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Original Contributions

Influence of dietary carnitine on lipid and carbohydrate metabolism in rats

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Summary: Dietary saturated fatty acids, when compared with polyunsaturated fatty acids, increase plasma triglyceride and cholesterol concentrations, possibly because saturated fatty acids are preferentially converted into triglycerides. Carnitine is known to stimulate the oxidation of fatty acids and the formation of ketone bodies, and thus divert fatty acids from the pathway of esterification and triglyceride synthesis. In rats fed semipurified diets, we tested the hypothesis that carnitine counteracts the increase in plasma cholesterol and triglycerides seen after the feeding of saturated fatty acids. Indeed, saturated fatty acids in the form of coconut fat were found to increase plasma triglyceride and cholesterol concentrations when compared with polyunsaturated fatty acids fed as corn oil. The addition of carnitine to the diet (1 %, w/w) did not affect this differential fat effect. Thus the hypothesis would be disproved. However, it cannot be excluded that the experimental conditions were improper, so as to demonstrate an interaction between dietary carnitine and saturated fatty acids: dietary carnitine per se did not influence the blood concentration of ketone bodies.

Zusammenfassung: Im Vergleich zu mehrfach ungesättigten Fettsäuren in der Nahrung erhöhen gesättigte Fettsäuren die Cholesterin- und Triglyceridekonzentrationen im Plasma, möglicherweise weil gesättigte Fettsäuren vorzugsweise in Triglyceride umgewandelt werden. Es ist wohlbekannt, daß Karnitin sowohl die Oxidation von Fettsäuren als auch die Ketonkörpersynthese anregt und damit die Veresterung und Triglyceridsynthese von Fettsäuren verhindert. Anhand von Ratten, denen halbgereinigte Diäten verabreicht wurden, haben wir die Hypothese geprüft, ob Karnitin der durch gesättigte Fettsäuren induzierten Erhöhung der Cholesterin- und Triglyceridspiegel entgegenwirkt. Gesättigte Fettsäuren in Form von Kokosöl erhöhten tatsächlich die Plasmakonzentrationen von Triglyceriden und Cholesterin im Vergleich mit mehrfach ungesättigten Fettsäuren in Form von Maisöl. Der Zusatz von Karnitin in der Diät hatte keinen Einfluß auf diesen Fetteffekt. Also ist die Hypothese abzulehnen. Es ist möglich, daß die experimentellen Konditionen nicht hinreichend waren, um eine gegenseitige Beeinflussung von Karnitin und gesättigten Fettsäuren in der Nahrung feststellen zu können: Nahrungskarnitin hatte keinen Effekt auf den Blutspiegel von Ketokörpern.

Key words: dietary carnitine; lipid metabolism; carbohydrate metabolism

Schlüsselwörter: diätetisches Karnitin; Lipidstoffwechsel; Karbohydratstoffwechsel

Introduction

Carnitine is essential for transport of activated long-chain fatty acids across the inner mitochondrial membrane to sites of beta-oxidation (6). Thus carnitine might play a key role in the regulation of fatty acid metabolism. In diet-induced hyperlipidemic rats, ingestion of carnitine produced a decrease in plasma triglycerides (8, 11, 12). Carnitine treatment also led to increases in plasma carnitine and acylcarnitine levels (8, 11), which may promote increased rates of beta-oxidation, as illustrated by a tendency towards raised ketone body levels in plasma (8). Possibly, carnitine supplementation induces the liver to preferentially convert fatty acids into ketone bodies instead of into triglycerides. We have proposed earlier (3), that the metabolic basis for plasma triglyceride lowering by dietary polyunsaturated fatty acids, when compared with saturated fatty acids, may involve preferential conversion of polyunsaturated fatty acids into ketone bodies. Consequently, there is a diminished synthesis of triglycerides and depressed formation of very-low-density lipoproteins and also of their products, low-density lipoproteins, which carry most of the cholesterol in plasma. Thus plasma cholesterol levels will be decreased. It could be hypothesized that carnitine counteracts the increase in plasma triglycerides and cholesterol as induced by dietary saturated fatty acids. In the present study, this hypothesis was tested using rats. Since fatty acid and ketone body metabolism are intimately related to carbohydrate metabolism, plasma glucose and liver glycogen concentrations were also measured.

Materials and Methods

Female, random-bred Wistar rats (Cpb/WU) were used. Until the beginning of the experiment (Day 0) the animals were maintained on a commercial pelleted rat diet (RMH-B^R, Hope Farms BV, Woerden, The Netherlands), which was provided as meal from Day -9. From the age of 5 weeks the rats were housed individually in cages (24×17×17 cm) constructed of stainless steel with wire mesh bases. The cages were located in a room with controlled lighting (12 h/day), constant temperature (20 °C), and relative humidity (55–65 %).

At Day 0 of the experiment, the rats, aged 7 weeks, were divided into eight dietary groups consisting of six animals each. The distributions of plasma cholesterol concentrations and body weights were similar for all groups. The compositions of the semipurified diets, which were provided in meal form, are given in Table I. The type of fat (corn oil versus coconut fat), the amount of cholesterol in the diet (essentially cholesterol-free versus 1 % cholesterol) and the amount of carnitine (essentially carnitine-free versus 1 % carnitine) were the only variables. The animals received the experimental diets for 21 days. Food and tap water were provided ad libitum.

Blood samples were taken into heparinized tubes after a 16-h fast by orbital puncture under light diethyl-ether anesthesia. At the end of the experiment the anesthetized animals were sacrificed by decapitation. The livers were removed, weighed and stored at −20 °C until analysis.

Crude fat concentrations and fatty acid composition of the whole diets were determined according to Folch et al. (5) and Metcalfe et al. (9), respectively. Plasma total cholesterol was measured enzymatically according to Siedel et al. (14), by using a test combination (Monotest). Plasma triglycerides were measured enzymati-

Table 1. Composition of the experimental diets.

	Diet							
	Corn oil	Coconut fat	Corn oil + carnitine	Coconut fat + carnitine	Corn oil + cholesterol	Coconut fat + cholesterol	Corn oil + cholesterol + carnitine	Coconut fat + cholesterol + carnitine
Ingredient (g/100 g)								
Corn oil	20.0	2.0	20.0	2.0	20.0	2.0	20.0	2.0
Coconut fat	–	18.0	–	18.0	–	18.0	–	18.0
D,L-carnitine	–	–	1.0	1.0	–	–	1.0	1.0
Cholesterol	–	–	–	–	1.0	1.0	1.0	1.0
Corn starch	35.0	35.0	34.0	34.0	34.0	34.0	33.0	33.0
Constant components[1)]	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0
Chemical analysis (g/100 g)								
Crude fat	19.8	19.9	20.2	19.9	20.5	20.9	21.1	21.0
Fatty acids (g/100 g fatty acids)								
C 12:0	–	35.1	–	39.1	0.2	35.1	0.2	37.0
C 14:0	–	15.5	–	15.4	0.1	16.2	0.1	16.2
C 16:0	10.7	10.0	10.4	9.3	10.1	10.4	10.3	10.0
C 18:0	2.1	3.3	2.0	2.7	2.0	3.3	2.0	3.0
C 18:1	28.9	11.5	28.4	10.1	29.1	12.3	28.9	11.0
C 18:2	54.4	9.1	54.9	8.3	55.8	9.6	55.2	9.0
Sat. total	13.3	76.3	13.0	79.5	13.0	77.7	13.1	79.1
Mono. total	29.4	11.9	28.6	10.1	29.6	12.5	29.6	11.2
Poly. total	55.4	9.5	56.0	8.4	56.9	9.7	56.1	9.2

[1)] The constant components consisted of (g): casein, 20; sucrose, 10; molasses, 5; sawdust, 2; dicalcium phosphate, 2.9; sodium chloride, 0.6; magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate 1.8; vitamin premix 1.2; mineral premix 1.0. The composition of the mineral and vitamin premixes have been described elsewhere (4).

cally with a test combination according to Sullivan et al. (15). Plasma glucose was measured enzymatically according to Schmidt (13), using a test combination (Gluco-quant). Analysis of acetoacetate and D-3-hydroxybutyrate in blood was performed as described earlier (10). All plasma and blood analyses were performed in duplicate on a Multistat III plus micro-centrifugal analyzer (Instrumentation Laboratory Inc., Lexington, MA, USA). All test combinations were purchased from Boehringer Mannheim GmbH, FRG. The livers were homogenized in distilled water, and cholesterol was extracted and analyzed according to Abell et al. (1). Liver homogenate samples were treated with Dreywood's anthron reagent and glycogen was determined as glucose according to Hassid and Abraham (7).

Results and Discussion

Table 2 shows that body weights and feed intakes tended to be somewhat lower in the groups fed carnitine. As would be anticipated (10), dietary cholesterol increased liver wet weight.

As documented in Table 3, dietary corn oil induced lower concentrations of triglycerides in plasma than did coconut fat. Dietary carnitine did not affect this difference, which disproves the hypothesis that triggered this investigation. The dietary effects on blood ketone bodies are not entirely in agreement with the hypothesis either. Indeed, coconut fat tended to decrease blood concentrations of acetoacetate, but dietary carnitine did not counteract this effect. Blood levels of D-3-hydroxybutyrate were not systematically influenced.

Dietary corn oil lowered plasma cholesterol concentrations when compared with coconut fat (Table 3). Dietary cholesterol increased plasma

Table 2. Body and liver weight, and feed intake.

Measure	Diet			
	Corn oil	Coconut fat	Corn oil + cholesterol	Coconut fat + cholesterol
Body weight (g)				
initial				
– carnitine	124 ± 9.8	125 ± 8.6	120 ± 10.0	118 ± 9.4
+ carnitine	122 ± 9.8	119 ± 11.4	118 ± 7.8	117 ± 7.9
final				
– carnitine	170 ± 14.2	185 ± 15.6	174 ± 17.2	168 ± 9.7
+ carnitine	153 ± 20.4	158 ± 11.4	161 ± 16.9	160 ± 10.7
Liver weight (g)				
– carnitine	5.6 ± 0.78	4.8 ± 0.44	7.4 ± 0.87[b]	5.8 ± 0.43[b]
+ carnitine	4.4 ± 0.51[a]	5.0 ± 0.32	6.8 ± 1.24[b]	5.8 ± 0.95
Feed intake (g/day)				
– carnitine	12.9 ± 1.46	12.3 ± 0.90	13.5 ± 1.64	13.2 ± 0.80
+ carnitine	12.0 ± 1.22	12.3 ± 0.82	12.6 ± 0.82	12.6 ± 0.59

Results are expressed as means ± SD for six animals in each group. The experiment lasted 21 days. [a] Significantly different from comparable group fed diet without carnitine and [b] significantly different from comparable group fed diet without cholesterol ($p < 0.05$; two-tailed Student's t-test).

cholesterol concentrations, but did not alter the lowering effect of corn oil. It is interesting to note that carnitine tended to lower plasma cholesterol when the diet contained corn oil, but not when it contained coconut fat. No satisfactory explanation can be given. In any event, it could imply that the cholesterol elevating action of coconut fat is not related to the combination of a diminished conversion of fatty acids into ketone bodies, and an enhanced synthesis of triglycerides. Again, our hypothesis is contested.

Cholesterol feeding caused a dramatic increase in liver cholesterol with all dietary fat-carnitine combinations, this effect being amplified by diet-

Table 3. Liver cholesterol and glycogen, plasma cholesterol, glucose, and triglycerides, and blood ketone bodies in rats fed the experimental diets.

Measure	Diet			
	Corn oil	Coconut fat	Corn oil + cholesterol	Coconut fat + cholesterol
Liver				
cholesterol (μmol/g)				
− carnitine	7.7 ± 2.56	6.3 ± 0.76	102.7 ± 19.9[b]	46.8 ± 7.17[b, c]
+ carnitine	7.4 ± 1.34	6.8 ± 1.02	100.1 ± 14.7[b]	42.8 ± 17.7[b, c]
glycogen (mg/g)				
− carnitine	1.47 ± 0.26	1.39 ± 0.18	2.54 ± 0.93[b]	2.02 ± 0.24[b]
+ carnitine	1.40 ± 0.21	1.48 ± 0.33	2.04 ± 1.04	2.24 ± 0.46[b]
Plasma				
cholesterol (mM)				
initial				
− carnitine	2.64 ± 0.36	2.64 ± 0.34	2.71 ± 0.33	2.70 ± 0.36
+ carnitine	2.61 ± 0.32	2.66 ± 0.30	2.60 ± 0.30	2.55 ± 0.29
final				
− carnitine	1.95 ± 0.43	2.26 ± 0.19	2.97 ± 1.24	3.28 ± 0.90[b]
+ carnitine	1.54 ± 0.37	2.68 ± 0.21[c]	2.86 ± 1.10[b]	3.38 ± 1.31
glucose (mM)				
− carnitine	6.0 ± 0.84	6.4 ± 0.63	6.1 ± 0.68	6.7 ± 1.01
+ carnitine	5.7 ± 0.58	7.3 ± 0.71[a]	5.6 ± 0.35	6.8 ± 0.41[c]
triglycerides (mM)				
− carnitine	0.45 ± 0.20	0.50 ± 0.09	0.28 ± 0.08	0.39 ± 0.22
+ carnitine	0.30 ± 0.04	0.52 ± 0.14[c]	0.24 ± 0.06	0.38 ± 0.25
Blood				
acetoacetate (mM)				
− carnitine	0.67 ± 0.20	0.63 ± 0.11	0.70 ± 0.19	0.50 ± 0.10[c]
+ carnitine	0.59 ± 0.06	0.47 ± 0.09[a, c]	0.73 ± 0.15	0.55 ± 0.11[c]
D-3-OH-butyrate (mM)				
− carnitine	1.19 ± 0.36	1.23 ± 0.26	1.07 ± 0.37	1.03 ± 0.25
+ carnitine	1.33 ± 0.21	0.86 ± 0.21[a, c]	1.15 ± 0.22	1.08 ± 0.24

Results are expressed as means ± SD for six animals in each group. The experiment lasted 21 days. [a] Significantly different from comparable group fed diet without carnitine, [b] significantly different from comparable group fed diet without cholesterol, and [c] significantly different from comparable group fed corn oil ($p < 0.05$; two-tailed Student's t-test).

ary corn oil when compared with coconut fat (Table 3). This effect of corn oil is consistent with earlier observations (2, 10). Dietary carnitine did not affect liver cholesterol concentrations.

In agreement with earlier work (10), dietary cholesterol elevated liver glycogen concentrations. The type of dietary fat and the amount of dietary carnitine did not modify this effect of dietary cholesterol (Table 3). Although not statistically significant, it would appear that corn oil produced higher liver glycogen concentrations than coconut fat, in the absence rather than in the presence of carnitine (Table 3). On the other hand, carnitine tended to increase liver glycogen levels on diets containing coconut fat. No such effect was seen with diets containing corn oil. Further studies are required on this point. Plasma glucose concentrations were somewhat increased with diets containing coconut fat compared with corn oil. Carnitine did not systematically influence plasma glucose.

We have investigated whether dietary carnitine would be able to antagonize the elevation of plasma triglycerides and cholesterol induced by dietary saturated fatty acids when compared with polyunsaturated fatty acids. It is clear that carnitine did not have such an effect. The implication is that this study does not lend support to the idea (3) that dietary saturated fatty acids increase plasma triglycerides and cholesterol because these fatty acids are preferentially used for triglyceride synthesis rather than oxidation and successive ketone body formation. It is possible however, that under the present conditions the hypothesis could not be tested, since carnitine was ineffective, possibly because the metabolism of the rats was already saturated with carnitine. This notion is derived from the observation that carnitine per se did not affect blood levels of ketone bodies. However, it should be realized that the flux through the pool of ketone bodies may increase without influencing pool size.

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