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Cardiac carnitine leakage is promoted by cardiomyopathy

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Abstract

Objective: We investigated whether a damaged heart with cardiomyopathy (CM) influences cardiac-stored carnitines.

Methods: A sensitive, specific, carnitine-requiring yeast was used to determine blood carnitine concentration in 116 healthy subjects. For comparison with blood carnitine concentrations from patients with CM, we selected 33 male patients, ages 29 to 67 y, with evidence of CM and 24 male patients, ages 31 to 66 y, with no CM as categorized by cardiac catheterization.

Results: During catheterization, significantly higher concentrations of arterial blood levels of carnitines leaked from hearts of patients specifically with CM; no arterial blood carnitines leaked from hearts of patients without CM. Venous blood carnitine concentration for all patients was within the normal range. Carnitine did not accumulate in venous blood and was not a source of large amounts of leaked blood carnitines in patients with CM.

Conclusion: CM causes leakage of carnitines from heart stores, possibly making cardiac tissue vulnerable to damage. We do not know whether cardiac carnitine leakage leads to CM or if established CM promotes cardiac carnitine leakage.

Keywords: Cardiac catheterization; Plasma carnitines; Heart; Cardiomyopathy

Introduction

Carnitine is biosynthesized in the body and supplied by diet, mostly from beef. Biosynthesis requires lysine, methionine, niacin, vitamins B6 and C, and iron [1,2]. The physiologic role of carnitine is to transfer long-chain fatty acids from cytosol to the mitochondrial matrix for β-oxidation of fatty acids that, rather than glucose, is used by cardiac and skeletal muscle as a major energy source [1–3]. Although carnitine is not synthesized by cardiac and skeletal muscle, both contain almost all body stores of carnitine. The liver, brain, and kidney are the only tissues able to synthesize carnitine for transport to muscle, heart, and other tissues [2]. Pathologic manifestations induced by carnitine fluctuations in humans and animals have been noted during advanced liver disease, renal insufficiency, severe protein malnutrition, and catabolic states exacerbated by urinary excretion of carnitine [4–13]. While studying blood micronutrients in patients with cardiovascular disease, we noted that some of these patients, when catheterized, had higher than normal circulating levels of free, acyl, and total carnitines in the arterial blood; results from patients who had cardiomyopathy (CM) stood out. For this reason, we examined the effect of CM on cardiac carnitine binding capacity.

Materials and methods

Study population

Blood (5 mL) for carnitine evaluation was collected from 116 healthy subjects ages 21 to 72 y who did not have heart disease from an antecubital vein into Vacutainers (Becton Dickinson, Sunnyvale, CA, USA) that contained ethylene-diaminetetra-acetic acid as an anticoagulant. This peripheral blood represents venous blood that enters the heart and serves as a marker for circulating venous blood concentrations of carnitine. One hundred seventy-seven patients registered to undergo diagnostic cardiac catheterization for suspected heart disease were

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surveyed. At registration all patients had blood drawn from an antecubital vein (see above) before catheterization. After catheterization, we selected blood for study from 33 male patients ages 29 to 67 y who had a specific diagnosis of CM, e.g., chronic and acute myocarditis, dilated cardiopathy, hypertrophic cardiomyopathy, ischemic cardiomyopathy, as confirmed by cardiac catheterization. This diagnosis was made for patients who had left ventricular ejection fraction less than or equal to 35% by left ventriculography; patients who had valvular heart disease, congenital heart defects, or coronary artery bypass surgery were excluded. We also selected blood from 24 catheterized male patients ages 31 to 66 y who showed no specific evidence of CM after catheterization to compare blood concentrations of carnitine with those from 33 patients who had CM. During cardiac catheterization, 5 mL of blood was withdrawn specifically from the aortic root into Vacutainers (see CARDIAC CATHETERIZATION) to represent arterial blood *exiting* heart. All subjects gave informed consent to participate in this study, which was approved by the committee on human research.

Carnitine analysis

Many assay methods have been reported to determine carnitine concentration using radioenzymes or other radiolabeled substitutes, radio counters, high-performance liquid chromatography, ion-exchange binding, or spectroscopy; as reviewed, these assays are unmanageable for routine use [14,15]. A spontaneous carnitine-requiring strain of the yeast *Torulopsis bovina* (ATCC 26014, American Type Culture Collection, Rockville, MD, USA) was used for carnitine assays as in previous studies [14,15]. The yeast growth can be measured turbidimetrically in a chemically defined medium after carnitine is added from *plasma* extract to the medium as a standard for gauging growth of the organism. The growth turbidity is linearly proportional to carnitine concentration in blood or from a carnitine standard over a workable range of 0.6 to 60 pM/mL [14]. The standard used is synthetic L-carnitine HCL (Sigma Chemical, St. Louis, MO, USA).

Because we and other investigators do not know the exact molecularity of all free and fatty acid complexed carnitines liberated from blood, we used the terms *free* to denote acid-soluble carnitines and *acyl* to denote alkali-liberated carnitines from fatty acids [15,16]. The sum of free and acyl carnitines yields total (free and fatty acid complexed) blood carnitine concentration. Deviations (mean ± standard deviation) in carnitine concentration in plasma were calculated by standard methods; Student's *t* test was applied to estimate the significance differences between means.

Cardiac catheterization

Catheterization was done by using Seldinger's basic technique [17]. During catheterization, blood for carnitine analyses was purposely collected from the *aortic root* because it yields an exclusive sample, of *arterial blood leaving the heart*. In contrast, venous blood from antecubital veins of 116 healthy ambulatory subjects and 57 patients with CM represents blood *entering* the heart from the peripheral circulation (Table 1).

Table 1
Free, acyl, and total carnitine concentrations in blood from subjects with or without cardiomyopathy*

Blood	Carnitine (μM/L)		
	Free	Acyl	Total
Entering heart (venous)			
Healthy subjects (n = 116)	40.9 ± 4.9	6.8 ± 5.5	45.9 ± 7.4
Patients (n = 57)			
Without CM (n = 24)	43.2 ± 5.1	8.1 ± 4.6	47.2 ± 6.9
With CM (n = 33)	46.2 ± 4.9	8.4 ± 4.4	49.3 ± 7.8
Exiting heart (arterial)			
Patients			
Without CM (n = 24)	39.1 ± 5.0	8.7 ± 3.7	47.7 ± 5.6
With CM (n = 33)	58.9 ± 16.1	14.3 ± 6.8	73.2 ± 12.1
$P^{\dagger}$	<0.0001	<0.0003	<0.0001

CM, cardiomyopathy.
* Values are mean ± standard deviation.
† Arterial blood values in subjects with CM versus those without CM.

Results

Before cardiac catheterization, patients with or without CM (as later diagnosed by catheterization) had similar venous carnitine concentrations as healthy subjects. Table 1 also indicates that catheterized patients with CM have significantly higher arterial blood concentrations ($P < 0.0001$) of carnitines that leak from the heart than do patients without CM.

Discussion

The striking finding of this study is that significantly higher concentrations of carnitine in arterial blood leak from damaged hearts of patients with CM; it does not occur in patients without CM (Table 1). No change in carnitine levels was seen in venous blood from any patient compared with venous blood from healthy subjects (Table 1). This indicates that the high concentration of cardiac-leaked arterial carnitines is not due to supplementation from the venous system; the carnitine comes directly from cardiac tissue leakage. The cardiac-leaked arterial blood carnitines are probably redistributed to tissue without accumulating in venous blood. These results are not due to the catheterization procedure because exiting arterial blood from catheterized patients without CM had no increased blood levels of carnitine (Table 1) despite catheterization. Carnitine loss from cardiac tissue (Table 1) may be responsible for promoting CM by damaging cellular metabolism [5,7,11,

18,19]. This could further myocardial damage by facilitating free radical toxicity. As an antioxidant, increased cardiac-bound carnitine might eliminate this toxicity [5,11,20]. It would be prudent to replace the loss of cardiac-leaked carnitine in cardiac tissue in patients with CM by using the oral or parenteral (intravenous) route. However, long-term mass oral L-carnitine therapy of patients with CM rarely produces a favorable effect in reversing myocardial damage [5,11,13,18,19]. Oral carnitine loading is therefore not reliable for increasing blood and tissue levels of carnitine [13,15,18,19] because ingested carnitine supplements are rapidly destroyed by bacterial action in the gut [15]. The intravenous route seems more reliable for flooding of tissue with carnitine by mass action, which should increase blood carnitine and tissue utilization [18]. This would bypass the gut, where much bioavailable carnitine is misused or incompletely absorbed [5,15,18]. Studies have indicate that intravenous carnitine administered in large doses can saturate cardiac tissue with carnitine made deficient by CM [5,8,11,13,19].

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