Effects of short- and long-term feeding of L-carnitine and congeners on the production of eicosanoids from rat peritoneal leucocytes

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The effect of short- and long-term feeding with L-carnitine, L-acetyl carnitine and L-propionyl carnitine on the production of eicosanoids from *in vitro* stimulated carrageenan-induced rat peritoneal macrophages was investigated. Both young (4 weeks) and old (18 months) rats were used. A lower number of cells was isolated from the peritonea of treated than control young rats after 4 d feeding, but after 60 d no differences were observed. A similar reduction in cell number was found when old animals were given L-acetyl carnitine or L-propionyl carnitine (acutely) or L-acetyl carnitine or L-carnitine (chronically). Plasma carnitine levels were higher in young rats given carnitine both chronically and acutely. Carnitine derivatives were without effect. In contrast, levels of total carnitine in the plasma of old rats given L-carnitine and L-acetyl carnitine for 4 d and 60 d were higher than in controls. There was no correlation between total plasma carnitine level and effects on prostaglandin, thromboxane and leukotriene B_4 (LTB_4) production. In young rats the most important changes were observed in relation to the production of prostacyclin (PGI_2), measured as 6 keto-prostaglandin $F_{1\alpha}$. Prostacyclin production was higher in the groups given carnitine or its derivatives. The net result of the changes in PGI_2 was that the 6 keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 and the 6 keto-prostaglandin $F_{1\alpha}$:LTB_4 ratios tended to be higher in cells from young animals following short-term feeding with L-carnitine. When young rats were given carnitine compounds for 60 d PGI_2 production was lower in cells from L-acetyl carnitine- and L-propionyl carnitine-fed animals. The net result of the changes in PGI_2 was that the 6 keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 and the 6 keto-prostaglandin $F_{1\alpha}$:LTB_4 ratios were lower in cells from animals fed with carnitine compounds. In old rats the PGI_2 production was lower after short-term feeding with carnitine compounds and was higher after long-term feeding. LTB_4 production was lower after L-carnitine and L-acetyl carnitine treatment for 4 d and also lower after 60 d treatment with L-acetyl carnitine. The net results of the changes in PGI_2 were that the 6 keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 and the 6 keto-prostaglandin $F_{1\alpha}$:LTB_4 ratios were lower after short-term feeding of all three compounds and higher after the long-term treatment with L-acetyl carnitine and L-propionyl carnitine in old rats. By long-term treatment with low-dose aspirin of patients with heart failure and claudication, the 6 keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 ratio is positively increased, which is a beneficial cardioprotective effect. The mechanism of action of carnitine in heart failure and claudication could also be achieved by an increase of this ratio. Our results suggest that elderly patients could be treated chronically by carnitine to obtain this beneficial effect.

L-Carnitine: Peritoneal macrophages: Eicosanoids

Before long-chain fatty acids are oxidized in mitochondria they are activated by acyl coenzyme A (acyl CoA) synthetase (*EC* 6.2.1.3) and carried across the mitochondrial

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inner membrane by L-carnitine (β-hydroxy-(γ-*N*-trimethylamino)-butyrate). Carnitine deficiency, defined as a decrease in intracellular carnitine, leads to the accumulation of acyl CoA esters within the cell and an inhibition of acyl transport through the mitochondrial inner membrane. Inhibition of the mitochondrial β-oxidation results in fatty acid infiltration and, eventually, heart or liver failure (Barth *et al.* 1983; Scholte *et al.* 1989, 1990; Wanner *et al.* 1989). Carnitine deficiency can be due to a genetic deficiency, or may occur as a side-effect of dialysis treatment. For example, serum carnitine and leucine levels were significantly decreased in patients on continuous ambulatory peritoneal dialysis (CAPD) for more than 4 months. This observation suggests that malabsorption plays a role in the reduction of serum carnitine levels in patients receiving CAPD (Murakami *et al.* 1990). The clinical conditions of infants and children with a variety of diagnoses associated with low circulating carnitine concentrations improved after carnitine supplementation, and carnitine has been reported to have several beneficial effects on ischaemic heart diseases and arrhythmias in man, where it augments the ischaemic heart tolerance to stress (Schinetti & Mazzini, 1986).

Carnitine administration may also have beneficial effects in patients with disorders of NH_3 metabolism (Ohtsuka & Griffith, 1991; Rebouche, 1992). Interestingly, adults suffering from chronic diseases associated with ageing, such as heart disease or cardiomyopathy due to carnitine loss, are improved by treatment with L-carnitine, L-acetyl carnitine or L-propionyl carnitine (Winter *et al.* 1987; Scholte *et al.* 1989, 1990; Wanner *et al.* 1989; Rebouche, 1992).

Besides its stimulation of fatty acid oxidation, L-carnitine has also been shown to enhance the formation of arachidonic acid from linoleic acid by isolated hepatocytes. It was suggested that this was due to the removal of the inhibitory effect of long-chain acyl CoA on acetyl CoA carboxylase (*EC* 6.4.1.2), a regulating enzyme in fatty acid synthesis, by long-chain acyl carnitine (McGarry *et al.* 1975; Christophersen & Norseth, 1981; Rebouche, 1986; Stryer, 1988; Conte *et al.* 1992). This increased arachidonic acid synthesis could be important, as eicosanoids, metabolites of arachidonic acid, are important local modulators of inflammation. For example, the cyclooxygenase product prostaglandin E_2 (PGE_2) is recognized as an important inhibitor of both cell migration and mediator formation, whereas leukotriene B_4 (LTB_4), a lipoxygenase (*EC* 1.13.11.12) product, is a potent chemotactic agent which promotes the formation of mediators at inflammatory sites (Lehmann *et al.* 1988; Renz *et al.* 1988; Schepers *et al.* 1988; Yamaoka *et al.* 1988). Furthermore, the ratio between the vasoconstrictive prostanoid thromboxane A_2 (TxA_2), synthesized by platelets, and the vasodilative prostacyclin (prostaglandin I_2; PGI_2), synthesized by endothelial cells, is of importance in controlling platelet aggregation and adherence to blood vessels (Campbell, 1990). CoA also plays a role in the formation and breakdown of products from both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (Conte *et al.* 1992). Recently the oxidation of PGE_2 in rat liver peroxisomes and mitochondria was shown to require the activation to PGE_2 CoA by microsomal PGE_2 CoA synthetase (Schepers *et al.* 1988). Furthermore, LTB_4 is degraded through ω- and β-oxidation in neutrophils and liver cells. The formation of LTB_4 CoA esters which occurs in rat-liver microsomes may be essential for LTB_4 β-oxidation (Yamaoka *et al.* 1988). While LTB_4 is a potent chemotactic agent and promotes the formation of mediators at inflammatory sites, its degradation products are less potent than the parent compound. Hence the formation of LTB_4 CoA esters and their subsequent β-oxidation might be of importance in limiting LTB_4 activity at the sites of inflammation. Thus, carnitine could stimulate both the synthesis and breakdown of eicosanoids by the same mechanism, i.e. reducing the concentration of long-chain acyl CoA within the cell.

Recently we showed that feeding young rats (3–4 months) with L-carnitine or various

derivatives resulted in a preferential higher production of PGI_2 by peritoneal macrophages (Elliott *et al.* 1990). Our results could provide the basis for a possible explanation for the beneficial effects of carnitine and its derivatives in cardiovascular diseases. Such investigations into the mechanisms of L-carnitine and its derivatives may lead to the identification of new roles for these compounds.

In the present study we extended our investigations by examining the effect of L-carnitine, L-acetyl carnitine (formed during β-oxidation of even-chain fatty acids) and L-propionyl carnitine (formed during β-oxidation of uneven-chain fatty acids) on eicosanoid production by carrageenan-induced peritoneal cells of young and old rats after 4 or 60 d exposure. Earlier results obtained using young rats given carnitine compounds for 4 d are shown for ease of comparison (Elliott *et al.* 1990).

MATERIALS AND METHODS

Materials

L-Carnitine, L-acetyl carnitine, and L-propionyl carnitine were gifts from Sigma-Tau Pharmaceutical Co. (Rome, Italy). Carrageenan was purchased from Marine Colloids Inc (Springfield, NJ, USA). Lymphoprep was from Nyegaard Diagnostica (Oslo, Norway) and Dulbecco's modified Eagle's medium was obtained from Life Technologies (Breda, The Netherlands). A23187 was from Sigma (Axel, The Netherlands). ^{3}H-labelled eicosanoids were from Amersham ('s-Hertogenbosch, The Netherlands) and antibodies from Advanced Magnetics Inc. (Cambridge, MA, USA). Other chemicals were purchased from Merck (Amsterdam, The Netherlands) and Sigma.

Animals

Male Wistar rats were used in all experiments. The animals were housed in groups of three in polyethylene cages with food and water *ad lib*. Artificial light was supplied in a 12 h light–dark cycle.

Treatment

Two series of experiments were carried out.

Short-term treatment. Young rats (3–4 months, nine rats per group) and old rats (16–18 months, five rats per group) were given by intubation on days 1–4, 300 mg L-carnitine or carnitine equivalents (L-acetyl carnitine and L-propionyl carnitine)/kg dissolved in 1 ml distilled water. Control animals were given distilled water. All animals were injected intraperitoneally with 2 ml of a carrageenan solution (1 mg/ml) on day 1 (Elliott *et al.* 1990).

Long-term treatment. Young rats (3–4 months, nine rats per group) and old rats (16–18 months, five rats per group) were given in their drinking water on days 1–60, 50 mg L-carnitine or carnitine equivalents (L-acetyl carnitine and L-propionyl carnitine)/kg. Control animals were given water. All animals were injected intraperitoneally with 2 ml of a carrageenan solution (1 mg/ml) on day 56 (4 d before the end of the experiment).

Isolation and incubation of peritoneal cells

On day 4 or 60, 1 h after the last administration of L-carnitine or its derivatives, cells were isolated from pooled Gey's balanced-salt solution washes of the peritonea of the rats by density-gradient centrifugation over Lymphoprep and suspended in Dulbecco's modified Eagle's medium (2×10^6 nucleated cells/ml). The harvested peritoneal cell population consisted of $> 85\%$ macrophages and $> 10\%$ polymorphonuclear leucocytes. A 1 ml portion of cell suspension was incubated at 37° for either 2 h without ionophore (basal

Table 1. *Effect of short- and long-term feeding of* L-*carnitine or its congeners on the absolute cell number* ($\times 10^6$) *isolated from peritonea of young and old rats*†

(Values are means and standard deviations for nine (young) and five (old) rats)

		Control		L-Carnitine		L-Acetyl carnitine		L-Propionyl carnitine	
	Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Young rats	Short-term	13·33	3·73	6·34*	1·95	4·91*	0·38	6·48*	1·29
	Long-term	13·92	3·22	12·94	2·32	14·52	5·31	13·01	3·11
Old rats	Short-term	13·80	1·55	12·44	1·86	6·28*	0·51	7·89*	1·37
	Long-term	15·30	2·59	8·41*	2·07	8·39*	0·38	14·29	2·40

Mean values were significantly different from control values: * $P < 0.05$ (Students t test).
† For details of procedures, see pp. 787–788.

production) or 30 min with 10^{-6} M-A23187 (ionophore-stimulated production). The cell suspensions were then centrifuged and the supernatant fractions analysed for production of LTB_4, PGE_2, TxB_2, and 6 keto-prostaglandin $F_{1\alpha}$ (6 keto-$PGF_{1\alpha}$) by radioimmunoassays (Zijlstra & Vincent, 1984; Elliott *et al.* 1990). The net eicosanoid production was calculated by subtraction of the basal production from the ionophore-stimulated production. Blood samples were taken for measurement of total plasma carnitine levels (Pande & Caramancion, 1981; Rössle *et al.* 1985).

Statistical evaluation

In the experiments the values are given as means and standard deviations for either nine or five rats per group. Statistical significance was calculated using the Student's t test.

RESULTS

Results obtained using young rats fed with carnitine compounds for 4 d have been previously published (Elliott *et al.* 1990) and are given here solely for the purposes of comparison.

Cell number

Young rats, short-term. All three compounds significantly reduced the number of nucleated cells isolated from peritonea 4 d after an intraperitoneal injection of carrageenan.

Young rats, long-term. No differences were found between the groups with respect to the number of nucleated peritoneal cells isolated after 60 d.

Old rats, short-term. L-Acetyl carnitine and L-propionyl carnitine significantly reduced the number of nucleated cells isolated from the peritonea after 4 d treatment.

Old rats, long-term. L-Carnitine and L-acetyl carnitine significantly reduced the number of nucleated cells isolated from the peritonea 60 d after treatment (Table 1).

Plasma levels of carnitine

Of the three compounds used, only in the L-carnitine group was the total L-carnitine concentration in plasma higher in all experiments in comparison with the controls (Table 2). In the L-acetyl carnitine group the total L-carnitine concentration in the plasma of old rats (chronic and acute) was higher than in controls.

Table 2. *Effect of short- and long-term feeding of* L-*carnitine or its congeners on the plasma levels (μmol/l) of carnitine in young and old rats*†

(Values are means and standard deviations for nine (young) and five (old) rats)

		Control		L-Carnitine		L-Acetyl carnitine		L-Propionyl carnitine	
	Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Young rats	Short-term	66·3	4·5	102·4*	18·7	75·3	11·2	76·1	19·9
	Long-term	76·7	9·1	125·3*	14·2	82·6	15·3	86·0	14·6
Old rats	Short-term	81·6	17·5	138·8*	20·0	114·2*	11·4	109·3	23·7
	Long-term	69·2	15·2	95·9*	9·9	98·4*	18·0	88·5	24·8

Mean values were significantly different from control values: * $P < 0.05$ (Students t test).
† For details of procedures, see pp. 787–788.

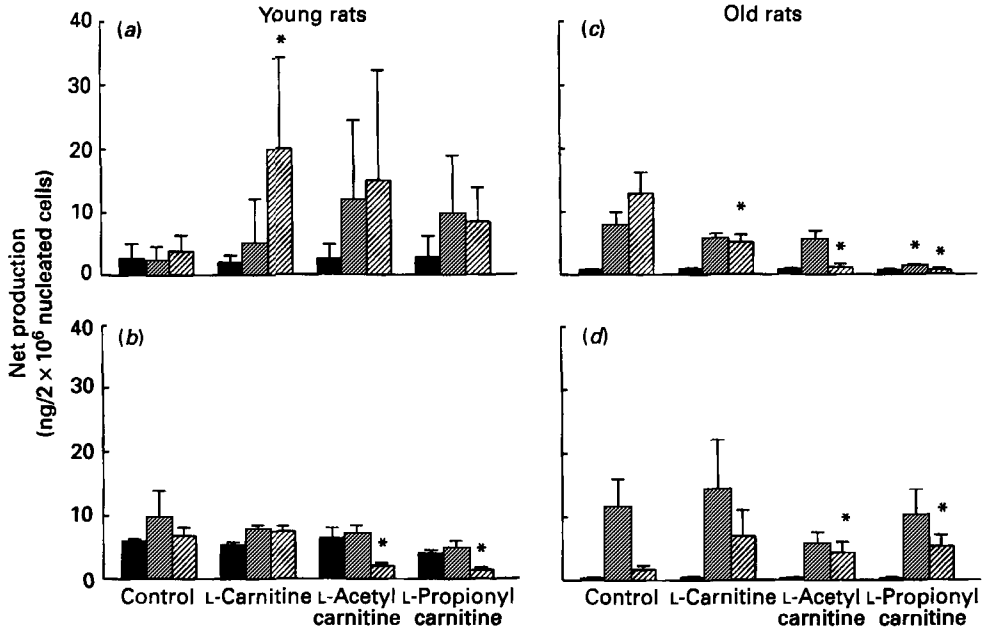

Fig. 1. Effect of short- (4 d; panels (*a*) and (*c*)) and long-term (60 d; panels (*b*) and (*d*)) feeding of L-carnitine, L-acetyl carnitine and L-propionyl carnitine on the production of prostaglandin E_2 (■), thromboxane B_2 (▨), and 6 keto-prostaglandin $F_{1\alpha}$ (▨) from carrageenan-induced peritoneal cells of young and old rats. Values are means with standard deviations indicated by vertical bars. Mean values were significantly different from control values: * $P < 0.05$ (Student's t test). For details of procedures, see pp. 787–788.

Production of eicosanoids from carrageenan-induced peritoneal cells

Young rats. In the short-term experiments the production of 6 keto-$PGF_{1\alpha}$ and TxB_2 tended to increase with L-acetyl carnitine and L-propionyl carnitine (Fig. 1). Production of 6 keto-$PGF_{1\alpha}$ was significantly higher and that of TxB_2 tended to be higher after feeding with L-carnitine. LTB_4 production was significantly higher in all three treatment groups (Table 3). However, no effects on PGE_2 formation were detected. The 6 keto-$PGF_{1\alpha}$:TxB_2

Table 3. *Effect of short- and long-term feeding of* L-*carnitine or its congeners on the net production of leukotriene* B_4 *(ng/2 × 10⁶ macrophages) from peritoneal cells in young and old rats*†

(Values are means and standard deviations for nine (young) and five (old) rats)

		Control		L-Carnitine		L-Acetyl carnitine		L-Propionyl carnitine	
	Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Young rats	Short-term	0·77	0·36	1·54*	0·87	1·72*	1·19	1·19*	0·72
	Long-term	0·37	0·19	0·63	0·70	0·31	0·40	0·25*	0·23
Old rats	Short-term	1·42	0·21	0·93*	0·30	1·17*	0·35	1·03	0·60
	Long-term	0·33	0·13	0·38	0·17	0·05*	0·03	0·26	0·14

Mean values were significantly different from control values: * $P < 0·05$ (Students t test).
† For details of procedures, see pp. 787–788.

Table 4. *Effect of short- and long-term feeding of* L-*carnitine or its congeners on the 6 keto-prostaglandin* $F_{1\alpha}$*: thromboxane* B_2 *ratio of peritoneal cells from young and old rats*†

(Values are means and standard deviations for nine (young) and five (old) rats)

		Control		L-Carnitine		L-Acetyl carnitine		L-Propionyl carnitine	
	Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Young rats	Short-term	1·72	1·55	3·80	4·05	1·26	1·38	0·87	0·70
	Long-term	0·71	0·23	0·97*	0·11	0·29*	0·06	0·28*	0·08
Old rats	Short-term	1·65	0·43	0·90*	0·16	0·22*	0·08	0·46*	0·17
	Long-term	0·15	0·06	0·48*	0·25	0·76*	0·27	0·52*	0·19

Mean values were significantly different from control values: * $P < 0·05$ (Students t test).
† For details of procedures, see pp. 787–788.

and the 6 keto-$PGF_{1\alpha}$:LTB_4 ratios, calculated from the net values, tended to be higher with L-carnitine treatment (Tables 4 and 5). This was due to the fact that the stimulation of 6 keto-$PGF_{1\alpha}$ production was greater than that of the other eicosanoids measured (Elliott *et al.* 1990).

In the long-term experiments the 6 keto-$PGF_{1\alpha}$ production was significantly lower in the L-acetyl carnitine and L-propionyl carnitine groups, while it was not in the L-carnitine group. No changes in PGE_2 production were detected (Fig. 1). LTB_4 production was significantly lower after feeding L-propionyl carnitine (Table 3). This resulted in significantly lower 6 keto-$PGF_{1\alpha}$:TxB_2 and 6 keto-$PGF_{1\alpha}$:LTB_4 ratios after treatment with L-acetyl carnitine and L-propionyl carnitine, but a significantly higher 6 keto-$PGF_{1\alpha}$:TxB_2 ratio in the L-carnitine group compared with controls (Tables 4 and 5).

Old rats. In the short-term experiments the production of 6 keto-$PGF_{1\alpha}$ was significantly lower in all carnitine groups and the TxB_2 production was significantly lower in the L-propionyl carnitine group. No changes in PGE_2 production were detected (Fig. 1). LTB_4 production was significantly lower after L-carnitine and L-acetyl carnitine treatments. The 6 keto-$PGF_{1\alpha}$:TxB_2 and 6 keto-$PGF_{1\alpha}$:LTB_4 ratios were significantly lower in all three treatment groups than in the control group.

Table 5. *Effect of short- and long-term feeding of* L-*carnitine or its congeners on the 6 keto-prostaglandin* $F_{1\alpha}$*: leukotriene* B_4 *ratio of peritoneal cells from young and old rats*†

(Values are means and standard deviations for nine (young) and five (old) rats)

		Control		L-Carnitine		L-Acetyl carnitine		L-Propionyl carnitine	
	Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Young rats	Short-term	4·88	2·90	12·99	8·35	8·74	8·29	7·13	4·47
	Long-term	18·32	7·25	11·83	9·20	6·55*	6·15	5·48*	3·67
Old rats	Short-term	9·16	1·86	5·53*	1·56	1·10*	0·40	0·61*	0·32
	Long-term	5·21	1·99	18·32	9·53	87·86*	38·22	20·56*	9·69

Mean values were significantly different from control values: * $P < 0{\cdot}05$ (Students t test).
† For details of procedures, see pp. 787–788.

In the long-term experiments no significant effects on PGE_2 and TxB_2 production were detected. Only in the L-acetyl carnitine and the L-propionyl carnitine groups was a significantly lower production of 6 keto-$PGF_{1\alpha}$ found (Fig. 1). LTB_4 production was significantly lower in the L-acetyl carnitine group after chronic exposure (Table 3). The 6 keto-$PGF_{1\alpha}$:TxB_2 ratio was significantly higher in all three treatment groups and the 6 keto-$PGF_{1\alpha}$:LTB_4 ratio was significantly higher in the chronic experiments after treatment with L-acetyl carnitine and L-propionyl carnitine (Tables 4 and 5).

DISCUSSION

Only exposure to L-carnitine resulted in an elevation of total plasma L-carnitine in young rats (acute and chronic exposure). Both L-carnitine and acetyl carnitine treatment elevated total plasma L-carnitine in old rats. However, in view of the lack of a correlation between plasma L-carnitine and other variables measured, the significance of these increases is not clear. One effect of feeding L-carnitine or its congeners to young rats for 4 d was the much lower number of carrageenan-elicited macrophages isolated from the peritonea of the treated animals. In contrast there was no effect when young rats were treated chronically. The effect of carnitine compounds on the number of elicited macrophages isolated from old rats was variable. Only in the L-acetyl carnitine group was a lower number of elicited macrophages found after both chronic and acute exposure. These results indicate that some L-carnitine compounds can modify the macrophage response to chemotactic signals generated when carrageenan is injected intraperitoneally. The exact effect depends on the age of the animal and time of exposure. A reduction of 50% in the number of macrophages at an inflammatory site could well be beneficial, for example in chronic immuno-inflammatory diseases.

We previously demonstrated that PGI_2 production by rat and rabbit aortas increased with age (Adolfs & Elliott, 1982; Elliott & Adolfs, 1984; Vincent & Zijlstra, 1986). In the present study we also show that eicosanoid production by macrophages is increased with age. It now appears that an increase in the turnover of arachidonic acid could be a characteristic of ageing. From the short-term feeding experiments with older rats we conclude, in general, that L-carnitine and its derivatives inhibited PGI_2 production. In contrast, the production of PGI_2 after long-term feeding was stimulated. The net results of the changes in PGI_2 were that the 6 keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 and the 6 keto-prostaglandin $F_{1\alpha}$:LTB_4 ratios were lower after short-term feeding of all three

compounds and higher in the long-term treatment with L-acetyl carnitine and L-propionyl carnitine in comparison with controls. The production of PGI_2 and TxB_2 from macrophages isolated from young test animals was higher than that from control rats after short-term feeding with L-carnitine and its derivatives. The results of these changes were that the 6 keto-$PGF_{1\alpha}$:TxB_2 ratio and 6 keto-$PGF_{1\alpha}$:TxB_2 ratio were higher after short-term L-carnitine treatment. After long-term treatment with L-acetyl carnitine and L-propionyl carnitine a lower production of PGI_2 was observed. The net results were that the 6 keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$:LTB_4 ratios were lower after feeding with L-acetyl carnitine and L-propionyl carnitine.

In man the 6 keto-$PGF_{1\alpha}$:TxB_2 ratio is positively increased by long-term low-dose aspirin treatment, despite the fact that aspirin inhibits prostaglandin formation. This is because although aspirin inhibits the synthesis of platelet thromboxane A_2, a vasoconstrictor and platelet activator, and of vascular endothelium prostacyclin synthesis, a vasodilator and platelet inhibitor (Haslam & McClenaghan, 1981; Weksler *et al.* 1983; Kallmann *et al.* 1987), the effect of aspirin on endothelium cyclooxygenase is much less than the effect on the platelets. The mechanism of action of carnitine in heart failure and claudication could be achieved by an increase in the 6 keto-$PGF_{1\alpha}$:TxB_2 ratio. Our results suggest that elderly patients could be treated chronically with carnitine to obtain this beneficial effect. Further clinical trials, in which both plasma eicosanoid levels and prostacyclin production are monitored, should be performed to test this hypothesis.

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