Lipid Metabolism and Cardiovascular Morbidity and Mortality in Hemodialysis Patients: Role of Factors Modulating Cytosolic Calcium

Animal studies indicate that insulin resistance and glucose intolerance leading to dyslipidemia in uremic rats are associated with increased cytosolic calcium ([Ca++i]). The resistance and intolerance are reversed with verapamil, but recur after its discontinuation. This finding suggests that hyperparathyroid-induced [Ca++i] increase is responsible for the metabolic derangement.

We retrospectively examined, over a 12-year period, the effects of factors that lower [Ca++i] on total serum cholesterol and triglycerides in 332 hemodialysis (HD) patients. Because the study was retrospective, detailed lipid profiles were not available. We therefore relied on morbidity and mortality outcomes related to atherosclerotic vascular disease. Patients with diabetes mellitus were excluded, because their dyslipidemia and vascular disease are mediated via a different mechanism.

Four groups emerged: group I [high parathormone (PTH) in the absence of calcium channel blockers (CCBs), n = 107], representing the highest [Ca++i]; group II (high PTH in the presence of CCBs, n = 76) and group III (lower PTH in the absence of CCBs, n = 66), representing intermediate [Ca++i]; and group IV (lower PTH in the presence of CCBs, n = 83) representing the lowest [Ca++i]. The theoretically lower [Ca++i] was achieved via CCB therapy or lower PTH, or both.

The mean serum cholesterol in group I was 322 ± 24 mg/dL and the level of triglycerides was 398 ± 34 mg/dL. Group II had mean serum cholesterol of 196 ± 16 mg/dL and triglycerides of 157 ± 17 mg/dL. Group III had a mean serum cholesterol of 202 ± 19 mg/dL and triglycerides of 160 ± 15 mg/dL. Group IV had a mean serum cholesterol of 183 ± 9 mg/dL and triglycerides of 94 ± 6 mg/dL. The differences in cholesterol and triglyceride levels among four groups were significant (p < 0.001) by one-way analysis of variance (ANOVA). The incidence of cardiovascular morbidity and mortality events was 61% in group I, 24% in group II, 28% in group III, and 18% in group IV ($\chi^2 = 47.7$, p < 0.001).

We conclude that, in non diabetic HD patients, hyperparathyroidism, especially in the absence of CCBs, is associated with severe dyslipidemia and increased risk of cardiovascular morbidity and mortality. Dyslipidemia may be related to a hyperparathyroid-induced increase in cytosolic calcium [Ca++i]. Lowering [Ca++i] by decreasing PTH or by blocking calcium entry into cells (via CCBs), or both, is associated with less dyslipidemia and improved long-term cardiovascular morbidity and mortality. Prospective randomized studies, with actual measurement of [Ca++i], are needed to verify the results of this study.

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Key words
Uremia, lipid metabolism, hyperparathyroidism, calcium channel blocker, calcitriol, cardiovascular mortality, cardiovascular morbidity

Introduction
Carbohydrate intolerance is common in patients with chronic renal failure (CRF) [1–5]. This abnormality is due to impaired insulin secretion [3,6–8] superimposed on a state of peripheral tissue resistance to the action of insulin [5,9,10]. Although many metabolic and hormonal disturbances—and uremic toxins—have been implicated [1–10], the exact mechanisms underlying peripheral resistance to insulin remain poorly defined.

On the other hand, impairment of insulin secretion has been extensively studied, and the mechanisms of impaired insulin secretion from the pancreatic islets are much better outlined [6–8]. Rats with CRF of six weeks’ duration display significant abnormalities in the 1-hour intravenous glucose tolerance test. Pancreatic islets from these rats show significant suppression of both the first and second phases of glucose-induced insulin secretion [6,7].

Because PTH has an acute calcium ionophoric action, it results in sustained elevation of intracellular calcium in the pancreatic islets [11]. The state of persistent secondary hyperparathyroidism in patients and animals with CRF may lead to increased calcium entry and excessive calcium accumulation in the pancreatic islets, resulting in functional impairment of the organ. Indeed, intracellular calcium concentration ([Ca++i]) was found to be significantly increased in pancreatic islets.
isolated from rats with CRF and secondary hyperparathyroidism. Surgical removal of the parathyroid gland prevented the accumulation and rise in basal levels of \([\text{Ca}^{++}]_{i}\) and was associated with normal glucose tolerance and insulin secretion [11].

Many studies, in animals and in humans, have shown that hyperparathyroidism increases \([\text{Ca}^{++}]_{i}\) and can adversely affect lipid metabolism and cause hyperlipidemia in CRF [12–20]. Treatment with calcium channel blockers has been shown to block PTH-induced calcium entry and to restore normal cytosolic calcium levels [12–16,21–23]. Baczynski et al. [24] and Bogin et al. [25] have confirmed these findings in isolated myocardial mitochondria.

To our knowledge, no large, long-term studies have examined the role of hyperparathyroid-induced increase in cytosolic calcium on lipid metabolism and cardiovascular morbidity and mortality in the hemodialysis population. Similarly, the possible beneficial role that calcium channel blockers may have in mitigating hyperlipidemia of uremia (by establishing long-term blockade of calcium entry into cells), and their possible efficacy in improving cardiovascular morbidity and mortality in HD patients has not been examined.

**Material and methods**

**Patients**

We retrospectively examined the records of patients from two large, university-based dialysis programs. We examined the charts of all patients enrolled in hemodialysis over a 12-year period (January 1989 to December 2000). Demographic data (age, sex, race, weight, body mass index, primary disease, comorbid conditions), presence of hypertension, degree of blood pressure control, history of smoking, and family history of cardiovascular disease were recorded. Mean serum cholesterol, triglycerides, parathyroid hormone levels, serum calcium, and serum phosphorus were recorded for all patients. Medications that alter lipid metabolism, primarily anti-hyperlipidemic agents and beta blockers, were included in the data analysis.

**Exclusion criteria**

We used these exclusion criteria:

- Diabetes (because dyslipidemia and cardiovascular morbidity and mortality in diabetic patients are mediated via mechanisms that are independent of cytosolic calcium and hyperparathyroidism)
- Long-term steroid use (same reason as for diabetes)
- Vasculitides (for example, systemic lupus erythematosus—because vascular injury is directly related to autoimmune–mediated inflammatory vasculitis)
- Pre-existing (prior to initiation of HD) vascular disease (myocardial infarction, documented coronary artery disease by EKG or cardiac catheterization, cerebrovascular accident, and peripheral vascular disease, including amputation or carotid artery stenosis documented by Doppler ultrasound)
- Use of anti-hyperlipidemic agents

**Study design**

Because the study was retrospective, routine direct measurements of cytosolic calcium over the study period were not available. The study aimed to examine the factors that, based on previous studies, lower cytosolic calcium [11–25]:

- Long-term treatment with calcium channel blockers (CCBs)
- Lower parathyroid hormone (PTH) level post parathyroidectomy
- Lower parathyroid hormone level owing to suppression of PTH with either intravenous or oral calcitriol

The patients were therefore divided into four groups: group I (high PTH in the absence of CCBs), theoretically representing the highest \([\text{Ca}^{++}]_{i}\); group II (high PTH in the presence of CCBs) and group III (lower PTH in the absence of CCBs), theoretically representing intermediate \([\text{Ca}^{++}]_{i}\); and group IV (lower PTH in the presence of CCBs), theoretically representing the lowest \([\text{Ca}^{++}]_{i}\). Intact PTH was measured by immunoradiometric assay. The PTH level was considered high if it was \(\geq 3.0\) times the maximum normal value and lower if it was \(< 3.0\) times the maximum normal value. The type and dose of CCBs were recorded. In addition, the average duration of CCB therapy was recorded. (Some patients were on and off CCBs.) To assess the cumulative effect of CCB therapy on cardiovascular morbidity and mortality, the total exposure of each patient to CCBs was determined. The duration of CCB therapy was calculated from the time the records showed a creatinine clearance < 30 mL/min to the end of the study.

Because no studies were available to determine the duration of CCB therapy that would have an effect on long-term cardiovascular morbidity and mortality, an arbitrary duration was determined as significant. Only patients receiving more than 6 cumulative years of CCB therapy were classified as a CCB-treated group (group II and group IV). Patients receiving fewer than 2 cumulative years of CCB therapy were classified as CCB-untreated (group I and group III). Data from these patients were included in the final analysis. Patients receiving cumulative doses of CCBs for more than 2 years, but fewer than 6 years, were excluded from the study analysis.

**Assessment of lipid metabolism and cardiovascular morbidity and mortality**

Because the study was retrospective, very few patients had detailed lipid profiles. Because detailed lipid profiles were not available (only total serum cholesterol and triglycerides were available), we relied on the morbidity and mortality outcomes related to atherosclerotic vascular disease. Charts were reviewed for fatal and non-fatal cardiovascular events. These included the diagnosis of coronary artery disease by...
serial cardiac enzymes and isoenzymes, serial EKGs, cardiac catherization, occurrence of myocardial infarction, cerebrovascular accidents, documented peripheral vascular disease by Doppler ultrasound studies on carotid arteries or extremities, and amputation or carotid endarterectomy related to atherosclerotic vascular disease.

**Statistical analysis**

Data are reported as mean ± standard error where appropriate. The data were analyzed among the groups using the statistical method of chi-square test for nominal variables, and the Student t-test and one-way analysis of variance (ANOVA) for continuous variables. Values of \( p \) less than 0.05 were considered significant.

Logistic regression analysis was also performed to examine the relationship between the presence of hyperlipidemia, as the dependent variable, and the occurrence of cardiovascular morbidity and mortality events. Among the four groups, analysis of the cumulative risk of combined morbidity and mortality, and morbidity alone and mortality alone, were determined by the Kaplan–Meier cumulative hazard method. The Kaplan–Meier cumulative hazard curves were compared using the Breslow–Gehan–Wilcoxon test.

**Results**

The study qualified 332 patients for enrollment in the analysis. Table I presents the demographic data for the four study groups. No statistically significant difference was seen among the four groups in regard to age, sex, race, body weight, cause of renal disease, or duration of dialysis. Furthermore, no statistically significant difference was seen among the four groups in regard to the presence of a family history of cardiovascular disease, the degree or duration of smoking, mean systolic blood pressure, mean diastolic blood pressure, nutritional status (as measured by mean serum albumin), or efficiency of dialysis (as measured by the urea reduction rate (URR) and \( Kt/V_{urea} \)).

Of the study patients treated with CCBs, 67% were on long-acting nifedipine for at least 35% of the study period. In the last 6 years of the study period, 71% were on amlodipine, 9% on felodipine, 8% on long-acting diltiazem, 8% on long-acting verapamil, and 4% on other CCBs. Approximately 75% of the patients changed the type of CCB at some point during the 12-year period. Patients classified under a CCB-treated group (groups II and IV) tended to be on a CCB for most of the study period (mean duration: 8.6 ± 1.5 years). Patients not classified under a CCB-treated group (groups I and III) tended to be on a CCB mostly in the last 2 years of the study period (mean duration: 1.7 ± 0.3 years; \( p = 0.0001 \)). Parathyroid hormone levels were obtained every 3 months, and the PTH level for each patient represented the mean level for the entire study period. Patients classified as having higher PTH levels (groups I and II) had considerably higher PTH levels than the patients classified as having lower PTH levels (groups III and IV), \( p < 0.04 \) (Table II).

<table>
<thead>
<tr>
<th>Group</th>
<th>HD patients (n)</th>
<th>Age in years</th>
<th>Sex [male:female (%)]</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>107</td>
<td>49±7</td>
<td>49:51</td>
<td>79.2±9</td>
</tr>
<tr>
<td>II</td>
<td>76</td>
<td>51±9</td>
<td>58:60</td>
<td>80.2±9</td>
</tr>
<tr>
<td>III</td>
<td>66</td>
<td>48:8</td>
<td>46:54</td>
<td>77.9±8</td>
</tr>
<tr>
<td>IV</td>
<td>83</td>
<td>53±9</td>
<td>53:47</td>
<td>80.4±9</td>
</tr>
</tbody>
</table>

All values are presented as mean ± standard error.

Group I = high parathyroid hormone, no calcium channel blockers; Group II = high parathyroid hormone, calcium channel blockers present; Group III = lower parathyroid hormone, no calcium channel blockers; Group IV = lower parathyroid hormone, calcium channel blockers present; HD = hemodialysis; PCKD = polycystic kidney disease.

Table II summarizes the results among the four groups. Fig. 1 shows a simplified bar graph of the mean serum cholesterol and triglyceride levels in the four groups. Group I (high PTH in the absence of CCBs, \( n = 107 \)) had a mean serum cholesterol of 322 ± 24 mg/dL and serum triglycerides of 398 ± 34 mg/dL. Group II (high PTH in the presence of CCBs, \( n = 76 \)) had a mean serum cholesterol of 196 ± 16 mg/dL and serum triglycerides of 157 ± 17 mg/dL. Group III (lower PTH in the presence of CCBs, \( n = 66 \)) had a mean serum cholesterol of 202 ± 19 mg/dL and serum triglycerides of 160 ± 15 mg/dL. Group IV (lower PTH in the presence of CCBs, \( n = 83 \)) had a mean serum cholesterol of 183 ± 9 mg/dL and serum triglycerides of 94 ± 6 mg/dL.

Cholesterol and triglycerides levels among four groups were significantly different (\( p < 0.001 \)) by one-way ANOVA. In group I, the cardiovascular morbidity and mortality incidence was 61%; in group II, it was 24%; in group III, 28%; and in group IV, 18% (\( \chi^2 = 47.7, p < 0.001 \)).

Fig. 2 shows the combined cardiovascular morbidity and mortality cumulative hazard plot. Fig. 3 shows the cumulative hazard plot for morbidity alone, excluding mortality; and Fig. 4, the cumulative hazard plot for mortality alone, excluding morbidity (cardiovascular diagnosis or event occurring without the patient’s death).

Logistic regression analysis showed that the presumed cytosolic calcium levels in the four groups (highest in group I, intermediate in groups II and III, and lowest in group IV), were statistically significant predictors of the occurrence of hypercholesterolemia (\( p = 0.005 \)), hypertriglyceridemia (\( p = 0.001 \)), and cardiovascular morbidity and mortality events (\( p = 0.001 \)). No statistically significant difference was seen in the mean serum cholesterol and triglyceride or cardiovascular morbidity and mortality between groups II and III, suggesting that these groups had a similar, lower level of cytosolic
Cytosolic Calcium and Dyslipidemia in Hemodialysis

**TABLE II** Summary of results in the four study groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients (n)</td>
<td>107</td>
<td>76</td>
<td>66</td>
<td>83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>322±24</td>
<td>196±16</td>
<td>202±19</td>
<td>183±9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>398±34</td>
<td>157±17</td>
<td>160±15</td>
<td>94±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>692±190</td>
<td>732±205</td>
<td>246±99</td>
<td>231±96</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dL)</td>
<td>5.9±1.2</td>
<td>5.7±1.0</td>
<td>4.9±1.0</td>
<td>5.5±1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.4±1.5</td>
<td>9.2±1.7</td>
<td>9.7±1.6</td>
<td>9.5±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>(\text{Ca}^{++} \times \text{PO}_4^{-})</td>
<td>56.4±4.2</td>
<td>53.1±4.6</td>
<td>50.4±5.0</td>
<td>51.4±5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>146±10</td>
<td>148±14</td>
<td>140±12</td>
<td>144±11</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>89±9</td>
<td>92±9</td>
<td>90±8</td>
<td>88±9</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>3.8±0.3</td>
<td>3.6±0.1</td>
<td>3.7±0.2</td>
<td>3.8±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>URR</td>
<td>71±4</td>
<td>68±3</td>
<td>69±5</td>
<td>72±5</td>
<td>NS</td>
</tr>
<tr>
<td>(\text{Kt/V}_{urea})</td>
<td>1.7±0.2</td>
<td>1.5±0.1</td>
<td>1.6±0.2</td>
<td>1.7±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiovascular M/M</td>
<td>61%</td>
<td>24%</td>
<td>28%</td>
<td>18%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are presented as mean ± standard error. Serum albumin is used as a measure of nutrition status; URR (urea reduction rate) and \(\text{Kt/V}\) are used as measures of adequacy of dialysis. Differences among groups were tested by one-way ANOVA, except cardiovascular M/M, which was tested by chi-square test (47.7), and intact parathormone level, where the unpaired \(t\)-test was used to compare combined groups I and II vs combined groups III and IV.

Group I = high parathyroid hormone, no calcium channel blockers; Group II = high parathyroid hormone, calcium channel blockers present; Group III = lower parathyroid hormone, no calcium channel blockers; Group IV = lower parathyroid hormone, calcium channel blockers present; PTH = intact parathormone; NS = nonsignificant; \(\text{Ca}^{++} \times \text{PO}_4^{-}\) = serum calcium by phosphorus product; URR = urea reduction rate; M/M = morbidity/mortality.

**FIGURE 1** Cholesterol (left bars) and triglyceride (right bars) levels in the four groups (Gr I – Gr IV).

**FIGURE 2** Combined cardiovascular morbidity and mortality cumulative hazard plot. Analysis of the cumulative risk of combined morbidity and mortality among the four groups (Gr-I – Gr-IV) was determined by the Kaplan–Meier cumulative hazard method. The \(p\) values from the Kaplan–Meier cumulative hazard curves were compared by Breslow–Gehan–Wilcoxon test (\(p = 0.001\)).

Discussion

Chronic exposure to high PTH levels in the uremic state results in sustained elevations in basal levels of \([\text{Ca}^{++}]_i\), and this abnormal intracellular calcium homeostasis is believed to be the basis for multiple organ dysfunctions [21]. Continuous PTH-mediated calcium entry into the cell is theorized to lead to inhibition of mitochondrial oxidation and of ATP pro-
production. This situation, in turn, leads to a reduction in active calcium extrusion and an accumulation of intracellular calcium. Treatment with calcium channel blockers has been shown to block PTH-induced calcium entry and to restore normal [Ca^{++}i] levels [21–23]. Baczynski et al. [24] and Bogin et al. [25] have confirmed these findings in isolated myocardial mitochondria.

Fadda et al. [12,13] showed that 5 of 6 nephrectomized rats with advanced CRF and hyperparathyroidism developed increased [Ca^{++}i], insulin resistance, glucose intolerance, and dyslipidemia. In these studies, the rats were treated daily with the calcium channel blocker verapamil. This therapy resulted in correction of the 1-hour intravenous glucose tolerance test and offered a protective effect on the pancreatic islets with maintenance of normal glucose-induced insulin secretion [12]. Other studies [13] clearly showed that the chronic use of calcium channel blockade prevented the rise in the islets’ basal [Ca^{++}i] despite the chronic increase in calcium entry induced by PTH.

Excess PTH has also been proposed to adversely affect lipid metabolism and cause hyperlipidemia in CRF [16–20]. Because glucose metabolism and lipid metabolism are closely interlinked, and both are dependent on plasma insulin levels, the severe hyperlipidemia seen in uremic patients may be indirectly related to insulin resistance resulting from hyperparathyroidism and increased [Ca^{++}i].

Cerebrovascular and cardiovascular diseases are important predictors of survival of dialysis patients and account for about half of the deaths in these patients [26]. Increased serum cholesterol [27,28], increased low-density lipoprotein (LDL) [29], and decreased high-density lipoprotein (HDL) [30] are associated with increased cardiovascular morbidity and mortality. The plasma profile in uremic patients is abnormal [31–33], including hypertriglyceridemia [34], hypercholesterolemia [35], and low HDL [36–38].

Parathyroid hormone may contribute to cardiovascular morbidity and mortality in several ways, including a permissive role in arteriolar wall thickening and myocardial interstitial fibrosis (promoting an increase in triglycerides and LDL cholesterol levels) and by contributing to hypertension [39–41]. In cultured adipocytes, vitamin D_{3} increased, and PTH reduced, lipoprotein lipase activity. The effects of PTH were prevented by the calcium channel blocker verapamil, again suggesting a role for increased [Ca^{++}i] in the development of dyslipidemia [20].

Based on the large body of evidence that increased PTH in uremic patients increases [Ca^{++}i], which in turn causes the metabolic derangement of insulin resistance, glucose intolerance, and dyslipidemia, we divided the patients in the current study into four groups. The groups were designed to hypothetically represent high, intermediate, and low [Ca^{++}i] levels according to the presence of factors that lower [Ca^{++}i]. These factors were (a) long-term treatment with CCBs; (b) lower PTH level post parathyroidectomy; (c) lower parathyroid hormone level by suppression with either intravenous or oral calcitriol. Group I (high PTH in the absence of CCBs) theoretically represented the highest [Ca^{++}i]. Group II (high PTH in the presence of CCBs) and group III (lower PTH in the absence of CCBs) theoretically represented intermediate [Ca^{++}i]. Group IV (lower PTH in the presence of CCBs) theoretically represented the lowest [Ca^{++}i].

The results of our study support previous animal and human studies indicating that a high PTH level is associated with increased [Ca^{++}i], which in turn causes the metabolic
derangement that leads to dyslipidemia, insulin resistance, glucose intolerance, and increased risk of cardiovascular morbidity and mortality. Group I, with presumably the highest [Ca++]i, demonstrated this point, showing the worst degree of dyslipidemia and the highest cardiovascular morbidity and mortality. Groups II and III showed no statistically significant difference in cholesterol and triglyceride levels or risk of cardiovascular morbidity and mortality. This result suggests that, even in the presence of high PTH (group II), CCBs were able to block calcium entry and to reduce [Ca++]i to a level at least equal to that in patients who had lower PTH levels (group III). Results for patients in group IV, with presumably the lowest [Ca++]i, suggest that the lower the level of [Ca++]i, the lower the risk of dyslipidemia and cardiovascular morbidity and mortality. It should be emphasized, however, that in this study no direct [Ca++]i measurements were available. The relationship between theoretically lower [Ca++]i in groups II, III, and IV and better lipid profile and improved cardiovascular morbidity and mortality can only be indirectly inferred, based on previous studies.

Conclusion

In non diabetic hemodialysis patients who are not receiving steroids or other agents capable of altering lipid metabolism, hyperparathyroidism, especially in the absence of CCBs, is associated with severe dyslipidemia and increased risk of cardiovascular morbidity and mortality. Dyslipidemia may be related to a hyperparathyroid-induced increase in [Ca++]i. Reducing [Ca++]i by decreasing PTH or by blockade of calcium entry into cells (via CCBs), or both, is associated with less dyslipidemia and improved long-term cardiovascular morbidity and mortality. Prospective randomized studies, with actual measurement of [Ca++]i, are needed to verify the results of the present study.

References

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