Calcium metabolism and hypertension

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Case presentation

A 28-year-old black male was referred to the Hypertension Clinic at New England Medical Center for evaluation of high blood pressure. Two days earlier, when he was seen in the Emergency Room with an upper respiratory tract infection, his blood pressure was 140/116 mm Hg. Treatment was prescribed for the respiratory symptoms. No laboratory studies other than a throat culture were performed. When he was seen 2 days later in the Hypertension Clinic, he was feeling well.

Three years earlier the patient had been told he had elevated blood pressure during an insurance physical examination. He could not recall the level of his blood pressure; the insurance was approved, however. No antihypertensive therapy was prescribed during the intervening period. The only other significant medical history was related to proteinuria, which had been detected during a military induction physical 10 years earlier. No protein was found in the urine at the time of the insurance examination. The patient described himself as a healthy, active individual without medical complaints. He had no weight loss, anxiety episodes, depression, night sweats, easy fatigability, or persistent respiratory complaints. He had never had an abnormal chest x-ray, and he had never used vitamin D preparations or thiazide diuretics. He was employed full time as a shipping clerk; he did not smoke, but he consumed large amounts of alcohol intermittently.

Physical examination revealed a thin, well-developed, well-nourished, young black male in no acute distress. The blood pressure was 148/100 mm Hg in the right arm when he was recumbent and 150/105 mm Hg in the right arm when he stood. Blood pressures in the left arm were similar. His pulse was 100 beats/min and regular. Pertinent physical findings included normal eye grounds, except for some early arteriolar narrowing. The thyroid was barely palpable, and no other masses or nodes were detected. The chest was clear to auscultation and percussion. Cardiac examination disclosed a normal PMI, and normal sounds were noted except for a grade I/VI systolic ejection murmur at the left sternal border radiating to the apex. The abdomen was flat and nontender with no organomegaly; normal bowel sounds were present. No bruits were noted. The genitourinary examination revealed a normally developed male. Musculoskeletal and neurologic examinations were within normal limits. No clubbing, edema, or cyanosis was evident in the extremities. His skin was warm, dry, and without any lesions. No lymphadenopathy was detected.

Urinalysis showed a pH of 6, trace protein, negative glucose, negative blood, and a benign sediment. Serum creatinine was 1.1 mg/dl; BUN, 22 mg/dl; sodium, 136 mEq/liter; potassium, 4.4 mEq/liter; chloride, 95 mEq/liter; and carbon dioxide, 24 mEq/liter. Serum total calcium was 11.6 mg/dl; phosphorus, 3.3 mg/dl; fasting glucose, 80 mg/dl; uric acid, 9.6 g/dl; albumin, 4.2 g/dl; cholesterol, 240 mg/dl; and triglycerides, 75 mg/dl. The T4 was 165 mg/dl (normal, 70—170 mg/dl); T3, 3.8 mg/dl; alkaline phosphatase, 2.2 (Bodansky units); hematocrit, 48.2%; hemoglobin, 16.8; and white blood cell count, 10,700 mm3 with a normal differential. The 24-hour urine protein excretion was 17—26 mg. A 24-hour urine collection contained 117 mEq of sodium and 14 mEq of calcium. The PTH level was 94 pg/ml (normal 15—30, N-terminal). Serum protein electrophoresis was normal. The PPD was negative. Two plain chest films were negative. An intravenous pyelogram was normal. Electrocardiogram was unrevealing. Plain films of the hands revealed no evidence of subperiosteal resorption.

Over the next 5 months, the blood pressure ranged between 125—145/85—95 mm Hg. No other medical problems were identified, except for persistent elevation of the serum total calcium. The repeat values for the serum calcium and phosphorus concentrations, respectively, were: 10.8 mg/dl and 2.6 mg/dl; 11.3 and 3.1 mg/dl; 11.8 and 2.6 mg/dl; 10.9 and 2.1 mg/dl.

Because of persistent hypercalcemia, elevated PTH levels, and a failure to identify an alternative basis for his hypercalcemia, the patient was admitted to the Surgical Service for surgery. At the time of admission, laboratory studies revealed: sodium, 138 mEq/liter; potassium, 4.2 mEq/liter; chloride, 97 mEq/liter; bicarbonate, 27 mEq/liter; serum total calcium, 11.1 mg/dl; phosphorus, 3.6 mg/dl; albumin, 4.3 g/dl; and a normal complete blood count. The left superior and inferior parathyroid glands were identified and biopsied. The right inferior parathyroid gland also was identified, and was excised along with the right lobe of the thyroid. The latter was removed in an attempt to locate the right superior parathyroid gland. Examination of the pathologic specimens revealed tissue from four parathyroid glands. Adenomatous hyperplasia was not mentioned, and the total volume of the excised gland mass was not recorded.

During the first 6 postoperative months, serum calcium concentrations ranged between 9.0 mg/dl and 10.2 mg/dl. Serum phosphorus values were between 3.2 mg/dl and 4.1 mg/dl. Six months following the neck exploration, the PTH again was measured. The reported value was 188 μEq/ml (normal, up to 85 μEq/ml; mixed N- and C-terminal assay), with a simultaneous serum total calcium of 9.9 mg/dl. Repeated PTH determinations 6 and 12 months later were 138 μEq/ml and 87 μEq/ml respectively; concurrent serum total calcium levels were 10.0 mg/dl and 10.2 mg/dl.

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During the first 4 years after subtotal parathyroidectomy, the patient's blood pressure was recorded in the 130-150/90-100 mm Hg range. He was treated with hydrochlorothiazide, 50 mg/day, for a short period during his second postoperative year, but on that regimen he developed acute gout and the hydrochlorothiazide was discontinued. Propranolol, 40 mg twice daily, was started, and the blood pressure fell to 110-130/82-90 mm Hg.

Discussion

DR. DAVID A. MCCARRON (Professor of Medicine and Co-head of the Division of Nephrology and Hypertension, and Director of the National Dairy Board Institute for Nutrition and Cardiovascular Research, Department of Medicine, Oregon Health Sciences University, Portland, Oregon): This patient's presentation and clinical course provided both a historic and pathophysiologic perspective on what has emerged as one of the most active [1] yet controversial areas of cardiovascular research [2, 3]: the relationship of calcium metabolism to the regulation of arterial pressure [4]. This young black male, who was referred to the Hypertension Clinic at New England Medical Center over 10 years ago with chronic hypercalcemia and hypertension, was responsible for initiating my interest in the possibility that disregulation of the PTH-Ca\(^{2+}\)-vitamin D axis was a factor in the development of hypertension in humans. At the time of his presentation, a search of the medical literature for information on this topic failed to identify a critical body of data and prompted the design of what I believe was the first controlled clinical study of the association between blood pressure status and parameters of calcium metabolism [5]. The findings of that investigation and the subsequent research by our laboratory at Oregon, as well as from many others over the past decade, form the basis for considering the following question: do dietary and physiologic factors that influence calcium homeostasis contribute to the modulation of arterial pressure in humans?

In attempting to answer that question, I will review a variety of observations relevant to this patient's medical disorders. I will return to this case after summarizing the current status of research in this field. I will begin with observations derived from epidemiologic studies of the interrelationships among arterial pressure, dietary calcium intake, and calcium metabolism in humans. I then will turn to laboratory investigations that addressed many of the same issues in experimental models. I will examine the clinical data and discuss the arterial pressure effects of increasing calcium intake in humans, as well as whether there are predictors of antihypertensive response to this intervention. Finally, I will summarize our current understanding of the mechanisms that have been invoked to explain how modifying calcium metabolism changes blood pressure in humans and in the experimental animal.

Historic perspective

Unlike some areas of new investigation, the concepts relating calcium to high blood pressure were introduced against a background of historic facts and beliefs that would, at least superficially, argue strongly against the possibility that chronic depletion of calcium, and/or altered cell regulation of calcium resulting in a functional depletion of the cation, raise arterial pressure. These observations include the following seven points. (1) Acute hypercalcemia was known to produce a rise in arterial pressure in normal subjects and in individuals with renal disease [6]. (2) An association between high blood pressure and primary hyperparathyroidism was well established [7]. (3) By the 1970s vascular smooth muscle contraction was known to be critically dependent on the release of intracellular calcium stores [8]. (4) A circulating inhibitor of sodium transport was thought to exist in at least a subset of hypertensive humans and in some animal models of high blood pressure, and this notion had come to dominate efforts to explain how intracellular free calcium might be elevated [9]. (5) In large part because of the sodium inhibitor hypothesis, some workers theorized that intracellular free calcium was elevated in the vascular tissue of experimental models and in humans with high blood pressure [10]. (6) Agents called "calcium antagonists" or "calcium channel blockers" had been identified as potentially effective antihypertensive agents. For obvious semantic reasons, if a calcium antagonist lowered blood pressure, it was difficult to reconcile how a calcium-deficient state, particularly at a cellular level, could contribute to the rise in arterial pressure. (7) From a nutritional standpoint, interest in diet and heart disease was principally focused on dietary excesses rather than on dietary deficiencies. In the domain of hypertension, no concept was more tightly embraced than that of excessive dietary sodium intake being a primary factor in the development of high blood pressure in humans [11].

Thus, in 1976, from virtually every vantage point (epidemiology, physiology, cell biology, pharmacology, and nutrition), data emerged that led to the hypothesis that calcium metabolism was linked to the development of high blood pressure. The corollary followed that exposing vascular tissue to excessive amounts of calcium was responsible for hypertension. In fact, when the initial clinical study was undertaken at New England Medical Center that year, our working hypothesis was precisely that. As the data from clinical and laboratory investigations have accumulated over the past 13 years, that original hypothesis became no longer tenable. In fact, the findings demanded that new hypotheses relating calcium and blood pressure be formulated.

Epidemiology

Biochemical parameters. In our initial clinical studies, we found an 18% higher level of circulating parathyroid hormone levels in patients with untreated essential hypertension than in normotensive subjects with similar levels of serum total calcium [5]. The higher parathyroid hormone levels could not be attributed to renal dysfunction, but were associated with an increase in urinary calcium excretion (when indexed against simultaneous urinary sodium excretion). This latter finding was interpreted as representing a renal "calcium leak" in hypertension. In a subsequent metabolic balance study of the renal handling of calcium in hypertension, we confirmed that urinary cAMP excretion was elevated in hypertensive patients [12]. In a study of young males, Grobbee and colleagues later documented that parathyroid hormone values directly correlated with blood pressure, after adjustment for body weight [13]. Strazzullo and coworkers also reported higher levels of parathyroid hormone, as well as higher levels of urinary cAMP excretion, in patients with high blood pressure compared with normals [14]. Using metabolic clearance studies, Strazzullo also documented higher values for renal calcium clearance in patients with mild essential hypertension when compared with appropriate control
subjects. An association between increased urinary calcium excretion and high blood pressure also was reported in a large population survey [15]. This relationship was interpreted as reflecting an increase in dietary calcium intake in hypertensive patients, although calcium intake was not directly assessed in this study. In our first intervention trial at Oregon, we found that the urinary calcium excretion rate was somewhat higher, even though dietary calcium intake was somewhat lower, in hypertensive subjects compared with normotensive individuals; however, these differences were not statistically significant [16]. We interpreted these observations as being more in keeping with a "renal calcium leak" than with an excessive intake of calcium as an explanation for the high urinary calcium excretion previously noted in hypertensive humans.

In several epidemiologic surveys, serum total calcium concentration has been directly associated with blood pressure, although in some studies this relationship might reflect differences in serum albumin concentration [15, 17]. In our index report of the relationship of serum ionized calcium to blood pressure, hypertensive patients had lower serum ionized calcium values than did the control group [18]. In subsequent reports, serum ionized calcium values have been either identical or modestly reduced in subjects with high blood pressure compared with those with normal blood pressure [19, 20]. In a carefully investigated population in Minnesota, ultrafilterable calcium (P = .01) and serum ionized calcium (P = .07) were lower in individuals with increased arterial pressure [21]. Resnick et al have reported that this relationship depends on renin status; patients with low renin activity have lower ionized calcium, whereas patients with high renin activity have higher ionized calcium values [20]. In collaboration with the Cornell group, we recently confirmed the stratification of serum ionized calcium by renin status [22]. Strazzullo and coworkers noted that serum ionized calcium at baseline was nearly identical in normotensive and hypertensive subjects but that, following an acute calcium infusion, serum ionized calcium increased to a significantly lesser degree in the hypertensives [14]. Two studies have reported higher levels of serum ionized calcium concentration in hypertensive subjects, but in both, thiazide diuretics (which increase serum ionized calcium values when used chronically) had been administered to many of the subjects [23, 24].

The relationship of serum phosphorus to high blood pressure has been much more consistent than has that of serum calcium to high blood pressure. Virtually all reports in the medical literature have identified either lower serum phosphorus values in hypertensive patients or an inverse correlation between serum phosphorus and arterial pressure in the population studied [5, 25].

In our recent study of serum 1,25 dihydroxyvitamin D₃ levels in normotensive individuals and in those with untreated hypertension, we found lower levels of 1,25(OH)₂D₃ in the hypertensive subjects [26]. We simultaneously measured serum phosphorus, serum ionized calcium, and serum parathyroid hormone levels using a two-site, intact-molecule chemiluminescent immunoassay [27]. Among males, parathyroid hormone levels correlated significantly and directly with both supine and standing systolic, diastolic, and mean arterial pressure. Among females, no such correlation was detected. Likewise, serum phosphorus values significantly and inversely correlated with arterial pressure in males but not in females. Serum ionized and total calcium values did not, however, correlate with blood pressure in the male subjects. Somewhat confusingly, serum total calcium correlated significantly and directly with blood pressure in females. What has emerged from this study is a picture of increased parathyroid hormone function in male hypertensives (with concurrent reductions in serum phosphorus values) and minimal, if any, reductions in serum ionized calcium values. Although this constellation of biochemical findings in male subjects normally would evoke an increase in circulating 1,25(OH)₂D₃ levels, precisely the opposite was observed [26].

I speculate that the pattern of biochemical parameters of calcium metabolism associated with hypertension is most consistent with a subtle degree of whole-organism calcium depletion with a secondary increase in parathyroid gland function. However, these defects in calcium homeostasis relate to blood pressure primarily in male subjects. This most recent observation may account for the inconsistencies in measured markers of calcium metabolism in hypertensive individuals in epidemiologic reports.

Dietary calcium. One of the questions prompted by our initial studies was whether dietary calcium intake was different in patients with hypertension than in normotensive individuals. The results from a pilot study in 1982 provided the first evidence that reduced dietary calcium intake was associated with hypertension [28]. Of 19 nutrients measured, only a 22% reduction in calcium intake distinguished the hypertensive from the normotensive subjects. We then turned to the National Health and Nutrition Examination Survey I (NHANES I) data base to determine whether such a relationship was evident in a representative population of adult Americans [29]. Figure 1 derives from our analysis of those data from the National Center for Health Statistics. As we reported in 1984, of the nutritional factors assessed in that survey, dietary calcium was the best single predictor of an individual's blood pressure. The more dietary calcium consumed, the lower an individual's blood pressure and the lower the probability of having blood pressure in the hypertensive range.

At least 23 subsequent publications in the medical literature have verified the finding of an inverse relationship between dietary calcium intake and blood pressure. Those studies are summarized in Table 1 [16, 21, 28–50]. In addition to our assessment of NHANES I, six other groups have utilized the same data base; five of these groups have reported similar findings even though sample sizes, definition of blood pressure status, and statistical techniques varied greatly [51]. The authors of the one discrepant report recently acknowledged that an alternative analysis did reveal a relationship between lower dietary calcium intake and higher blood pressure dependent on consumption of a high-sodium diet [35].

Whereas all other reports are based on cross-sectional population studies, Witteman et al utilized a survey of 58,218 females in the United States to determine prospectively the frequency with which hypertension was diagnosed over a 4-year period [49]. After adjustment for age, body weight, and alcohol consumption, a logistic regression model showed dietary calcium and dietary magnesium to have independent, inverse associations with hypertension. The relative risk for the development of hypertension was 0.78 at a dietary calcium
intake greater than or equal to 800 mg/day compared with an intake less than 400 mg/day. Thus, differences in calcium intake well within the range observed in the U.S. could account for 22% of the new cases of hypertension detected in this large, prospectively studied population of American women.

Several conclusions can be reached from these epidemiologic studies [36]. The inverse correlation of dietary calcium with blood pressure appears to be independent of age, gender, race, socioeconomic status, weight, geography, cigarette consumption, alcohol intake, estrogen status, and other dietary constituents. The association tends to be stronger with systolic than with diastolic pressures. Most important, there is a suggestion

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**Table 1. Epidemiologic studies of calcium and hypertension**

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Dietary data</th>
<th>Calcium source</th>
<th>Subjects</th>
<th>Sex</th>
<th>Other dietary components</th>
</tr>
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<tr>
<td>McCarron, 1982</td>
<td>Portland, OR</td>
<td>24-hr Diet</td>
<td></td>
<td>96</td>
<td>MF</td>
<td>Magnesium</td>
</tr>
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<td>NHANES I</td>
<td>24-hr Diet</td>
<td></td>
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<td>Potassium, vitamins C, A</td>
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<tr>
<td>Ackley, 1983</td>
<td>San Diego, CA</td>
<td>Quest Dairy products</td>
<td>5,050</td>
<td>30-79</td>
<td>MF</td>
<td></td>
</tr>
<tr>
<td>Garcia-Palmieri, 1984</td>
<td>Puerto Rico</td>
<td>24-hr Diet/milk</td>
<td>7,932</td>
<td>45-64</td>
<td>M</td>
<td>Alcohol, coffee</td>
</tr>
<tr>
<td>Harlan, 1984</td>
<td>NHANES I</td>
<td>24-hr Diet</td>
<td></td>
<td>2,055</td>
<td>MF</td>
<td>Alcohol, phosphorus</td>
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<td>Richman, 1984</td>
<td>Chicago, IL</td>
<td>History Diet</td>
<td></td>
<td>1,652</td>
<td>MF</td>
<td>Polysaturated fatty acid, alcohol</td>
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<td>Gruchow, 1985</td>
<td>NHANES I</td>
<td>24-hr Diet</td>
<td></td>
<td>3,854</td>
<td>MF</td>
<td>Alcohol, sodium, potassium</td>
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<td>24-hr Diet</td>
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<td>5,305</td>
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<td>Johnson, 1985</td>
<td>Wisconsin</td>
<td>Record Diet</td>
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<td>F</td>
<td></td>
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<tr>
<td>Kromhout, 1985</td>
<td>Netherlands</td>
<td>History Diet</td>
<td></td>
<td>605</td>
<td>M</td>
<td>Phosphorus, alcohol</td>
</tr>
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<td>McCarron, 1985</td>
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<td>24-hr Diet</td>
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<td>Reed, 1984</td>
<td>Honolulu, HI</td>
<td>24-hr Diet/milk</td>
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<td>46-65</td>
<td>M</td>
<td>Phosphorus, protein</td>
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<td>24-hr Diet</td>
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<td>Liebman, 1986</td>
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<td>24-hr Dairy products, diet</td>
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<td>615</td>
<td>M</td>
<td>Magnesium, fiber, potassium</td>
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<td>Witteman, 1987</td>
<td>US nurses study</td>
<td>24-hr Diet</td>
<td></td>
<td>58,218</td>
<td>F</td>
<td>Magnesium, alcohol</td>
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<tr>
<td>McGarvey, 1988</td>
<td>Providence, RI</td>
<td>Quest Maternal diet</td>
<td>212</td>
<td>0-01</td>
<td>MF</td>
<td>Magnesium, potassium</td>
</tr>
</tbody>
</table>

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*a Dietary data: 24-hr = 24-hour recall; ques = questionnaire; history = dietary history; freq = food frequency; record = food record.
that a threshold for dietary calcium intake exists at about 400 to 500 mg per day, below which the prevalence of hypertension can increase by about 25% [51, 50]. In a prospective study, Witteman et al found approximately a 20% reduction in the development of hypertension in women with a calcium intake greater than 800 mg/day [51].

Collectively, these biochemical, nutritional, and clinical observations suggest that in settings where there is evidence of failure to adequately regulate calcium homeostasis, hypertension is more likely to be present. These findings, however, do not support a cause-and-effect relationship; a causal association can be established only on the basis of clinical intervention trials, animal investigations involving experimental models of hypertension, and ultimately, cellular and subcellular experiments that identify a mechanism or mechanisms.

**Disturbances of calcium metabolism in animal models of hypertension**

Although our initial observations suggested that a relationship exists between disturbed calcium metabolism and hypertension in humans, we could not exclude the possibility that the calcium abnormalities were only an epiphenomenon. Accordingly, we turned to laboratory models of hypertension to address several essential points. If investigations of animal models of hypertension identified a biochemical pattern consistent with altered calcium metabolism, then characterizing the organs and cell types involved in the manifestation of these disturbances of calcium metabolism might provide critical insights into the mechanisms involved. Experiments in animal models provide a controlled setting in which to determine whether modifying dietary calcium intake changes blood pressure. Furthermore, with animal studies it should be possible to assess whether calcium’s effect on blood pressure is a nonspecific one observed only in certain forms of experimental hypertension, or a more generic one, modifying the course of blood pressure development in divergent models that share only one common characteristic, altered vascular resistance.

The information that has accumulated on animal studies of calcium and hypertension is characterized by remarkable consistency in some areas and by apparent discrepancies in others. The inconsistencies have been the subject of several analyses [52, 53]; we recently reviewed the findings from most of the reports and offered possible explanations for many of the earlier inconsistencies [53].

Evidence of disturbed calcium homeostasis can be identified in many experimental models of hypertension including the male spontaneously hypertensive rat (SHR), the salt-sensitive strain of the SHR [54], the Milan strain of the SHR [55], the Dahl sodium-chloride-dependent genetic rat model [56, 57], the DOCA-saline rat model (a mineralocorticoid/sodium-chloride-dependent model) [56, 58], and the renal vascular rat model (the “two-kidney, one-clip” rat model) [59]. In each instance, evidence favoring disturbed calcium metabolism has been adduced principally in the male.

**Biochemical defects.** The Aoki-Okamoto SHR displays a variety of biochemical defects in calcium metabolism previously noted in humans [53]. These defects include elevated circulating parathyroid hormone levels [60], which are evident as early as 6 weeks of age [61]. Hyperactivity of the parathyroid glands in these animals has been verified by a recent report by Merke and colleagues, who observed an increase in parathyroid gland mass in animals 11 weeks of age [62].

Serum ionized calcium values, with few exceptions, are reduced by 5% to 7%; this finding is similar to that in humans [53, 60, 63]. Serum phosphorus values are low and serum total calcium values do not differ significantly from controls [60]. The SHR has low circulating 1,25 dihydroxyvitamin D₃ levels which, in the setting of elevated PTH and low serum ionized calcium and phosphorus, is compelling evidence for a disregulation of 1,25 dihydroxyvitamin D₃ metabolism [64—66]. Elevated calcitonin levels have been found in the 8-week-old [67] and the 16-week-old SHR [68]. This finding supports the hypothesis that the calcium-regulating hormone axis is disturbed in the setting of experimental hypertension.

In young animals, urinary calcium excretion is normal or lower in the SHR compared with the Wistar-Kyoto rat (WKY) [67, 69]. From mid-adolescence (16–17 weeks of age) onward, however, urinary calcium excretion is increased in the SHR compared with the WKY [60]. Urinary cAMP excretion is normal or slightly below normal in the SHR; with chronic stimulation by parathyroid hormone infusion, the SHR appears unable to sustain the same degree of urinary cAMP production or calcium mobilization as the WKY [70].

Low serum ionized calcium values also have been found in the Dahl salt-sensitive, the DOCA-saline, and the two-kidney, one-clip rat models [59]; on average, the ionized serum calcium level is 5% to 7% less in hypertensive rats than in normotensive controls. Serum total calcium values do not differ from those in normotensive animals [53, 59]. Urinary calcium excretion has been reported as elevated compared with the control animal for the Milan strain of the SHR [55], the DOCA-saline rat, and the Dahl sodium-chloride-dependent rat model [56].

The perturbations of calcium metabolism that are consistent with a calcium deficiency are evident principally in the male animal; an exception to this general statement is the increased urinary calcium excretion (UₐCaV) and decreased serum ionized calcium that occur in both sexes [53]. The female SHR does exhibit abnormal parameters of calcium metabolism, but the abnormalities are the obverse of those noted in males. This contrast between sexes is similar to what is observed in humans [71]. Furthermore, and potentially of critical importance, the emergence of these biochemical defects appears to follow an age-dependent pattern [53]. Parathyroid gland function is increased and serum calcium decreased early in the life of the SHR. Parathyroid hyperplasia has been demonstrated in the 11-week-old SHR [62]. Not until later in life (15 to 20 weeks of age), however, do consistent differences in UₐCaV emerge. Thus, age- and gender-dependent differences might account for some of the discrepancies in the literature regarding the presence or absence of these biochemical abnormalities in the SHR [52, 53].

**Organ defects.** Several defects related to either the regulation of calcium homeostasis or organ responsiveness to the intracellular calcium signal have been identified in experimental models. The SHR again has been the subject of the most intense investigation, although several other animal models also have been studied. The organs involved include the proximal duodenum, kidney, bone, parathyroid glands, and vascular smooth muscle.
The status of calcium transport by the proximal duodenum in hypertensive animal models is controversial. Multiple techniques have been utilized to assess calcium handling by the duodenum. Investigators have reported normal absorption as well as increased or decreased calcium transport, not only using different but sometimes the same techniques [53, 64, 72–79]. Studies utilizing only the male SHR and intact duodenal tissue under controlled electrical conditions (Ussing chamber) consistently have reported low levels of net intestinal calcium transport ($J_{\text{net}}$) in 12-week-old rats [64, 74] and also in 24-week-old rats fed a low-calcium diet [74]. Balance studies have suggested abnormally high levels of absorption in the 4-week-old SHR [69, 78], but by the 12th week of age, absorption is lower in the SHR than in the WKY [78]. These age-related differences in intestinal calcium absorption are consistent with the hypothesis that in experimental hypertension disturbances of calcium metabolism evolve as the animal matures and hypertension emerges.

In the kidney, the important finding in the SHR is that 1,25 dihydroxyvitamin D$_3$ synthesis [79] is decreased in conjunction with increased urinary calcium clearance [80]. Bone studies have identified a reduction in density and calcium content in the male SHR (older than 24 weeks) [64, 81], with increased bone density in females at one year of age [74]. Reduced bone density has been reported in a recent study of the salt-sensitive Dahl rat [82]. Reduced bone density is the ultimate evidence for a failure of the hypertensive animal to retain an appropriate amount of calcium.

In vascular smooth muscle, a variety of alterations related to calcium metabolism have been reported, including altered sensitivity to calcium-regulating hormones (Bukoski RD, unpublished observations), increased membrane permeability to calcium [83, 84], and greater dependence on extracellular calcium for membrane stabilization [85].

In the Milan strain of SHR, decreased renal calcium reabsorption has been linked to a decrease in the renal epithelial cell calcium ATPase activity [55]. Likewise, in the DOCA-saline rat model and in the Dahl sodium-chloride-dependent genetic model, increases in urinary calcium excretion have been characterized [56]. Failure to regulate calcium homeostasis is characteristic of experimental hypertension even when the initiating event may differ. An inability to absorb dietary calcium from the intestine or reabsorb filtered Ca$^{2+}$ in the nephron appear to be common threads in the link of disturbed calcium metabolism to elevated arterial pressure. Since the normal maintenance of calcium balance by these two organs is dependent on active Ca$^{2+}$ transport, the implications for a cellular defect in such a primary process is evident.

**Defects in cell calcium metabolism.** Cellular calcium-handling defects have been identified in experimental models of hypertension, principally in the SHR. Of greatest importance is the multitude of abnormalities described in the vascular smooth muscle cell isolated from the SHR [53]. The presence of potentially important defects of cell regulation in several cell types suggests, at least in the genetic model of the SHR, that a fundamental disturbance in either calcium binding or calcium transport is programmed into the cells. Of particular note are the observations that enterocyte influx [86] and efflux [87] of calcium are reduced in the SHR and that renal intracellular calcium may be elevated [88]. This latter finding might account for the alterations in 1,25 dihydroxyvitamin D$_3$ production by the proximal renal tubular cell and for the impairment of intestinal calcium transport. Other investigators, however, have found either no differences or a decrease in renal tubular intracellular free calcium [89, 90]. Probably of less importance in the pathophysiology of hypertension are the observations in the SHR that intracellular calcium may be elevated and/or that membrane-bound calcium may be decreased in red cells [91], lymphocytes [92], and platelets [93]. These observations do suggest, however, that a generalized alteration in the regulation of intracellular calcium metabolism in this particular model of experimental hypertension does exist.

**Intracellular calcium disturbances.** As I noted, several investigators have reported increases in intracellular free calcium (as measured by calcium-sensitive dyes) in platelets, lymphocytes, and proximal tubular cells of the SHR. Recent studies have used the more sensitive fura-2 calcium indicator. This technique makes it possible to study intact resistance vessels and to measure intracellular calcium both in vascular tissue, where the endothelial layer is intact, and in isolated, cultured, single vascular smooth muscle cells. Using the fura-2 technique on cultured vascular smooth muscle cells isolated from the SHR, we have not been able to identify an increase in basal intracellular free calcium [94]. In isolated mesenteric resistance vessels from the SHR and WKY, Bukoski found decreased calcium transients in vascular tissue derived from the hypertensive animals as compared with normotensive controls (unpublished observations). The finding of normal basal levels and a decreased calcium transient in the vascular tissue of the SHR is in direct opposition to the increase in intracellular free calcium of vascular tissue, as originally hypothesized by Blaustein [10].

Calcium fluxes have been measured in vascular smooth muscle cells [95] and in isolated duodenal enterocytes of the SHR [86]. In smooth muscle cells, influx is increased; in enterocytes, precisely the opposite has been observed. Enterocytes exhibit not only a low influx rate, but a diminished intracellular calcium pool (both bound and free). Calcium efflux rate constants also are decreased in SHR enterocytes, probably as a functional response to the decrease in the calcium influx rate [87]. The differences in measured Ca$^{2+}$ influx between these two cell types likely reflect the fundamental variations in the techniques used in these experiments and do not necessarily indicate opposite effects in these two systems. Both could reflect a defect in the intracellular transport of calcium.

Magnesium-calcium-ATPase pump activity is decreased in the vascular tissue of the SHR [96]. The observation that calcium uptake by vesicles from isolated vascular smooth muscle is decreased in the SHR led to the assumption that this decrease reflects an abnormality of the calcium pump. Calmodulin activity has been studied in the brain of the SHR [97]. Compared with the WKY control, no differences exist in the activation of the protein by calcium or in its ability to stimulate phosphodiesterase activity. However, Nojima, Kishi, and Sako identified a pseudocalmodulin protein in the brain of the SHR that exhibits an amino-acid-sequence defect in one of the four critical calcium-binding regions of the calmodulin molecule [98]. Calmodulin-binding-protein content is reduced in renal tubular and proximal renal tubular cells of the SHR. Recent studies have suggested that renal intracellular calcium may be elevated in red cells [91], lymphocytes [92], and platelets [93]. These observations do suggest, however, that a generalized alteration in the regulation of intracellular calcium metabolism in this particular model of experimental hypertension does exist.
calmodulin in the heart, kidney, and vascular tissue of the SHR [100].

These observations point to important cellular calcium defects in experimental hypertension (Fig. 2). These defects could represent multiple disturbances in different cell lines or might reflect an effect of a single, primary defect in the regulation of the calcium pump by either calcium or calmodulin, in the binding of calcium to its critical intracellular binding protein, or in the response to a local circulating factor that activates calmodulin. Such a defect or defects in vascular tissue could result in either increased reactivity or abnormal cell growth, either one of which would cause peripheral resistance to rise and arterial pressure to be elevated. Much additional investigative work is needed to clarify the issues surrounding the pathophysiology of disturbed cellular calcium metabolism that is associated with elevated arterial pressure.

Figure 3 provides a theoretical depiction of the way in which data from studies of human and experimental hypertension might be tied together. This schema suggests that afflicted individuals might encounter a problem in the presence of reduced dietary calcium intake because of a simultaneous impairment in their ability to retain calcium. One could hypothesize that, either because of a genetic defect or an acquired effect stemming from inadequate intake of calcium itself, a disorder of cell calcium transport appears to develop that produces submaximal intestinal absorption and inadequate renal reabsorption of calcium. As a consequence, the alteration in biochemical parameters portrayed in Figure 3 would occur, and retained calcium (and thus bone density) would be reduced. In the vascular smooth muscle cell, fundamental shifts in cell calcium permeability, transport, storage, and release might develop that potentially would modify optimal vascular smooth
muscle function and result in an increase in peripheral resistance and arterial pressure through subcellular mechanisms that remain to be determined.

**Blood pressure response to increased calcium intake**

*Experimental studies.* The antihypertensive effect of increased dietary calcium has been studied in five hypertensive models and in several normotensive strains. Animals that are responsive to increased dietary calcium include the following models: SHR [60, 61, 101], salt-sensitive SHR (Taconic Farms) [54], DOCA-saline rat model [102, 103], Dahl sodium-chloride-dependent genetic model [104], and the two-kidney, one-clip renovascular model [105]. In addition, increased dietary calcium also lowers blood pressure in normotensive rats including the WKY [106], Wistar [107], and Fischer 344 strains [108]. Several studies also have examined the effects of calcium restriction on blood pressure [60, 106]. The calcium content of a standard rat diet is considered to be 0.8% to 1.0% by weight. Our initial studies showed a reduction in blood pressure when we fed rats a high-calcium (4%) diet [60]. We subsequently used a less elevated level of dietary calcium, approximately 2%, and documented a similar blood-pressure-lowering effect [74, 109]. Karanja et al from our laboratory recently reported a dose-response curve for dietary calcium in the SHR: 90% of the antihypertensive effect was reached with a diet of 1.5%; only a minimal further reduction in blood pressure was achieved at higher dietary calcium levels [109].

Thus, the inverse relationship between dietary calcium content and blood pressure is nonlinear in the SHR as it is in humans [29, 48]. In the male SHR, the steepest portion of this curve lies between 0.25% and 0.5% dietary calcium [109]. Within this range the greatest change in blood pressure of the SHR will occur. We have not been able to identify a consistent antihypertensive effect in the female SHR, although a decrease in blood pressure was reported by Lau and coworkers [110].

Hatton and colleagues demonstrated an effect of reduced maternal calcium intake on the fetus and during the first 3 weeks postpartum [111]. These manipulations significantly affected the development of hypertension in the young SHR: besides the dose dependency, a time dependency of calcium’s antihypertensive effect has been identified. When the diet of the very young animal is supplemented with calcium, the antihypertensive effects emerge more rapidly. If supplemental calcium is introduced during adolescence, a longer period (up to 10 weeks) may be necessary for an antihypertensive effect to emerge.

Calcium’s effect on blood pressure in the SHR is influenced importantly by the accompanying level of sodium intake. Sodium restriction impairs the antihypertensive effect of increased dietary calcium, whereas modest sodium supplementation enhances calcium’s antihypertensive action [74]. Of the various calcium salts studied, the carbonate has been used most commonly; however, calcium phosphate [107], lactate, gluconate, citrate, and chloride [112] also lower blood pressure in experimental models.

The rise in blood pressure typically observed in the Dahl sodium-chloride-dependent genetic model of hypertension is prevented by providing a 2% calcium diet [101]. In the DOCA-saline model of experimental hypertension, supplemental calcium also prevents the rise in blood pressure typically induced by a high sodium-chloride intake and by administration of mineralocorticoids [102, 103]. Similarly, in the renovascular model (the two-kidney, one-clip hypertensive rat), calcium supplementation over a relatively short period (4 to 6 weeks) prevents blood pressure from rising [105]. Furthermore, supplementing the diet with calcium reverses a number of the hormonal changes characteristic of this model [60, 61]. The blood pressure in the salt-sensitive SHR does not rise when the sodium chloride content of the diet is increased, if calcium is also added to the diet [54]. Not only does supplemental calcium result in normal blood pressure regulation in this model, but the defects in central nervous system regulation of blood pressure noted by previous workers are prevented by this intervention [54].

These observations from hypertensive and normotensive animals, in which genetic backgrounds differ, and in which the primary pathophysiologic mechanisms vary widely, suggest that calcium lowers blood pressure by a generic action. Peripheral vascular resistance is increased in all these models, and increasing calcium intake lowers blood pressure in each case. It is reasonable to suggest, therefore, that dietary calcium either has a salutary effect on circulating factors that control peripheral vascular tone, or that it has a direct effect on the response of vascular smooth muscle cells to circulating factors. An additional possibility is that the dietary calcium corrects a primary defect in intracellular calcium metabolism. How increasing dietary calcium might reverse such a cellular defect is a matter for speculation.

*Human studies.* Whereas epidemiologic studies have demonstrated that dietary calcium intake is lower in humans with hypertension than in normal individuals and that many hypertensive patients exhibit biochemical and metabolic evidence of calcium depletion, the critical test of the hypothesis that failure to maintain a positive calcium balance will result in an increase in blood pressure is whether changes in dietary calcium intake influence blood pressure. Although animal studies suggest that modification of dietary calcium changes blood pressure, the clinical relevance of these observations ultimately depends on whether similar findings are documented in controlled clinical studies. Table 2 summarizes the 20 trials of calcium supplementation that have been published or presented at scientific meetings to date [16, 38, 113–129]. Three of these trials were not properly controlled with a placebo phase or a control group, but 13 included both random patient allocations and a placebo control group. The level of calcium supplementation employed in the various studies ranged from 0.4 to 2.0 g/day and most commonly were 1.0 to 1.5 g/day. Most studies used calcium carbonate, but some used calcium citrate, gluconate, or lactogluconate, and one used yeast; in 2, a dietary source of calcium was employed. The length of supplementation ranged from 5 days to 1 year.

Blood pressure fell significantly in at least a subset of the population studied in three-quarters of these trials; no significant blood pressure changes were reported in the other one-fourth. In no study was a significant increase in blood pressure detected for the population under observation. Blood pressure reductions with calcium supplementation ranged from 2 mm Hg to 10 mm Hg, with a median of 5 mm Hg. The studies reporting the most convincing blood pressure reductions involved random patient allocation, a placebo control period, and larger...
Adverse reactions to the calcium supplements and for the lack of studies are particularly important because of the absence of diastolic in one and by 3.1 mm Hg diastolic in the other. These placebo control groups by 2.8 mm Hg systolic and 2.3 mm Hg pressure was lowered in the calcium-supplemented (versus either males alone [121] or predominantly males [117]; blood pressure falls with increased calcium intake in both sexes, males experience somewhat greater reductions than females, particularly males who are normotensive to start with, although this difference is not firmly established. In one trial that had ample numbers of black and white subjects, no difference in blood pressure response was found between the two races [121]. Ethnic origin similarly does not appear to be a predictor of a favorable blood pressure response. As I said, males appear to be slightly more responsive than females, particularly males who will have a beneficial response to calcium supplementation.

Of the studies that did detect a blood pressure reduction, all but 2 used at least 8 weeks of supplementation. By contrast, 2 negative trials, one by Bloomfield [120] and one by Cappuccio [127], used calcium supplements for only 4 weeks. Two of the positive trials were also of short duration (1 to 2 weeks), but significant blood pressure reductions probably were detected in these studies either because of a relatively high level of supplementation [113] or because of intense observation in a metabolic ward of a hospital [118].

Supplementing the diet with calcium has been reported to reduce blood pressure both in hypertensive and normotensive populations. With the exception of the report by Tabuchi [119], studies of hypertensives have, for the most part, focused on individuals with borderline to mild hypertension. Not surprisingly, somewhat greater blood pressure reductions have been achieved in hypertensive than in normotensive populations. Although blood pressure falls with increased calcium intake in both sexes, males experience somewhat greater reductions [128]. Two of the best-controlled and largest trials enrolled either males alone [121] or predominantly males [117]; blood pressure was lowered in the calcium-supplemented (versus placebo control) group by 2.8 mm Hg systolic and 2.3 mm Hg diastolic in one and by 3.1 mm Hg diastolic in the other. These studies are particularly important because of the absence of adverse reactions to the calcium supplements and for the lack of patient dropout due to side effects. It is noteworthy that no study subject has been reported as having developed renal calculi during calcium supplementation.

Although the reductions in blood pressure observed in these clinical trials have been modest (3 mm Hg to 6 mm Hg), such changes, extrapolated to the general population, could yield significant benefits with respect to overall cardiovascular morbidity and mortality [130]. Moreover, the modest reductions represent average changes in blood pressure and include some individuals who experienced a fall in systolic blood pressure of greater than 20 mm Hg compared with values recorded during placebo control [16]. Similarly, as with most antihypertensive interventions, some individuals have no apparent response to the treatment and some sustain a rise in blood pressure [16, 126]. This heterogeneous response to calcium supplementation is similar to that observed in clinical trials of sodium restriction [131-133] and potassium supplementation [134].

### Predictors of a blood pressure response in humans

Many parameters have been analyzed in an effort to predict who will have a beneficial response to calcium supplementation. As I said, males appear to be slightly more responsive than females, particularly males who are normotensive to start with, although this difference is not firmly established. In one trial that had ample numbers of black and white subjects, no difference in blood pressure response was found between the two races [121]. Ethnic origin similarly does not appear to be a predictor of response.

Among various biochemical parameters, serum ionized calcium has not been a predictor of a favorable blood pressure response, although in our initial trial the individuals who

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects</th>
<th>Design</th>
<th>Dose of calcium</th>
<th>Significant BP response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belizan, 1983</td>
<td>57</td>
<td>18-35</td>
<td>1 g (CaCO3) × 22 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Resnick, 1983</td>
<td>10</td>
<td>—</td>
<td>2 g (CaCO3) × 5 days</td>
<td>Yes</td>
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<tr>
<td>Johnson, 1985</td>
<td>95</td>
<td>35-65, F</td>
<td>1.5 g (CaCO3, citrate) × 8 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>McCarron, 1985</td>
<td>80</td>
<td>22-70</td>
<td>1 g (CaCO3, citrate) × 8 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Morris, 1985</td>
<td>16</td>
<td>30-65</td>
<td>1 g (CaCO3, citrate) × 16 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Meese, 1986</td>
<td>27</td>
<td>—</td>
<td>800 mg (Ca²⁺, citrate) × 8 wk</td>
<td>No</td>
</tr>
<tr>
<td>Grobbee, 1986</td>
<td>90</td>
<td>18-27</td>
<td>1 g (CaCO3) × 12 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Luft, 1986</td>
<td>16</td>
<td>22-56</td>
<td>1 g (CaCO3) × 8 days</td>
<td>Yes</td>
</tr>
<tr>
<td>Tabuchi, 1986</td>
<td>8</td>
<td>8 = 73</td>
<td>2 g (gluconate) × 2 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Morris (unpublished)</td>
<td>86</td>
<td>50-80</td>
<td>1 g (CaCO3) × 12 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Bloomfield, 1986</td>
<td>32</td>
<td>18-75</td>
<td>1.5 g (CaCO3) × 4 wk</td>
<td>No</td>
</tr>
<tr>
<td>Lyle, 1987</td>
<td>75</td>
<td>19-52, M</td>
<td>1.5 g (CaCO3) × 12 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Strazzullo, 1986</td>
<td>17</td>
<td>38 = 43, M</td>
<td>1 g (lactoglucolone + carbonate) × 15 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Bierenbaum, 1987</td>
<td>200</td>
<td>21-56</td>
<td>1.4 g (milk) × 6 mo</td>
<td>Yes</td>
</tr>
<tr>
<td>Gilliland, 1987</td>
<td>24</td>
<td>57±10</td>
<td>400 mg (CaCO3) × 24 wk</td>
<td>No</td>
</tr>
<tr>
<td>Thomsen, 1987</td>
<td>28</td>
<td>—</td>
<td>2000 mg × 1 yr</td>
<td>No</td>
</tr>
<tr>
<td>Vinson, 1987</td>
<td>15</td>
<td>19-24, M</td>
<td>500 mg (Ca gluconate) or 500 mg (Ca yeast) × 7</td>
<td>Yes</td>
</tr>
<tr>
<td>Cappuccio, 1987</td>
<td>18</td>
<td>28-65</td>
<td>1600 mg/day (Ca lactate glucose) × 4 wk</td>
<td>No</td>
</tr>
<tr>
<td>Morris, 1987</td>
<td>133</td>
<td>18-70</td>
<td>1.0 g (CaCO3) or 1.5 g (dietary Ca) × 12 wk</td>
<td>Yes</td>
</tr>
<tr>
<td>Van Beresteyn, 1986</td>
<td>58</td>
<td>20-23, F</td>
<td>1500 mg (CaCO3, powder) × 6 wk on Ca-restricted diet</td>
<td>No</td>
</tr>
</tbody>
</table>
experienced the smallest change in ionized calcium also had the largest drop in blood pressure [16]. Resnick et al reported that patients with low-renin hypertension have the most notable response. In this subgroup, these investigators found relatively low levels of serum ionized calcium [20]. Some patients with high-renin hypertension experience a minimal pressor effect [114]. Grobbee and Hofman reported that subjects whose serum total calcium level was below the median had a greater blood pressure response compared with those whose levels were above the median [117]. Similarly, people who were above the median for parathyroid hormone level had greater reductions in blood pressure.

We have been unable to identify a predictive value for circulating serum 1,25 dihydroxyvitamin D3 levels (Morris CD, personal communication), and we were unable to verify the predictive value of plasma renin status reported by Resnick et al [20, 135]. In our experience, urinary calcium excretion has not been a predictor of blood pressure response [16]; however, Strazzullo et al did find a correlation between urinary calcium excretion during the placebo phase and a greater blood pressure reduction with calcium supplementation [122]. Finally, baseline dietary calcium intake has not been predictive of a blood pressure response in any of our clinical trials [16, 115, 128]. Grobbee et al reported that parathyroid hormone and total serum calcium levels might be modestly predictive of a blood pressure response [117]. The predictive value, however, is not of sufficient magnitude to enable the clinician to identify individual patients who might benefit.

Mechanisms of action

Several potential mechanisms have been postulated to explain how dietary calcium affects blood pressure. Figure 4 represents a number of these mechanisms. I principally will focus on the ways in which dietary calcium might affect the intrinsic properties of the resistance arterioles or circulating factors that modify vascular smooth muscle contractility. I also will discuss briefly several nonvascular mechanisms.

Vascular smooth muscle. Early work by Bohr et al [136] prompted us to postulate a membrane-stabilizing effect of dietary calcium that would reduce vascular sensitivity to circulating vasoconstrictors [28]. This postulate was subsequently tested in our laboratory [137, 138]. No consistent alteration in the sensitivity of either aortic or mesenteric vascular rings to exogenous norepinephrine was identified in animals fed a calcium-supplemented diet. In the course of those studies, we also assessed whether dietary calcium impairs maximal contractility of the vasculature; such an effect also might result in a lowering of blood pressure. These studies have demonstrated that quite the reverse is true. In the SHR, calcium supplementation produces an increase in maximal contractility, thereby reversing the impaired maximal contractility in this model.

Using isolated, cultured, aortic vascular smooth muscle cells from the WKY and SHR, we assessed the effect of long-term exposure to increased extracellular calcium within the physiologic range on cell 45Ca2+ uptake [139]. Prolonged exposure to higher extracellular calcium produces a greater 45Ca uptake of the isolated vascular smooth muscle cells from the SHR. No effect was observed in the WKY. More recently, we assessed prostacyclin production by aortic rings isolated from SHR fed a calcium-supplemented diet from 3 to 12 weeks of age [140]. Compared with animals maintained on low-calcium diets, the vascular tissue of the calcium-supplemented animals exhibited a significant increase in production of 6-keto-PGF1α, an indication that production of this vasodilator, prostacyclin, was increased by the vascular tissue.

Passive properties of the vascular smooth muscle also must be considered. Vascular smooth muscle hypertrophy long has been known to develop in the hypertensive animal in concert with a rise in blood pressure [141]. The SHR's vascular smooth muscle cells proliferate faster than do those of the WKY. No data regarding the effects of calcium supplementation on cell growth and hypertrophy are available, however. Passive elastic properties of the vasculature also contribute to blood pressure regulation, particularly basal blood pressure. Vascular aortic rings isolated from the calcium-supplemented SHR exhibit an improvement in passive-elastic properties [137]. The calcium-supplemented animal does not experience the typical age-related reduction in vessel compliance. This effect on vessel
compliance might not only explain reductions in basal blood pressures, but could prove important in the progression of vascular disease if similar beneficial effects on compliance are documented in resistance vessels. These observations suggest several possible mechanisms, although it is premature to draw any conclusions about how dietary calcium might act on vascular smooth muscle to lower arterial pressure.

**Calcium-regulating hormone.** Vascular tissue contains receptors for PTH and 1,25(OH)₂ vitamin D [142, 143], and circulating levels of both these hormones are modified by changes in dietary calcium intake. Thus, calcium-regulating hormones must be considered potentially important mediators of dietary calcium’s antihypertensive effect [135, 144]. Recent studies in our laboratory have demonstrated a direct effect on calcium regulation in vascular smooth muscle cells by both of these calcium-regulating hormones [144]. The incubation of isolated vascular smooth muscle cells with either parathyroid hormone or vitamin D stimulates ⁴⁵Ca²⁺ uptake [139]. Of possibly greater importance is the observation that short-term incubation of intact mesenteric resistance vessels with either 1,25(OH)₂ vitamin D₃ or PTH significantly modifies the calcium transient (as measured by fura-2) evoked by exposure to norepinephrine (Bukoski RD, personal communication). In vessels from normotensive animals, 1,25 dihydroxyvitamin D₃ dampens the calcium transient and increases the transient in vessels isolated from the SHR. Likewise, PTH reduces the calcium transient in the WKY, but exhibits only minimal effects on the calcium transient in the SHR.

These findings suggest a direct intracellular effect of the calcium-regulating hormones on calcium release and possibly storage by intact resistance vessels and/or cultured vascular smooth muscle cells derived from experimental animals. The data further suggest that the observed response to the calcium-regulating hormones differs based on whether the vascular tissue is isolated from hypertensive or from normal animals. The mechanisms by which 1,25(OH)₂ vitamin D₃ and PTH modify cellular calcium uptake or calcium transients are unknown. A vasodilatory action of parathyroid hormone has long been recognized [145–147]. The observations I have noted in isolated vascular tissue along with the identification of PTH receptors in vascular tissue represent the first evidence that PTH’s effects on blood pressure might be mediated through a direct action on the intracellular handling of calcium by resistance vessels.

**Central-nervous-system effects.** Several lines of evidence suggest that dietary calcium supplementation modifies central-nervous-system sympathetic outflow [148, 149]. Hatton and colleagues demonstrated that both acute [148] and chronic stress-induced elevations [149] in blood pressure were dampened by dietary calcium supplementation in the SHR. Likewise, Yang and coworkers reported that the alterations in central-nervous-system response to sodium-chloride loading in the salt-sensitive SHR are obviated by dietary calcium supplementation [54]. Finally, Peuler et al demonstrated in the Dahl salt-sensitive rat that calcium supplementation reduces sympathetic outflow concurrent with a reduction in blood pressure [104]. Scrogin and coworkers measured peripheral catecholamine levels in the calcium-loaded SHR and observed that circulating catecholamine levels are, if anything, slightly elevated on the higher-calcium diets [150]. This finding must be reconciled with the observation that central-nervous-system sympathetic outflow is reduced following dietary calcium supplementation in the salt-sensitive SHR and Dahl rats [54, 104]. Scrogin et al suggested that calcium loading affects catecholamine-receptor binding in vascular tissue without necessarily altering peripheral catecholamine release [150].

**Phosphate depletion.** Lau and colleagues proposed that phosphate depletion, induced by a high calcium intake, impairs cardiac and vascular contractility, thereby accounting for the hydropoietic effects of dietary calcium [151]. Although neither changes in phosphate balance nor intracellular phosphate-dependent energetics have been assessed thoroughly, several lines of evidence argue against this theory. First, at levels of supplemental calcium in the relatively low range of 2%, serum phosphorus concentrations, although lower than control, remain well within the normal range and are not consistent with the degree of hypophosphatemia typically associated with impaired cardiovascular response [74]. Second, the observation that maximal vascular contractility is improved in the vascular tissue of the calcium-supplemented hypertensive animal indicates that vascular smooth-muscle contraction is not diminished on a high-calcium diet [137]. Third, Bindels et al demonstrated that dietary phosphate supplements in the SHR reduce blood pressure during mid-adolescence [152]. This last observation runs contrary to the notion that phosphate depletion accounts for the blood-pressure-lowering effects of dietary calcium. Nevertheless, given the critical role of dietary phosphorus in modulating calcium homeostasis as well as the synthesis and release of a number of the calcium-regulating hormones, the role of phosphate metabolism requires further study.

**Nonvascular mechanisms.** Nonvascular mechanisms for the antihypertensive effects of dietary calcium also must be considered. First, a reduction in intravascular volume as a consequence of a natriuretic action of calcium supplementation has been proposed and is based on observations by several groups [2, 61]. This hypothesis has been addressed directly in humans by Luft and coworkers, who were unable to demonstrate any change in sodium balance in a metabolic study of normal individuals and hypertensive patients whose diets were supplemented with calcium for one to two weeks [118]. Second, Lau et al observed that vascular volume is increased in the calcium-supplemented SHR in which blood pressure has been reduced [151]. In younger animals fed additional calcium, Hatton et al found no change in plasma volume [153]. The most compelling evidence against a significant natriuretic action of calcium comes from two reports that indicate that calcium’s antihypertensive action is enhanced by simultaneous sodium chloride supplementation [74, 154]. Collectively, these observations strongly argue against a reduction in intravascular volume, and thereby overall sodium balance, being induced by calcium supplementation.

A change in blood viscosity is another nonvascular mechanism by which dietary calcium might affect blood pressure. Hatton and colleagues measured changes in hematocrit that occur with calcium supplementation as an indirect assessment of viscosity [155]. The changes in blood pressure observed with the higher calcium intake were independent of changes in the animal’s hematocrit.

Thus, central as well as peripheral mechanisms appear to be
involved in the blood pressure reduction noted with calcium supplementation. The central mechanisms might include a reduction in central sympathtic outflow; the peripheral mechanisms could reflect changes in circulating calcium-regulating hormones that appear to have direct vascular actions. Calcium supplementation probably affects calcium metabolism in vascular smooth muscle and also might alter passive-elastic properties of vascular smooth muscle. Finally, changes in prostaglandin metabolism suggest that membrane phospholipid metabolism is another potentially productive area for further investigation of the mechanisms of dietary calcium’s blood-pressure-lowering effect.

Potential areas of clinical application

The relationship of dietary calcium to blood pressure might be particularly pertinent to several subpopulations at risk of developing hypertensive cardiovascular disease. Kurtz and coworkers demonstrated that sodium-chloride-sensitive hypertensive men manifest increased urinary calcium excretion [156], reminiscent of the hypercalciuria observed in the DOCA-saline and Dahl sodium-chloride-dependent experimental models [57]. The observation that calcium supplementation lowers blood pressure in various animal models of sodium-chloride-related hypertension [102, 104] should encourage further exploration of the link between calcium homeostasis and sodium chloride sensitivity in human hypertension.

Pregnancy-induced hypertension is another important clinical problem for which the concepts outlined in this review could have future application. Several disturbances of calcium metabolism, some similar to those observed in essential hypertension, have been identified in pregnant women [157–159]. Furthermore, several intervention trials already have demonstrated that during the third trimester of pregnancy calcium supplementation significantly reduces the incidence of pregnancy-induced hypertension [160–162].

Hypertension is common in type-II diabetes mellitus, and a number of the calcium disturbances associated with essential hypertension also have been described in this disorder [163–165]. Gaboury et al [166] and Mondon and Reaven [167] recently reported that the spontaneously hypertensive rat (the best-characterized experimental model of calcium-mediated hypertension) also exhibits biochemical and humoral characteristics consistent with type-II or insulin-resistant diabetes. Preshad Singh and Kurtz also have provided a theoretical pathophysiologic association between the increased insulin levels observed in hypertensives and cellular calcium metabolism [168].

Alcohol-associated hypertension offers yet another provocative example of an association worth exploring. Chronic alcohol ingestion induces several alterations in cell calcium homeostasis [169, 170] and ultimately results in significant bone mineral loss [171]. An interaction among alcohol consumption, dietary calcium intake, and blood pressure has been reported in two epidemiologic studies [34, 39]. In addition, Criqui et al reported that, compared with other relationships between nutrients and blood pressure, the association of dietary calcium to blood pressure was evident only at low levels of alcohol intake. Their findings suggest a unique influence of alcohol on the way in which dietary calcium affects blood pressure [172].

Systolic hypertension in the elderly may be another clinical setting of interest. Dietary calcium intake declines significantly with age [36]. Age-related abnormalities in calcium metabolism parallel changes observed in hypertensive patients [173–175]. In a current trial in which we are assessing the effects of calcium supplementation on blood pressure in older individuals, we have observed potentially important age-related blood pressure reductions [176].

Alterations in calcium metabolism may be particularly important for the pathogenesis of hypertension in blacks. Dietary calcium intake among blacks in the United States is significantly less than that observed among whites [46, 47]. One epidemiologic study demonstrated that differences in dietary calcium intake account for most of the variation in blood pressure between adolescent white and adolescent black females [46]. Perhaps the most important implication of the hypothesis that maintaining a net positive calcium balance will protect against the development of hypertension is the demonstration that maintenance of the recommended daily allowance for calcium—the 800 to 1000 mg/day suggested by the U.S. National Academy of Sciences—will prevent the blood pressure increase that occurs with age in industrialized societies, and the attendant premature morbidity and mortality. The prospective data from Witteman et al [49], and the blood pressure reductions achieved in some of the normotensive individuals studied in the intervention trials, suggest that increasing dietary calcium intake can attenuate the rise in blood pressure and reduce the prevalence of hypertension that emerges with aging. This may be particularly true in groups with chronic underconsumption of dietary calcium, such as women and blacks.

Summary

Returning to the patient presented today, perhaps we can now understand some of his findings. As I noted, men are more likely to demonstrate alterations in calcium metabolism associated with elevations in blood pressure. Furthermore, blacks are more likely than whites to develop hyperparathyroidism [177], particularly in the third and fourth decades of life. It is unlikely, however, that parathyroid hormone was responsible for the increase in this patient’s arterial pressure because PTH has a vasodilating action. Moreover, the long-term response to parathyroidectomy is more likely to be an increase rather than a decrease in blood pressure [178]. It is also unlikely that the mild elevations in the serum total calcium observed in this patient were responsible for his hypertension. Correction of hypercalcemia by surgical intervention failed to improve the blood pressure. There is little evidence that mild, protracted hypercalcemia can account for increases in arterial pressure. Finally, the patient’s alcohol abuse might have contributed to his elevated blood pressure; it is possible that his hypertension was in part a reflection of the abnormal calcium metabolism he developed as a consequence of the alcohol abuse.

Answers to some questions we faced when we first studied this patient more than a decade ago can be provided by the wealth of basic research and clinical investigation that has occurred since. We now know that calcium metabolism is a factor in blood pressure regulation in some humans and in some experimental models. Epidemiologic studies document a consistent association between lower dietary calcium intake and
higher blood pressures in humans. An additional non-pharmacologic approach has been identified that can produce a modest but important lowering of blood pressure in a subset of hypertensive individuals. Much data show that calcium-regulating hormones have important cardiovascular actions that might account for some of the mechanisms by which increased dietary calcium lowers blood pressure. Research in this area also has set the stage for exploring another theoretical mechanism for sodium-chloride-sensitive hypertension. Finally, a theoretical mechanism(s) has emerged that could provide a pathophysiologic link between hypertension and certain high-risk populations such as blacks, the elderly, type-II diabetics, and pregnant women.

The principal clinical implication derived from this work to date is the following: In patients with mild to moderate hypertension, the level of dietary calcium intake should be assessed. Patients whose intake is deficient should be encouraged simply to maintain calcium intake at 800 to 1000 mg/day. Our challenge is to attempt to prevent calcium deficiency in patients with essential hypertension. We need not at this time strive to increase dietary calcium to a level greater than the current recommended daily allowance of the National Academy of Sciences [3, 29].

Questions and answers

**DR. MARSHALL LINDHEIMER (Renal Section, Mitchell Hospital, Chicago, Illinois):** If I understand correctly, females appear to be more resistant than are males to the antihypertensive action of supplemental calcium, and further, women display less frequent abnormalities in parathyroid hormone and urinary calcium excretion when hypertensive. But you noted that pregnancy-induced hypertension was one condition in which calcium might prove therapeutic, because the data suggest that hypocalciumia accompanies pre-eclampsia, and calcium supplementation may decrease blood pressure in normotensive gravidas. Why does pregnancy make a woman's vasculature respond as if it were a man's?

**DR. MCCARRON:** In general, younger females are less likely to exhibit a relationship between calcium metabolism and blood pressure. This is not an absolute, however, because in some premenopausal hypertensive women, the blood pressure falls when calcium intake is increased [16]. The pregnant female, especially if she is black, appears to be an exception, particularly at the extremes of the reproductive years. Based on HANES I and II data, younger females in this country on average have dietary calcium intakes in the range of 300 to 500 mg/day [29]. During the second and particularly the third trimesters of pregnancy, significant metabolic demands are placed on the pregnant subject's calcium-regulating system. These demands reflect the calcium needed to mineralize the fetal skeleton and to provide for fetal growth in utero. Dietary calcium requirements during pregnancy and lactation have been estimated as exceeding 1500 mg/day [179]. If dietary calcium intake is significantly below this level, calcium depletion is likely to develop in the pregnant individual. As I discussed, blood pressure increases either as a direct reponse of the vasculature or as a reflection of the cardiovascular responses to increases in parathyroid hormone and vitamin D. Not enough is known for me to be more specific. However, from the intervention studies published to date, it is evident that increasing calcium intake during pregnancy can lower the risk of developing gestational hypertension or pregnancy-induced hypertension [160, 180].

**DR. GARY TOBACK (Renal Section, Mitchell Hospital):** A simple hypothesis that might explain some of the information you presented is that in males who develop hypertension when subjected to a low-calcium diet, a sex-specific trait—perhaps related to an abnormal locus on the Y chromosome—accounts for the high blood pressure. Has a double-blind study been carried out on this set of patients to determine whether their elevated blood pressure is alleviated by treatment with a calcium-supplemented diet? Can hypertension be made to reappear in such patients by a return to the low-calcium diet? Do males who develop hypertension on a low-calcium diet differ in any way from males or females who do not develop hypertension on that diet? Is the natural history of the hypertension induced in males by a low-calcium diet different from that observed in other types of secondary hypertension?

**DR. MCCARRON:** Dr. Eric Young in our group at Oregon has metabolic studies underway that attempt to characterize the differences between the calcium-sensitive and calcium-insensitive patients. I might also note that distinguishing a nutritional factor from a genetic factor in a human population can be difficult because nutritional patterns are passed along in a family. We tend to eat as our parents do. If one doesn't keep this difficulty in mind, lifestyle behavior could be mistaken for a genetic trait.

**DR. DAVID BUSHINSKY (Renal Section, Mitchell Hospital):** Serum total calcium tends to be high in hypertensive patients, yet ionized calcium is low. This finding would imply an alteration in calcium binding and suggests an abnormality in albumin or another circulating protein. Has such an abnormality been described?

**DR. MCCARRON:** In our experience, the correlation of higher total serum calcium concentrations with higher blood pressure is found primarily in females. Why this gender difference exists for total serum calcium values is not known. The serum ionized calcium data, although not entirely consistent, are just the opposite; lower values are observed in hypertensives than in normal individuals. You are correct that this constellation of findings implies an alteration in the extracellular binding of calcium and suggests that there is a circulating calcium-binding protein that is increased in some hypertensive patients.

**DR. BUSHINSKY:** Do you believe that alterations in calcium metabolism are a final common pathway or simply one component, perhaps a small one, in the control of systemic blood pressure?

**DR. MCCARRON:** I subscribe to the view that hypertension is a heterogeneous disorder. We are never going to identify a single explanation that accounts for a disorder like high blood pressure that afflicts 40 million people in the United States. On the other hand, altered calcium metabolism may be a factor for 15% to 30% of the population, as Witteman's recent report suggests [49]. These prospectively gathered epidemiologic data are consistent with the experience in the intervention trials. As I tried to point out, somewhere between 30% and 45% of an unselected population of hypertensives will respond favorably to added dietary calcium in their daily regimen.

**DR. KAI LAU (Director, Division of Nephrology, Michael...**
...to contract. Also, you referred to data, perhaps unpublished, showing that vascular smooth muscle cells pretreated with parathyroid hormone or 1,25 dihydroxyvitamin D3 have an attenuated response. Several laboratories, including our own, have shown that cytosolic free-calcium concentrations almost invariably reflect the metabolic and calcium balance status. Data from Japan, the U.S., and Europe indicate that acute changes in buffer calcium concentration elicit directional changes in cytosolic free-calcium concentrations. Chronically, dietary calcium manipulations also alter intracellular ionic calcium levels in a directional fashion. Stated simply, cytosolic free-calcium concentration is a very good indicator of the status of calcium balance; it is high when an animal is fed a high-calcium diet, and low when the animal is given a low-calcium diet. The current accepted model of vascular smooth muscle contraction indicates that an increase in the cytosolic free-calcium level initiates and signals contraction. How do you reconcile your data with these considerations, especially given the observations that PTH and 1,25(OH)2D3 acutely raise levels in a directional fashion. Stated simply, cytosolic free-calcium concentrations of virtually all cell systems examined?

**DR. MCCARRON:** It may be relatively easy to reconcile these seemingly divergent observations. Observations by Dr. Bukoski at Oregon represent the first measurements of the calcium transient in an intact resistance vessel. Any comparison with other findings must be made cautiously. The observations you are referring to have been gathered in isolated cells, and in many cases the measurement of intracellular calcium has been carried out in suspended cells, which means you are dealing with a manipulated system. In the case of our data, the mesenteric vessel is freshly isolated, then exposed to the agonist, in this case, norepinephrine. Under these more physiologic conditions, which come closer to mimicking the conditions the vessel experiences in vivo, we have observed precisely what you predicted if intracellular calcium stores reflect dietary intake and metabolic status. As we have proposed, high blood pressure, in selected circumstances, is associated with the patient's failure to maintain adequate calcium balance. Based on that premise and your own observations, an attenuation of the calcium transient is exactly what you would predict and indeed what we have observed.

**DR. LAU:** If the calcium transient is related to the contractile event, I don't understand why the increased vasocostrictive tone, reactivity, and contractility that are characteristic of the hypertensive animal should not be associated with, or perhaps causally related to, a higher calcium transient. Yet you demonstrate an attenuated calcium transient.

**DR. MCCARRON:** Again, this is reconcilable. What you are not taking into account is the association of established high blood pressure with vascular smooth-muscle-cell hypertrophy. It would be consistent to record a lower transient but a greater pressor response simply because of structural changes. Remember, we are not saying that a rise in the intracellular calcium did not occur in the SHR; we agree that contraction is associated with a rise in the concentration of intracellular free calcium. The transient does not have to be greater, provided there are more cells to contract.

**DR. SATISH KUMAR (Renal Fellow, Joint Michael Reese/Northwestern University Medical School):** Have any studies showed a positive correlation between hypertension and increased calcium intake or increased calcium levels in plasma?

**DR. MCCARRON:** No epidemiologic reports have noted higher dietary calcium intakes in hypertensives. In fact, few studies have shown the absence of a negative relationship; more than 20 reports have detected lower calcium intakes in association with higher arterial pressure. Sempous et al claimed that the calcium intake was similar for hypertensive and normotensive subjects in their analysis of HANES I and HANES II data [47]. Subsequently, they acknowledged that in HANES I there was an inverse relationship based on an alternative statistical approach [185]. So the dietary data base in the epidemiologic literature is consistent. As for plasma calcium levels, the data are less consistent. Several studies have indicated that serum total calcium values are higher in hypertensive patients. In our own experience, this finding appears to be most evident in females. Other reports have suggested that serum total calcium values are lower in the hypertensive individual [117], a finding similar
to what has been observed for serum ionized levels, as I mentioned.

**DR. JORDAN J. COHEN (Dean of Medicine, State University of New York at Stony Brook, Stony Brook, New York):** In considering nutritional factors in the pathogenesis of hypertension, one must acknowledge the overriding importance of obesity. One of the most predictable ways to lower blood pressure is through weight loss. Do you have a hypothesis that would link calcium metabolism with this striking antihypertensive effect of weight loss?

**DR. McCARRON:** You are correct. If there is one demographic or physiologic variable that matches up with blood pressure in virtually all populations, it is weight or body mass index. Weight might account for as much as 70% of variation in blood pressure in a given population. In our report on the HANES I data, we noted an inverse relationship between dietary calcium intake and body mass index within the United States [29]. By that, I mean that greater consumption of dietary calcium was associated with a lesser body mass index. Such an association also has been noted by other investigators. These are simply correlations and should not be used to argue for a causal relationship. Recently Metz et al from our group reported that in normal and hypertensive animals, body fat content is reduced and lean body mass increased as dietary calcium intake is increased [186]. The pathophysiologic mechanisms that would account for this association among dietary calcium, blood pressure, and obesity are unknown but certainly worth exploring. This relationship might represent a central-nervous-system effect mediated through a change in thermogenesis [187].

**DR. BUSHINSKY:** Much of the data that you presented on the hypertensive rat indicate an inherent abnormality in calcium homeostasis. Yet you say that ingestion of more calcium will lower blood pressure. These observations seem paradoxical unless one argues that eating more dietary calcium overcomes the disorder of calcium homeostasis. Can you link any homeostatic abnormality with the results of dietary studies?

**DR. McCARRON:** Increasing dietary calcium has lowered blood pressure in at least three normotensive strains: the Wistar, the Wistar-Kyoto, and the Fischer 344 rat [106-108]. An experimental animal does not have to have an elevated blood pressure to exhibit a reduction in blood pressure when additional calcium is consumed. That has also been the experience in the human trials [121]. Furthermore, the influence of dietary calcium intake on blood pressure has been noted in non-genetic models of hypertension [105]. We need not speculate that feeding supplemental calcium to selected individuals or laboratory models actually influences a genetic defect. But such an intervention might modify systemic factors such as the calcium-regulating hormones which, in turn, modify the expression of a genetic defect. Based on our experience utilizing isolated enterocytes derived from the SHR and WKY, we have been able to demonstrate that a presumably genetically linked defect in cytosolic flux of calcium is reversed by the ingestion of additional dietary calcium [76]. We have speculated that reversion of this defect might reflect a change in the regulation of the Mg2+-Ca2+-ATPase that results from the increased exposure of the cell to calcium because that pump is regulated by both Ca2+ and calmodulin.

In a separate system, the cultured myocyte, Bukoski and coworkers demonstrated that changes in extracellular Ca2+ within the physiologic range stimulate 45Ca uptake by the aortic myocyte [139]. Independent of circulating factors, therefore, changes in extracellular Ca2+ appear to influence Ca2+ transport by the myocyte.

**DR. LAU:** I have some difficulty with the notion that there is a renal calcium leak in genetic hypertension. The Ackerman study showed a lower baseline calcium excretion rate in human hypertensives and noted a higher calcium clearance only with increased natriuresis during saline loading [188]. The same is true of the study published several years ago in Clinical Science [14]. Your own data could be criticized for comparing 2-hour urine chemistries in fasting normals with 24-hour urine chemistries in hypertensives allowed ad-libitum food intake. Our animal work is the only study using classic clearance techniques and shows a reduction in calcium clearance in fasted hypertensive rats compared with age-, gender- and diet-matched fasted normotensive controls [189]. More fundamentally, your laboratory [78] and that of Chen Hsu at Ann Arbor (personal communication) reproduced our findings that there is an age dependency in the changes in urinary calcium excretion rate [189]. Early on, these animals are actually hypocalciuric, not hypercalciuric. I have difficulty understanding why, in a genetically transmitted disorder, the renal calcium leak postulated to contribute to hypertension does not appear in the first 15 to 18 weeks of life, by which time hypertension is almost maximally established. In fact, hypocalciuria is invariably present in the younger animals. You and your coworkers recently published an abstract showing that external calcium balance is also increased at an early age in the spontaneously hypertensive rat. In view of these findings, how can we conclude that calcium deficiency is causally related to the genesis or maintenance of hypertension?

**DR. McCARRON:** These seemingly disparate findings may all be very consistent. We agree that the very young SHR may be in slightly greater net positive Ca2+ balance than the WKY. However, as Drieke and coworkers have demonstrated, the very same animals studied at 12 to 14 weeks of age show just the reverse; that is, the SHR is no longer retaining an equivalent amount of Ca2+ compared with the WKY [78]. I should add that this latter finding is consistent with your own report of Ca2+ balance in the 10-week-old male SHR [112]. The question is why are there these age-related transitions in the SHR’s metabolic systems that are responsible for maintaining calcium balance? Currently we are pursuing the theoretical possibility that the SHR at 4 weeks of age is able to maintain equal or greater Ca2+ retention than the WKY, but this comes at the price of increased circulating levels of the Ca2+-regulating hormones (PTH and calcitonin) that have been demonstrated during adolescence and the rapid growth that occurs at that time. Even with the increased levels of the Ca2+-regulating hormones, the SHR’s intestine and kidney are no longer able to respond adequately, and negative Ca2+ balance ensues. The important unknown here is what is the cellular defect that underlies the inability of the organs to respond appropriately? Of equal importance is to understand how this defect relates to the rapid rise in arterial pressure that also occurs during this period. Most of the disturbances of calcium metabolism emerge during the period when blood pressure is climbing most rapidly, between about 8 and 20 weeks. Blood pressure plateaus after 25
to 28 weeks, however, which is also when the animal stops growing. The urinary calcium leak persists though [62].

Dr. Lau: If there were a defect in calcium homeostasis, I don't see why we would expect to find an increase in net external calcium balance, even early on. That would imply, at best, that we should expect near-normal or slightly reduced calcium balance compared with the normotensive strain. In published work, we have done experiments in both the female and male and documented the higher blood pressure in female SHR than in female WKY [151]. Van Os' group has obtained the same results in the male. You have done it in the male. So I think gender is not an issue here, as long as we compare SHR with WKY rats of the same sex in a given study. In my view, any definitive, global interpretation of the cumulative data is somewhat premature.

Would you comment on the age-related changes in serum concentration and in the synthesis of 1,25(OH)2D3? What if we were to postulate that the apparent defect is actually a compensatory reduction in 1,25 dihydroxyvitamin D3 synthesis in response to increased cumulative external calcium balance and/or higher cumulative phosphorus balance? What if the intracellular cytosolic free calcium levels in renal tubules in SHR at 12 weeks of age were found to be increased, at an age when 1,25(OH)2D3 production is down, serum levels of 1,25(OH)2D3 are reduced, and the corresponding duodenal calcium transport is depressed? Couldn't the last three metabolic changes merely reflect physiologic down-regulation? Why couldn't we reconcile all these data, at least tentatively, by that kind of interpretation?

Dr. McCarron: I do not believe it is rational to treat male and female SHR as equivalent models of hypertension. They are not. Their blood pressure pattern is strikingly different, as is their calcium metabolism. I should add that the situation is the same in human hypertension. I would agree that one reasonable interpretation of the intestinal Ca2+ transport data and/or higher cumulative phosphorus balance? What if the intracellular cytosolic free calcium levels in renal tubules in SHR at 12 weeks of age were found to be increased, at an age when 1,25(OH)2D3 production is down, serum levels of 1,25(OH)2D3 are reduced, and the corresponding duodenal calcium transport is depressed? Couldn't the last three metabolic changes merely reflect physiologic down-regulation? Why couldn't we reconcile all these data, at least tentatively, by that kind of interpretation?

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