissues of camel (*Camelus dromedarius*). *Comp. Biochem. Physiol.*, **111B**, 441–445.
5. Al-Eissa, M. S. and Alhomida, A. S. (1997). A study of the distribution of total, free, short-chain acyl and long-chain acyl carnitine in whole-blood and plasma of Arabian sand gazelles (*Gazella subgutturosa marica*). *Comp. Haematol. Int.*, **1**, 65–69.
6. Alhomida, A. S., Al-Jafari, A. A., Junaid, M. A., Al-Whaiby, S. A. and Duhaiman, A. S. (1995). Age, sex and diabetes-related changes in total, free and acylcarnitine in human plasma. *Med. Sci. Res.*, **23**, 167–169.
7. Alhomida, A. S., Duhaiman, A. S., Al-Jafari, A. A., Sobki, S., Al-Sulaiman, M. and Al-Khadar, A. (1996). Serum total, free and acyl carnitine concentrations in chronic glomerulonephritis patients. *Med. Sci. Res.*, **24**, 495–498.
8. Alhomida, A. S. (1997). Effect of chronic renal hemodialysis on serum total, free and acyl carnitine concentrations in adult chronic pyelonephritis patients. *Arch. Med. Res.*, **28**, 101–107.
9. Alhomida, A. S., Sobki, S. H., Al-Sulaiman, M. H. and Al-Khadar, A. A. (1997). Influence of gender and chronic hemodialysis treatment on total, free and acyl carnitine concentrations in human serum. *Int. Urol. Nephrol.*, **29**, 479–487.
10. Bohles, H., Evangeliou, A., Bervoets, K., Eckert, I. and Sewell, A. (1994). Carnitine esters in metabolic diseases. *Eur. J. Pediatr.*, **153**, 557–561.
11. Aranda, J. V. and Turmen, T. (1979). Methylxanthines in apnea of prematurity. *Clin. Perinatol.*, **6**, 87–107.
12. Jacobs, M. H., Senior, R. M. and Kessler, G. (1976). Clinical experience with theophylline. Relationship between dosage, serum concentration and toxicity. *J. Am. Med. Assoc.*, **235**, 1983–1986.

13. Woo, O. F., Pond, S. M., Benowitz, N. J. and Olson, K. R. (1984). Benefit of hemoperfusion in acute theophylline intoxication. *Clin. Toxicol.*, **22**, 411–424.
14. Supinski, G. S., Chandler, D. and Kelson, S. G. (1984). The effects of caffeine and theophylline on diaphragm contractility. *Am. Rev. Respir. Des.*, **130**, 429–433.
15. Rall, T. W. (1985). Central nervous system stimulants: the methylxanthines. In: *The Pharmacological Basis of Therapeutics*. (Gilman, A. G., Goodman, L. S., Rall, T. W. and Murad, F., eds.) MacMillan Publishing Co: New York, pp. 589–595.
16. Scotini, E., Carpenedo, F. and Fassina, G. (1983). New derivatives of methylxanthines: Effects of thiocaffeine, thiotheophylline and 8-phenyltheophylline on lipolysis and on phosphodieterase activities. *Pharmacol. Res. Commun.*, **15**, 131–143.
17. Fredholm, B. B. and Lindgren, E. (1984). The effects of alkylxanthines and other phosphodiesterase inhibitors on adenosine-receptor mediated decrease in lipolysis and cyclic AMP accumulations in rat fat cells. *Acta Pharmacol. Toxicol.*, **54**, 64–71.
18. Fredholm, B. B. (1980). Theophylline actions on adenosine receptors. *Eur. J. Resp. Dis.*, **61**, 29–36.
19. Al-Jafari, A. A., Junaid, M. A. and Alhomida, A. S. (1966). Investigation of the influence of theophylline feeding on total, free, short-chain acyl and long-chain acyl carnitine levels in skeletal muscle and liver of rats. *In vivo*, **10**, 569–574.
20. Alhomida, A. S. (1997). Study of the effects of theophylline-related changes in total, free, short-chain acyl and long-chain acyl carnitine concentrations in rat heart. *Toxicol.*, **121**, 205–213.
21. Alhomida, A. S. (1996). Total, free, short-chain, long-chain acyl carnitine levels in Arabian camel milk (*Camelus dromedarius*). *Ann. Nurt. Metabol.*, **40**, 221–226.
22. Lilienthal, J. L., Zierler, K. L., Folk, B. P., Buka, R. and Riley, M. J. (1950). A reference base and system for analysis of muscle constituents. *J. Biol. Chem.*, **182**, 501–508.
23. Markwell, M. A. K., Haes, S. M., Tolbert, N. E. and Bieber, L. L. (1981). Protein determination in membrane and lipoprotein samples: manual and automated procedures. *Methods Enzymol.*, **72**, 269–303.
24. Al-Kholafi, A. M. and Alhomida, A. S. (1977). Evaluation of theophylline-induced changes on plasma total, free, short-chain acyl and long-chain acyl carnitine concentrations in rats. *Med. Sci. Res.*, **25**, 31–34.
25. Winer, B. J., Brown, D. R., Michels, K. M. (1991). *Statistical Principles in Experimental Design*. McGraw-Hill: New York.
26. Scammell, J. G. and Fregly, M. J. (1982). Effect of theophylline on thyroid status in the rat. *Pharmacol.*, **25**, 160–169.
27. Bukowiecki, L. J., Lupien, J., Follea, N. and Jahjah, L. (1983). Effects of sucrose, caffeine and cola beverages on obesity, cold resistance and adipose tissue cellularity. *Am. J. Physiol.*, **1244**, R500–R507.
28. Donowitz, M., Tai, Y.-H. and Asarkof, N. (1980). Effects of serotonin on active electrolyte transport in rabbit ileum, gall bladder and colon. *Am. J. Physiol.*, **239**, G463–G472.
29. Field, M. (1971). Intestinal secretion: effect of cyclic AMP and its role in cholera. *N. Engl. J. Med.*, **284**, 1137–1142.
30. Mitenko, P. and Ogilvie, R. (1973). Rational intravenous doses of theophylline. *N. Engl. J. Med.*, **289**, 600–603.
31. Rowe, D. J. F., Watson, I. D., Williams, J. and Berry, D. J. (1988). The clinical use and measurement of theophylline. *Ann. Clin. Biochem.*, **25**, 4–26.
32. Gross, C. J. and Henderson, L. M. (1984). Uptake of L-carnitine, D-carnitine and acetyl-L-carnitine by isolated guinea-pig enterocytes. *Biochem. Biophys. Acta*, **772**, 209–212.
33. Hokland, B. M. and Bremer, J. (1986). Metabolism and excretion of carnitine and acylcarnitine in the perfused rat kidney. *Biochem. Biophys. Acta*, **886**, 223–241.
34. Spindel, E. (1984). Action of the methylxanthines on the pituitary and pituitary-dependent hormones. In: *The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects*. Alan R. Liss, Inc: New York, pp. 331–355.
35. Brass, E. P. (1992). Carnitine transport. In: *L-Carnitine and its Role in Medicine: from Functions to Therapy*. (Ferrari, R., DiMauro, S. and Sherwood, G., eds.) Academic Press: California, pp. 21–36.
36. Rebouche, C. J. (1989). Carnitine transport and tissue carnitine accretion in rats. *Biochim. Biophys. Acta*, **1033**, 111–113.
37. Sartorelli, L., Mantovani, G. and Ciman, M. (1989). Carnitine and deoxycarnitine concentration in rat tissues and urine after their administration. *Biochim. Biophys. Acta*, **1006**, 15–18.
38. Alhomida, a. S., Duhaiman, A. S., Al-Jafari, A. A. and Junaid, M. A. (1996). Purification of carnitine acetyltransferase from the skeletal muscle of the camel (*Camelus dromedarius*). *Mol. Cell. Biochem.*, **165**, 95–101.
39. Alhomida, A. S., Al-Jafari, A. A., Duhaiman, A. S., Rabbani, N. and Junaid, M. A. (1996). Kinetic properties of purified carnitine acetyltransferase from the skeletal muscle of the Arabian camel (*Camelus dromedarius*). *Biochimie*, **78**, 204–208.
40. Alhomida, A. S. (1996). Inhibition studies of the carnitine acetyltransferase from the skeletal muscle of the camel (*Camelus dromedarius*) by sulfhydride reagents and metal ions. *Biochem. Mol. Biol. Int.*, **39**, 923–931.
41. Alhomida, A. S. (1997). Investigation of the effects of theophylline administration on carnitine acetyltransferase activity of rat heart. *J. Enz. Inhib.*, **12**, 291–301.