# Hepatic Carnitine Palmitoyltransferase Activity in Cattle

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ABSTRACT. A fluorometric assay for the determination of hepatic carnitine palmitoyltransferase (CPT) activity was slightly modified for use with cattle samples. With this assay, the $K_m$ value was 0.56 ± 0.10 mM with respect to L-carnitine (mean ± SD, n=4) and was 9.6 ± 2.2 $\mu$M (n=3) with respect to palmitoyl-CoA. The average hepatic CPT activity was 33.6 ± 2.0 $\mu$mol CoASH/min/g protein in 38 healthy cattle and was similar in both sexes and among breeds. Hepatic CPT activity showed no correlation with serum phospholipid, free fatty acid, triglyceride or total cholesterol concentrations. — KEY WORDS: carnitine palmitoyltransferase, cattle, fluorometric assay.

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Carnitine palmitoyltransferase (CPT), located on the mitochondrial membrane, is an enzyme that translocates long-chain fatty acids (LCFA) from the cytosol to the mitochondrial matrix [7, 15]. Since LCFA are unable to cross the mitochondrial membrane directly [6], they are activated to their coenzyme A esters by long-chain acyl-CoA synthetase situated on the outer mitochondrial membrane and are transported into the mitochondrial matrix in a process which is dependent on L-carnitine and carnitine acyltransferase [2, 11]. Hepatic CPT activity has therefore been examined in relation to fatty acid oxidation [2, 9]. CPT is thought to be a key enzyme in the development of bovine lipid metabolic disorders, for example, ketosis, hepatic lipidosis and fatty liver.

Usually CPT activity is determined by radiochemical assay or spectrophotometric assay. Although radiochemical assay is highly sensitive, it is complicated and it is necessary to use radioactivity. Spectrophotometric assay has low sensitivity. Shäfer *et al.* [14] described a rapid fluorometric method for the determination of CPT activity in rat and human livers. In their method, CPT activity was measured as the rate of coenzyme A (CoASH) release from palmitoyl-coenzyme A (palmitoyl-CoA) by CPT with *N*-(9-acridinyl)maleimide (NAM) to form a fluorescent product. We used this assay with minor modifications for cattle liver to determine the activity in samples from 38 healthy cattle.

The hepatic tissue samples and serum were collected from 38 healthy cattle (14 Japanese Black (JBl) × Holstein (H) crossbreed, 11 H, 7 JBl and 6 Japanese Brown) immediately after sacrifice, and were frozen until assayed. Hepatic tissue samples were homogenized in 10 vol. of a solution containing 100 mM sodium phosphate, 100 mM potassium phosphate, and 2 mM EDTA, pH 7.4. The homogenates were then sonicated (30W) on ice for 5 times in 5 seconds bursts with 1 min intervals. Homogenate protein was measured by the Coomassie Brilliant Blue assay (Bio-Rad protein assay, Bio-Rad Laboratories, CA, U.S.A.) with bovine serum albumin as the standard.

The assay medium contained 5-40 $\mu l$ of tissue homogenate, palmitoyl-CoA (0.1 mM), NAM (0.2 mM), sucrose (220 mM), potassium chloride (KCl, 40 mM), and buffer (50 mM Tris/HCl, pH 7.4) at a total volume of 1.8 m*l*. Fluorescence intensity, measured at an emission wavelength of 435 nm and an excitation wavelength of 360 nm, showed a linear increase dependent on CoASH release. The rate of fluorescence intensity increase was measured for approximately 4 min, and then the reaction was initiated by the addition of L-carnitine (5 mM). The rate of increase before the addition of carnitine was the activity of endogenous CoASH release in the absence of carnitine, and the rate of increase after the addition of carnitine was equal to the carnitine-dependent activity plus endogenous activity. Thus CPT activity was the difference between the rate after the addition of carnitine and the endogenous rate.

The $K_m$ value, calculated from the Lineweaver-Burk plot, is 0.56 ± 0.10 mM with respect to L-carnitine (n=4), and 9.6 ± 2.2 $\mu$M with respect to palmitoyl-CoA (n=3). A randomly selected cattle liver tissue sample yielded coefficients of variation (CV) of 8.8% (within-run) and 8.9% (between-run).

In the original assay, 0.5 mg/m*l* of BSA was added to the buffer to maintain the acyl-CoA concentration and to prevent the formation of palmitoyl-CoA micelles. Shäfer *et al.* showed a higher activity of CPT in the absence of 0.5 mg/m*l* BSA. With bovine samples, the addition of 0.5 mg/m*l* BSA to the reaction buffer was slightly increased in CPT activity or had no effect, whereas the addition of more BSA decreased CPT activity (Fig. 1). With BSA present, the true free concentration of palmitoyl-CoA is unknown, and the palmitoyl-CoA/albumin ratio affects the $K_m$ for L-carnitine [12]. Therefore, no BSA was added to our buffer. The formation of palmitoyl-CoA micelles in our reaction buffer was not found, but our sample homogenate had a high protein concentration (about 8 to 11 mg/m*l*, in the homogenate) that would play the role of albumin in preventing micelle formation.

The potassium concentration had no effect on this assay (Fig. 2), in contrast to previous reports starting that CPT activity was affected by the buffer potassium ion concentration and was decreased at high potassium buffer [13]. We added 40 mM KCl and 220 mM sucrose to the buffer. This technique is often used in radiochemical assay [5].

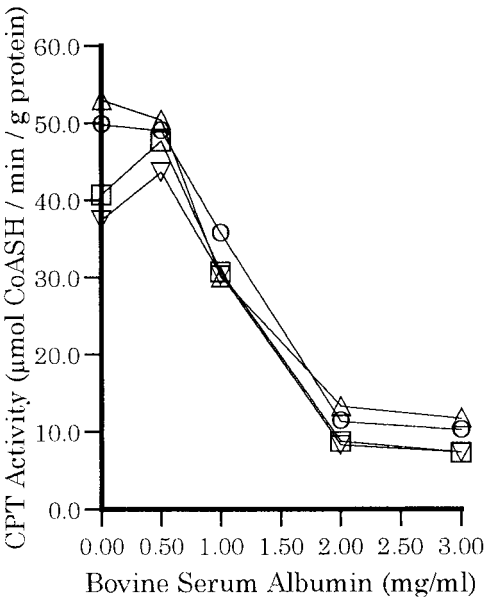


Fig. 1. Effect of the bovine serum albumin concentration on bovine hepatic CPT activity. Bovine samples were assayed in a final volume of 2.0 m*l* of 50 mM Tris-HCl, containing 220 mM sucrose, 40 mM KCl and 0.1 mM palmitoyl-CoA.

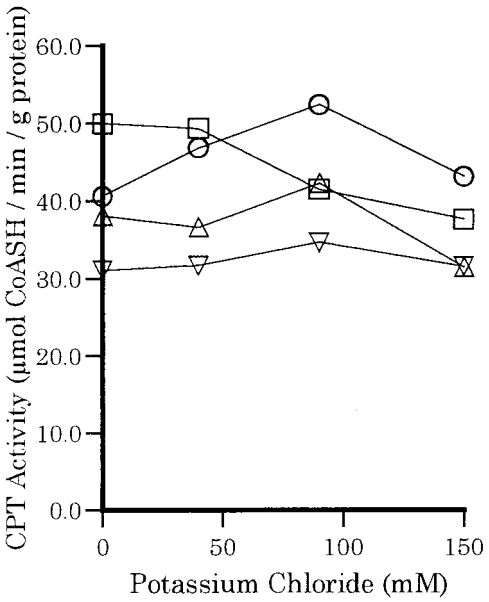


Fig. 2. Effect of the potassium concentration on bovine hepatic CPT activity. Randomly selected bovine samples were assayed in a final volume of 2.0 m*l* of 50 mM Tris-HCl, containing 0.1 mM palmitoyl-CoA. The proportions of sucrose and KCl together always provided for an osmolarity of 300 mosmal/L in the reagent buffer.

In our method, the $K_m$ value for L-carnitine and that for palmitoyl-CoA were in agreement with the results obtained for rat by spectrophotometric assay [2] and for cattle by radiochemical assay [8].

Table 1. Hepatic carnitine palmitoyltransferase (CPT) activity in cattle

| Breed | CPT activity mean ± SD, μmol CoASH release/min/g protein | | |
|---|---|---|---|
| | Male | Female | Total |
| H | 32.2 ± 5.9 (n=3) | 30.6 ± 5.5 (n=8) | 31.0 ± 1.6 (n=11) |
| H×JBl | 34.9 ± 8.2 (n=7) | 34.3 ± 23.2 (n=7) | 34.6 ± 4.5 (n=14) |
| JBI | ND | 34.0 ± 11.6 (n=7) | 34.0 ± 11.6 (n=7) |
| JBr | ND | 35.8 ± 13.8 (n=6) | 35.8 ± 13.8 (n=6) |
| Total | 34.1 ± 7.4 (n=10) | 33.5 ± 14.0 (n=28) | 33.6 ± 12.5 (n=38) |

H: Holstein. H×JBl: Horstein × Japanese Black cross breed. JBl: Japanese Black. JBr: Japanese Brown. ND: Not done.

Average hepatic CPT activity was 33.6 ± 2.0 μmol CoASH/g protein in 38 healthy cattle. There was no difference between sexes in H and H × JBl crossbreeds. In males, there was no difference among breeds (Table 1). These results suggested that hepatic CPT activity is unrelated to sex or breed. But the breeds used were all beef cattle, so they might have received high-energy feed with the result that there was low fat mobilization for energy. If milk cattle were studied, the results might be different.

Hepatic CPT activity showed no correlation with serum phospholipid, free fatty acid, triglyceride or total cholesterol concentrations (Fig. 3). It is generally accepted that some hormones, i.e. insulin [4, 10] and glucagon [10],and some tissue CoA esters, i.e. malonyl-CoA [1, 2, 3, 8,] and methylmalonyl-CoA [3, 15], regulate CPT activity. In our study, these factors were not measured and the effect of these factors on our samples is not known but we thought that CPT activity apparently was not directly regulated by serum lipids.

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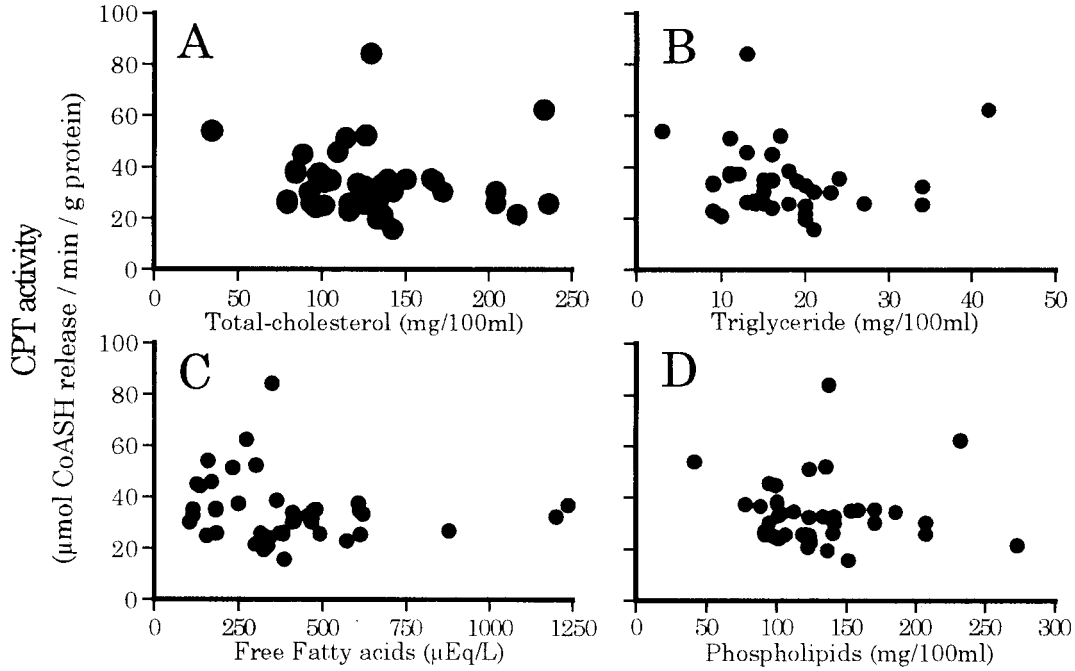


Fig. 3. Correlations between CPT activity and serum total-cholesterol (A), serum triglyceride(B), serum free fatty acids (C) and serum phospholipids (D).

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