Serum Calcium Fractions in Diabetes Mellitus
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Diabetes mellitus is associated with a decrease in bone mineral content and increased urinary excretion of calcium and phosphate. The purpose here was to elucidate the pathogenesis of these changes by comparing serum calcium fractions of diabetics and healthy controls. In a cross-sectional study, serum ionized calcium at pH 7.40, [Ca2+]i, of 46 insulin-treated diabetics was decreased in comparison with 44 healthy controls: mean 1.16 (SD 0.04) vs 1.21 (SD 0.03) mmol/L (p < 0.001). The decreased [Ca2+]i was associated with a higher concentration of ionized calcium in diabetic serum. The diabetics had higher concentrations of undetermined anions (p < 0.001) and this anion gap correlated negatively with [Ca2+]i (r = −0.43, p < 0.02). Serum [Mg2+] was decreased in the diabetics (p < 0.001). Values for venous acid–base status, serum creatinine, total and ultrafiltrable calcium, parathyroid, and inorganic phosphate were the same in the two groups. [Ca2+]i, but not serum total calcium, had increased by 0.04 mmol/L (p < 0.001) in the diabetics and by 0.02 mmol/L (p < 0.01) in the controls by 90 min after breakfast, with and without substitution insulin, respectively.

Insulin-dependent diabetes mellitus is associated with bone mineral loss and increased urinary excretion of calcium and phosphate (1–9). The changes relate to indices of poor metabolic control, but the underlying mechanism remains unknown (10). Data from the literature on serum calcium fractions in diabetes mellitus are conflicting, and the purpose of the present study was to re-examine serum calcium fractions in uncomplicated juvenile diabetes mellitus.

Materials and Methods
The insulin-dependent diabetic subjects were 26 women and 20 men, all ambulatory. Their mean age was 31 years (range 16–57 years). None had proteinuria, and serum urea and creatinine concentrations were normal. The duration of diabetes averaged nine years (range 1 to 31 years), and the mean insulin dose was 39 int. units/day (range 16–108 int. units/day).

We first examined 25 fasting diabetics and 23 age- and sex-matched controls. Later, we examined 21 diabetics and 21 healthy controls to see the effect of subcutaneous insulin injection and a standard breakfast. The samples for this experiment were drawn before and exactly 90 min after the insulin administration and the meal. All samples for each of the two experiments were treated alike. Serum was kept at −20 °C, and all analyses were performed on one day with the samples randomly ordered, to avoid day-to-day variation or bias.

The concentration of ionized calcium at pH 7.40 and 37 °C, [Ca2+]i, was determined with an ICA 1 ionized calcium analyzer (Radiometer) (11). Serum was equilibrated at 37 °C with two humidified CO2-air mixtures with pCO2 of 4 and 8 kPa. The apparatus measured pH and [Ca2+] simultaneously, and [Ca2+]i was determined by logarithmic interpolation to pH 7.40 with a calculator (Hewlett-Packard Model 85).

Total calcium and magnesium were determined by atomic absorption spectrophotometry with a Perkin-Elmer Model 403 atomic absorption spectrophotometer.

Serum ultrafiltrable calcium was determined after ultrafiltration at pCO2 = 0 and 37 °C. We added 25 μL of 1 mol/L hydrochloric acid to 1 mL serum, and the pCO2 approached zero after a 24-h incubation in open tubes at 4 °C. The ultrafiltrate of serum was made by placing 400 μL serum into a bent, clean Visking cellophane tubing, mounted in a test tube with the infusion above the bottom, and centrifuging at 37 °C for 1 h (12). We did not correct for the Donnan distribution or the mass concentration of water.

Venous plasma pH and pCO2 were determined with an ABL 2 (Radiometer), and actual bicarbonate concentration was calculated with the Henderson–Hassebalch equation.

Serum parathyroid (PTH) concentration was determined by radioimmunoassay with use of an antiserum to bovine C-terminal PTH (Institut National des Radioéléments, IRE, B-6220 Fleurus, Belgium).

Sodium and potassium ion concentrations were determined by flame emission photometry with a FLM 3 flame photometer (Radiometer), and chloride concentration was determined by coulometric titration with a CMT 10 chloride titrator (Radiometer). Inorganic phosphate (molybdate reaction), albumin (66 000 g/mol, bromresol green method), creatinine (picrate method), urea (diacetyl-monoxime method), and glucose (glucose dehydrogenase reaction, photometry at 340 nm) were determined by continuous-flow analysis (Technicon AutoAnalyzer).

The analytical imprecision, expressed as relative standard deviation, of duplicate measurements was 5.0% for PTH, 2.5% for ultrafiltrable calcium, and <2% for the other components.

Student's t-test was used to evaluate differences between means, and the paired t-test to compare paired data.

Results
The [Ca2+]i values for all the fasting insulin-dependent diabetics averaged 0.05 mmol/L less than the mean for the healthy controls. Figure 1 shows [Ca2+]i and PTH in the first experiment, in which 25 diabetics were compared with 23 healthy controls. Mean serum total calcium (not significant) and albumin (p < 0.01) of the diabetics were both decreased by 0.03 mmol/L, and with a substance ratio of 1:1 between albumin-bound calcium and albumin, the mean values for albumin-corrected total calcium were identical. Mean serum ultrafiltrable calcium concentration was also identical in the two groups, whereas serum magnesium was decreased in the diabetics [mean 0.75 (SD 0.06) vs 0.83 (SD 0.05) mmol/L, p < 0.001]. The mean serum pH before ultrafiltration was the same in the two groups. Serum ultrafiltrable calcium correlated negatively with pH (r = −0.59, p < 0.001), and a cor-

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Mean [Ca\(^{2+}\)]\(_{7.4}\) was 1.16 (SD 0.04) vs 1.20 (SD 0.03) mmol/L (p < 0.001); mean PTH was 2.5 (SD 0.9) vs 2.5 (SD 0.6) int. units/L (NS). [Ca\(^{2+}\)]\(_{7.4}\) and PTH did not correlate in either group. "U" in the figure is int. unit.

Correction to pH 7.40, made by using this correlation, still gave identical mean values.

The mean venous plasma pH of the diabetics and the healthy controls was 7.33 (SD 0.03) and 7.34 (SD 0.03), and the mean actual venous bicarbonate concentration was 22.7 (SD 2.5) vs 23.3 (SD 2.1) mmol/L, but the differences were not statistically significant. The pH dependency of [Ca\(^{2+}\)] in the diabetic sera was normal.

Fasting mean plasma glucose of the diabetics was 11.1 mmol/L (range 4.6-18.8 mmol/L). Glucose did not affect results obtained with the calcium-selective electrode, and in spite of sample handling the glucose concentration in the serum samples did not change.

The anion gap of the diabetic sera was on the average 3 mmol/L higher than that of the control sera (Figure 2), and it was negatively correlated with [Ca\(^{2+}\)]\(_{7.4}\) in the diabetics (r = -0.43, p < 0.02).

The usual insulin injection accompanied by a standard meal caused an increase of 0.04 mmol/L in [Ca\(^{2+}\)]\(_{7.4}\) after 90 min and a simultaneous decrease of 0.10 mmol/L in serum inorganic phosphate (Figure 3). A change was also observed in the healthy controls after the same standard meal without exogenous insulin (Figure 4), but the increase in [Ca\(^{2+}\)]\(_{7.4}\) averaged only 0.02 mmol/L. Serum inorganic phosphate correlated negatively with [Ca\(^{2+}\)]\(_{7.4}\) in both diabetics and healthy controls (Figure 5). The following components did not change significantly: serum albumin, total calcium, PTH, sodium or potassium ion. Acid-base status was not determined during the second experiment.

**Discussion**

The decreased [Ca\(^{2+}\)]\(_{7.4}\) in the present study agrees with Jasinski et al. (13), who found a decrease in [Ca\(^{2+}\)] of 0.18 and 0.38 mmol/L in 11 uncomplicated and 17 complicated cases of adult-onset diabetes, but they explained the decrease by increased calcium binding to the plasma proteins. That ob-

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![Figure 1](image1.png)

*Fig. 1. Serum parathyrin (PTH) vs serum ionized calcium at pH 7.40. [Ca\(^{2+}\)]\(_{7.4}\) of 25 diabetics (●) and 23 healthy controls (O). Mean [Ca\(^{2+}\)]\(_{7.4}\) was 1.16 (SD 0.04) vs 1.20 (SD 0.03) mmol/L (p < 0.001); mean PTH was 2.5 (SD 0.9) vs 2.5 (SD 0.6) int. units/L (NS). [Ca\(^{2+}\)]\(_{7.4}\) and PTH did not correlate in either group. "U" in the figure is int. unit.*

![Figure 2](image2.png)

*Fig. 2. The concentration of undetermined anions ("anion gap") in serum of 25 diabetics (●) and 23 healthy controls (O). Mean values 23.5 (SD 2.9) vs 20.5 (SD 2.8) mmol/L (p < 0.001).*

![Figure 3](image3.png)

*Fig. 3. [Ca\(^{2+}\)]\(_{7.4}\) and inorganic phosphate in serum of 21 diabetics before and 90 min after subcutaneous insulin and breakfast. Mean [Ca\(^{2+}\)]\(_{7.4}\) increased from 1.15 (SD 0.04) to 1.19 (SD 0.03) mmol/L (p < 0.001); mean inorganic phosphate declined from 1.29 (SD 0.22) to 1.19 (SD 0.20) mmol/L (p < 0.001), but the magnitudes of the changes were not correlated. The meal consisted of 80 g of bread, 25 g of butter, 25 g of sausage, 25 g of cheese, 25 g of marmalade, and 200 mL of milk.*

![Figure 4](image4.png)

*Fig. 4. [Ca\(^{2+}\)]\(_{7.4}\) and inorganic phosphate in serum of 21 healthy controls before and 90 min after breakfast. Mean [Ca\(^{2+}\)]\(_{7.4}\) rose from 1.22 (SD 0.03) to 1.24 (SD 0.03) mmol/L (p < 0.01); mean inorganic phosphate declined from 1.28 (SD 0.16) to 1.13 (SD 0.17) mmol/L (p < 0.001). The meal was the same as in Figure 3.*
A negative correlation was found for each group: $r = -0.55 (p < 0.005)$, and $r = -0.56 (p < 0.005)

The lowered [Ca$^{2+}$]$_{1,4}$ of the diabetics was associated with increased complexed calcium, and some of the calcium complexes may possibly have physiological importance, as suggested in ref. 15. Lactate, $\beta$-hydroxybutyrate, acetocacete, and non-esterified fatty acids were probably involved, as they are increased in diabetes mellitus (16–19). Non-esterified fatty acids increase the binding of calcium by albumin (20, 21); nevertheless, protein-bound calcium was normal. Hypomagnesemia probably increased calcium-binding by decreasing the binding competition between calcium and magnesium.

In spite of the lowered [Ca$^{2+}$]$_{1,4}$, the mean serum PTH concentration of the diabetics was normal, in agreement with earlier reports of low to normal PTH values in diabetes mellitus (3, 10, 22). It is inexplicable why the parathyroid glands did not increase the total calcium concentration in serum, but the hypomagnesemia may have contributed (23–25). The low serum magnesium concentration of the diabetics confirms other reports (26, 27). Others have examined 1,25-(OH)$_2$ vitamin D$_3$ and calcitonin (8, 10, 28), and so far human diabetes mellitus has not been linked to any dysfunction of the calcium regulatory hormones. A direct action of insulin on human bone has been suggested (10), and a pancreatic calcium elevating polypeptide (CEP), thought to be present in some commercial insulin preparations, has also been suggested (29–31).

The increase in [Ca$^{2+}$]$_{1,4}$ in both patients and controls during the second experiment could be due to a lower non-esterified fatty acids concentration postprandially (18, 19), because total calcium was constant. Serum inorganic phosphate concentration also decreased, but from the association constant of CaHPO$_4$ (32) and the calcium buffering capacity of plasma and interstitial fluid (33) it could only account for about 10% of the observed increase in [Ca$^{2+}$]$_{1,4}$. Others have found that the postprandial alkaline tide decreased [Ca$^{2+}$]$_{1,4}$ by an increase in the bicarbonate concentration (34, 35).

denson and Bowers (36) found a mean increase in [Ca$^{2+}$] of 0.04 mmol/L 1 to 2 h postprandially, in agreement with the present study.

We found an inverse correlation between [Ca$^{2+}$]$_{1,4}$ and inorganic phosphate in both diabetics and healthy controls. The decreased [Ca$^{2+}$]$_{1,4}$ would lower the activity product of bone salt and thereby favor bone resorption, but the importance is difficult to evaluate at present. Further studies on the serum calcium fractions in relation to insulin and various calcium complexing anions are needed to increase our understanding of the calcium metabolic changes in diabetes mellitus.

**References**