

## In Vitro Esterase Activity of Carbonic Anhydrase on Total Esterase Activity Level in Serum

Received: January 21, 2002

**Abstract:** The in vitro esterase activity of carbonic anhydrase on total esterase activity level was investigated. This effect was obtained by subtracting the value of net esterase from the value of total esterase in sera with normal ( $95.22 \pm 8.87$ ) and high glucose levels ( $208.58 \pm 87.90$ ). Total esterase activity, net esterase activity and esterase activity of carbonic anhydrase were  $20.2 \pm 1.9$ ,  $18.1 \pm 1.8$  and  $2.1 \pm 0.7$  U/min/mL serum in sera with a normal glucose level, and  $21.3 \pm 2.8$ ,  $17.9 \pm 2.9$  and  $3.4 \pm 1.2$  U/min/mL serum in sera with a high glucose level, respectively. Thus, the esterase activity of carbonic anhydrase was determined to be 10.4% in sera with a

normal glucose level, and to be 16% in sera with a high glucose level. The total esterase activity level of the sera with a high glucose level was statistically significant ( $p < 0.05$ ) compared to those with a normal glucose level, whereas the difference between net esterase activity levels was not significant. As seen, without taking into consideration the effect of the in vitro esterase activity of carbonic anhydrase, the total esterase levels would have been found significant under pathological conditions and this may lead us to interpret the in vivo results in error.

**Key Words:** Esterase activity, esterase, and carbonic anhydrase

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### Introduction

In mammalian serum, there are several enzymes, such as pseudocholinesterase, carboxylesterase, arylesterase and paraoxonase, which have shown esterase activity (1). Esterases are known to hydrolyse endogenous substrates, and the majority hydrolyse lipid ester substrates (1). In addition to these esterases, only under in vitro conditions, the esterase activity of carbonic anhydrase (CA) was determined (2). CA basically catalyses the reversible hydration of  $\text{CO}_2$  to  $\text{HCO}_3^-$  and  $\text{H}^+$  (2). CA, a family of zinc metal enzymes, is a well-characterized pH regulatory enzyme in most tissues. To date 12 isozymes of CA have been described in mammals (3).

Both in physiological and pathological conditions, some studies have been carried out to show a change in the activity levels of both esterase enzymes and CA (4-14). In some of these studies, for instance, it was found that serum paraoxonase activity changes in patients with diabetes, uraemia, myocardial infarction and familial hypercholesterolaemia and in those who have undergone a kidney transplant (4-7). In another study, it was determined that urinary cholinesterase activity varies in patients with insulin-dependent diabetes (8). In addition, it was observed that CA activity changes in patients with

diabetes, hypertension, muscle and liver disease, and colorectal cancer (9-14).

So far no report has dealt with the in vitro esterase activity of CA on total esterase activity level. Therefore, the aim of the present study was to investigate this possible role of CA in two different serum groups with normal and high glucose levels.

### Materials and Methods

In this study, sera were obtained from subjects with normal and high glucose (diabetic) levels in the Department of Internal Medicine, Medical School, Atatürk University. Forty normal subjects had a serum glucose level of 62–109 mg/dL (Mean  $\pm$  SD,  $95.22 \pm 8.87$  mg/dL) and 40 diabetic subjects had a serum glucose level of 114–518 mg/dL (Mean  $\pm$  SD,  $208.58 \pm 87.90$  mg/dL).

Esterase activity level was measured spectrophotometrically using p-nitrophenyl acetate as a substrate according to the method described by Armstrong et al. (15) with a slight modification. The assay system consisted of 0.1 mL of serum in a 1 cm spectrophotometric cell containing 1.4 mL of 0.05 M

Tris-H<sub>2</sub>SO<sub>4</sub> buffer (pH 7) and 1.5 mL of 3 mM p-nitrophenyl acetate. The change in absorbance at 348 nm was measured over 3 min, before and after adding serum. One unit of enzyme activity was expressed as 1 μmol p-nitrophenol released per minute at room temperature (11). In order to obtain the net esterase activity, this measurement was repeated in the presence of 0.1 mL of 0.1 M acetazolamide (AZM) (11,16). Therefore, the esterase activity of carbonic anhydrase was obtained by subtracting the value of net esterase from the value of total esterase. All data were expressed as the mean ± SD. Data were analysed using by paired-samples or independent-samples Student's t test.

### Results

In order to investigate the possible esterase role of CA on total esterase activity level, we studied two different serum groups with normal and high glucose levels. As seen from the Table, total esterase activity, net esterase activity, and esterase activity of carbonic anhydrase were determined to be 20.2 ± 1.9, 18.1 ± 1.8 and 2.1 ± 0.7 U/min/mL serum in sera with a normal glucose level, and to be 21.3 ± 2.8, 17.9 ± 2.9 and 3.4 ± 1.2 U/min/mL serum in sera with a high glucose level, respectively. The difference between net esterase activity and total esterase activity in the two serum groups was statistically significant (p < 0.01, paired-samples Student's t test), and this difference reflects the in vitro esterase activity of carbonic anhydrase. The total esterase activity level of the sera with a high glucose level was statistically significant (p < 0.05, independent-samples Student's t test) when compared to those with a normal glucose level, whereas the difference between net esterase activity levels was not

significant. The last result suggests that without taking into consideration the effect of the in vitro esterase activity of carbonic anhydrase, the total esterase levels would have been found significant under pathological conditions and this may lead us to interpret the in vivo results in error.

### Discussion

CA shows hydratase activity under in vivo conditions. In addition, it only shows esterase activity under in vitro conditions (2,7). This is the first report about the in vitro esterase activity of CA on total esterase activity level in serum. To investigate this possible role of CA, we measured the esterase activities of esterase and CA enzymes spectrophotometrically using p-nitrophenyl acetate as substrate. In previous studies, the carbonic anhydrase inhibitor, acetazolamide (AZM), was chosen to investigate the in vivo inhibition effect on CA (18,19) and the in vitro esterase activity of CA in erythrocyte (11,16). Therefore, in the present study, we took advantage of AZM because of its inhibition effect. We determined the esterase activity of CA by a double assay procedure in the presence and absence of AZM. In serum, we measured the esterase activity of CA by subtracting the value with AZM from the value without AZM.

Both in physiological and pathological conditions, some studies have been carried out to show a change in activity levels of both esterase and CA enzymes. For instance, it was found that serum paraoxonase activity changes in patients with diabetes, uraemia, myocardial infarction and familial hypercholesterolaemia and in those who have undergone a kidney transplant (4-7). In addition, it was observed that CA activity changes in

Table. Levels of esterase activities (Mean ± SD).

Serum Glucose level (mg/dL)	Total Esterase activity (U/min/mL Serum)	Net Esterase activity (U/min/mL Serum)	Esterase activity of Carbonic Anhydrase (U/min/mL Serum)	%
95.22 ± 8.87	20.2 ± 1.9	18.1 ± 1.8 <sup>c</sup>	2.1 ± 0.7	10.4
208.58 ± 87.90	21.3 ± 2.8 <sup>a</sup>	17.9 ± 2.9 <sup>b,c</sup>	3.4 ± 1.2 <sup>d</sup>	16.0

<sup>a</sup>p < 0.05, vs total esterase activity of the sera with a normal glucose level (independent Student's t test).

<sup>b</sup>p > 0.05, vs net esterase activity of the sera with a normal glucose level (independent Student's t test).

<sup>c</sup>p < 0.01, vs total esterase activity (paired-samples Student's t test).

<sup>d</sup>p < 0.001, vs CA activity of the sera with a normal glucose level (paired-samples Student's t test).

patients with diabetes, hypertension, muscle and liver disease, and colorectal cancer (9-14). In our study, the esterase activities of carbonic anhydrase were 10.4% in sera with a normal glucose level and 16.0% in sera with a high glucose level (Table). Therefore, the difference between total and net esterase activity was statistically significant ( $p < 0.01$ ) in these two different serum groups. The total esterase activity level of the sera with a high glucose level was statistically significant ( $p < 0.05$ ) compared to those with a normal glucose level, whereas the difference between net esterase activity levels was not significant. As seen, without taking into consideration the effect of in vitro esterase activity of carbonic anhydrase, the total esterase levels would have been found as significant under pathological conditions and this may lead us to interpret the in vivo results in error.

In conclusion, without taking into consideration some enzymes such as CA having in vitro, but not in vivo, esterase activity, total esterase activity levels may not be the only determining factor for activity of the known in vivo esterase enzymes in physiological and pathological conditions. Further studies are needed to investigate the degree of esterase activity of CA on each specific substrate of known esterase enzymes in order to determine the in vitro esterase activity of CA on total esterase activity levels.

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