

# Calcium and calcitonin responses to calcium infusion in type I diabetes mellitus

J.A. Amado, C. Gomez, S. Obaya<sup>1</sup>, M. Otero, J. Gonzalez-Macias

Endocrine Unit, Department of Internal Medicine and <sup>1</sup>Department of Clinical Biochemistry, Valdecilla Hospital School of Medicine, 39008 Santander, Spain.

**Summary:** We studied calcium and calcitonin responses to intravenous calcium infusion (3 mg of elemental calcium/kg of body weight in 10 minutes) in 21 type I diabetic males and 17 age-matched normal males. Baseline total calcium, parathyroid hormone and calcitonin levels were normal in the diabetic group, but ionized calcium was lowered. Cortical bone status and osteocalcin levels were normal, suggesting a normal osteoblastic function. Total calcium and ionized calcium responses to calcium infusion were lowered in the diabetic group. Despite these lowered calcaemic responses, calcitonin secretion was normal.

## Introduction

Several studies have shown a disturbed mineral metabolism and a decrease in bone mass in type I diabetic patients.<sup>1-4</sup> The mechanisms underlying these abnormalities are not well known. Calcitonin deficiency has been proposed as a pathogenetic factor in involutional osteoporosis.<sup>5</sup> In humans there are few and contradictory data on calcitonin secretion in diabetes. Schmitz *et al.*<sup>6</sup> found a normal calcitonin response to calcium infusion in a group of ten patients with type I diabetes of short duration, while Witt *et al.*<sup>4</sup> found a higher calcitonin response to a calcium meal in a group of type I diabetic children. In this study we evaluated serum calcium and calcitonin responses to intravenous calcium infusion in a group of well-characterized type I diabetic males.

## Materials and methods

The diabetic group consisted of 21 males aged  $32.2 \pm 6.2$  years (range 21–41 years) with a duration of the disease of  $92.2 \pm 64.6$  months (range 24–256 months) and an insulin dose of  $54 \pm 21.3$  U/day (range 16–80). All patients had a normal creatinine clearance and absence of significant proteinuria. Overt clinical

neuropathy was excluded in all of them. Their percentage of ideal body weight was  $113 \pm 8\%$ . None of them had diabetic ketoacidosis defined as ketonuria and pH lower than 7.25 in the last year. The control group consisted of 17 matched for height and weight normal males aged  $28.3 \pm 6.3$  years (range 24–43). Except for insulin in the diabetic group drug ingestion or diseases known to affect calcium metabolism were excluded in all individuals. The protocol of study was approved by the local investigation committee in accordance with the principles of the Declaration of Helsinki.

A ten minutes constant rate infusion of 3 mg of elemental calcium (as calcium gluconogalactonate, Calcium Sandoz TM) per kg of body weight, in 100 ml of isotonic saline, was begun at 0900 h after an overnight fast and before the morning insulin shot. Blood samples were obtained from the opposite arm at –5, 0, 10, 20 and 60 minutes for total calcium, ionized calcium and calcitonin. At 0 time we also measured glucose, HbA<sub>1c</sub>, phosphate, albumin, total proteins, creatinine, alkaline phosphatase, parathyroid hormone (PTH) and osteocalcin. Total calcium was determined using Corning 940 fluorometric titration (Corning Medical, Medfield MA). For ionized calcium measurement serum was obtained anaerobically and stored on ice until determined with a Space Stat ionized calcium analyzer (Orion Biomedical, Cambridge MA).<sup>7</sup> Interassay coefficient of variation was 1.3% at a concentration of 1.1 mmol/l. Glucose.

Correspondence: J.A. Amado, M.D.  
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phosphate, albumin, total proteins, alkaline phosphatase and creatinine were measured by Autoanalyzer (Technicon Corp., Terrytown NY). Total HbA<sub>1c</sub> was measured by chromatography (Isolab). Calcitonin, carboxyterminal PTH and osteocalcin were measured by direct RIA using kits furnished by Immunonuclear Corp., Stillwater MN. Intraassay and interassay coefficients of variation were respectively 5% and 14% for calcitonin, 4.5% and 13.3% for carboxyterminal PTH, and 6% and 8.6% for osteocalcin.

Cortical bone status was assessed as the ratio of cortical area (CA) to total area (TA) at the midpoint of the second metacarpal of the right hand.<sup>8</sup>

Results are given as mean  $\pm$  standard deviation. Statistical analysis was done using Student's test or Mann-Whitney's U test when appropriate, and lineal correlation coefficient. Results were considered significant if  $P < 0.05$ .

## Results

Table I shows fasting blood and cortical bone status studies. No significant differences were found for any parameter, apart from glucose and HbA<sub>1c</sub>. CA/TA was normal in the diabetic group.

Table II shows the responses to the calcium infusion. Baseline total calcium and calcitonin were normal in the diabetic group, but ionized calcium was significantly lower ( $P < 0.05$ ). After calcium infusion total calcium and ionized calcium were lower in the diabetic group. Likewise maximal increase in total and ionized calcium (calcium at 10 minutes minus calcium at 0 minutes) was lower in the diabetic group. Calcitonin response in the diabetic group was normal. We

found no significant correlation between baseline glucose or HbA<sub>1c</sub> and baseline or stimulated calcitonin. No correlation was found either between duration of the disease or CA/TA and calcitonin response.

## Discussion

Calcium homeostasis seems to be abnormal in human diabetes mellitus,<sup>1-4</sup> despite some negative reports.<sup>9</sup> In this study we found normal levels of total calcium, phosphate, alkaline phosphatase, osteocalcin and PTH in a group of diabetic males, but ionized calcium was lowered, in accordance with a recent report by Fogh-Andersen *et al.*<sup>10</sup> A low ionized calcium despite a normal total calcium may be due to increased calcium-binding properties of diabetic blood. Factors responsible for this are unknown.<sup>10</sup> In spite of a lowered ionized calcium, carboxyterminal PTH was normal. This agrees with previous reports of normal to lowered PTH levels in diabetes.<sup>1,9-12</sup> The lack of measurable response of the parathyroids to the lowered ionized calcium is unexplained though in this study we have not measured intact PTH, the most sensitive way of evaluating parathyroid gland function. Hypomagnesaemia, a frequent finding in diabetes, may be responsible.<sup>12</sup> Regrettably in this study we did not measure magnesium levels.

Osteopaenia, as assessed by photon absorptiometry, is found in diabetic patients of short duration (McNair *et al.*<sup>1</sup>) but in our group of patients cortical bone measured by CA/TA was normal. This could be due to the lower sensitivity of this method as compared to the other, or to the small number of patients studied.

Osteocalcin or bone Gla-protein is one of the most abundant proteins in the skeleton and it is synthesized

**Table I** Biochemical characterization of diabetic patients and control subjects

|  | Diabetic patients | Controls        | Statistical significance |
|--|-------------------|-----------------|--------------------------|
| Glucose (mmol/l)                       | 9.8 $\pm$ 5.5     | 5.4 $\pm$ 0.64  | $P < 0.01$               |
| HbA <sub>1c</sub> (%)                  | 11.9 $\pm$ 3.1    | 8.44 $\pm$ 1.32 | $P < 0.001$              |
| Inorganic phosphate (mmol/l)           | 1.20 $\pm$ 0.26   | 1.13 $\pm$ 0.11 | NS                       |
| Total calcium (mmol/l)                 | 2.25 $\pm$ 0.12   | 2.17 $\pm$ 0.09 | NS                       |
| Ionized calcium (mmol/l)               | 1.17 $\pm$ 0.02   | 1.13 $\pm$ 0.04 | $P < 0.05$               |
| Albumin (g/l)                          | 41.9 $\pm$ 3.4    | 43.2 $\pm$ 3.5  | NS                       |
| Total proteins (g/l)                   | 65.6 $\pm$ 3.5    | 66.9 $\pm$ 3.4  | NS                       |
| $\gamma$ glutamyl transpeptidase (U/l) | 23.1 $\pm$ 18.5   | 21.2 $\pm$ 11   | NS                       |
| Alkaline phosphatase (U/l)             | 79.6 $\pm$ 21.4   | 60.6 $\pm$ 15.2 | 0.05 $< P < 0.1$         |
| Creatinine ( $\mu$ mol/l)              | 80.4 $\pm$ 13.2   | 86.6 $\pm$ 13.2 | NS                       |
| PTH ( $\mu$ g/l)                       | 0.48 $\pm$ 0.20   | 0.47 $\pm$ 0.24 | NS                       |
| Osteocalcin ( $\mu$ g/l)               | 3.55 $\pm$ 1.13   | 3.79 $\pm$ 1.43 | NS                       |
| CA/TA                                  | 0.8 $\pm$ 0.09    | 0.78 $\pm$ 0.06 | NS                       |

NS = not significant.

**Table II** Total and ionized calcium and calcitonin responses to calcium infusion in diabetic patients and control subjects

|                          | Time (minutes) |              |              |             |             |                  |
|--------------------------|----------------|--------------|--------------|-------------|-------------|------------------|
|                          | -5             | 0            | 10           | 20          | 60          | Maximal increase |
| Total calcium (mmol/l)   |                |              |              |             |             |                  |
| Controls                 | 2.25 ± 0.12    | 2.19 ± 0.080 | 2.70 ± 0.13  | 2.52 ± 0.10 | 2.41 ± 0.10 | 0.52 ± 0.12      |
| Diabetic patients        | 2.17 ± 0.09    | 2.13 ± 0.10  | 2.54 ± 0.13  | 2.42 ± 0.11 | 2.32 ± 0.10 | 0.40 ± 0.08      |
| Statistical significance | NS             | NS           | <0.01        | <0.01       | <0.01       | <0.01            |
| Ionized calcium (mmol/l) |                |              |              |             |             |                  |
| Controls                 | 1.17 ± 0.02    | 1.16 ± 0.05  | 1.46 ± 0.06  | 1.34 ± 0.03 | 1.26 ± 0.05 | 0.30 ± 0.06      |
| Diabetic patients        | 1.13 ± 0.04    | 1.12 ± 0.04  | 1.36 ± 0.05  | 1.29 ± 0.05 | 1.23 ± 0.05 | 0.22 ± 0.04      |
| Statistical significance | <0.05          | <0.05        | <0.001       | <0.01       | NS          | <0.05            |
| Calcitonin (ng/l)        |                |              |              |             |             |                  |
| Controls                 | 50.3 ± 14.3    | 50.8 ± 21.6  | 95.5 ± 43.5  | 59.1 ± 27.9 | 54.4 ± 19.6 | 44.5 ± 37.2      |
| Diabetic patients        | 52.4 ± 26.3    | 53.4 ± 27    | 115.4 ± 53.4 | 81.1 ± 32.3 | 58.7 ± 26.5 | 64.9 ± 61.5      |
| Statistical significance | NS             | NS           | NS           | NS          | NS          | NS               |

NS = not significant

selectively by osteoblasts. A small fraction of newly synthesized protein is released directly into the blood, so serum osteocalcin levels reflect the amount of osteoblastic activity in the bone.<sup>13</sup> Our finding of normal levels of serum osteocalcin suggests that osteoblastic function is normal in diabetes and that high levels of alkaline phosphatase found in some diabetic patients are of hepatic rather than bony origin.<sup>14</sup>

Total calcium and ionized calcium reached lower levels in diabetics than in normals after calcium infusion. Although the lowered baseline ionized calcium levels may have contributed to this finding, the maximal increase was significantly lower in diabetics, suggesting that there is a lowered calcaemic response in diabetes. This could be due to renal hypercal-

ciuria.<sup>3,11</sup> Baseline calcitonin levels were normal in accordance with other studies.<sup>6,9</sup> Despite the lowered calcaemic response, calcitonin levels increased normally, as previously reported in a smaller group of recently diagnosed patients.<sup>6</sup> The degree of metabolic control as measured by glucose and HbA<sub>1c</sub>, or duration of the disease did not influence calcitonin response. In this group of type I diabetic patients calcitonin secretion was normal.

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