Increased Calcium Absorption in Prehypertensive Spontaneously Hypertensive Rat

Role of Serum 1,25-Dihydroxyvitamin D_3 Levels and Intestinal Brush Border Membrane Fluidity

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Abstract

Changes in Ca absorption have been described in the spontaneously hypertensive rat (SHR) compared with Wistar-Kyoto (WKy) rats. In 3.5-wk-old SHR and age-matched WKy controls, we measured direct arterial blood pressure, Ca absorption, and serum 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] levels and small intestine brush border membrane (BBM) fluidity and lipid composition. The two objectives were (*a*) to define the nature of the absorptive changes before detectable hypertension and (*b*) to evaluate the potential mechanism(s). We found that even at this normotensive stage (106±4 vs. 107±2 torr for the female and 109±3 vs. 104±3 torr for the male), the SHR (*a*) absorbed more Ca (1.46±0.06 vs. 1.14±0.08 mmol/d and 1.53±0.06 vs. 1.28±0.06 mmol/d, respectively) and retained more Ca, (*b*) had higher serum 1,25$(OH)_2D_3$ levels (340±36 vs. 160±18 pg/ml and 230±25 vs. 150±16 pg/ml, respectively), and (*c*) possessed BBM with increased fluidity and with reduced fatty acyl saturation index owing to decreased stearic (32.2±2.6% vs. 38.2±0.9%) but increased linoleic acids (12.2±2.0% vs. 7.6±1.6%). These results demonstrate increased Ca absorption in prehypertensive SHR associated with increased serum 1,25$(OH)_2D_3$ levels, increased intestinal BBM fluidity, and reduced saturation index, which singly or in combination could produce the changes in intestinal Ca transport.

Introduction

Previous balance studies by our laboratory in the adult spontaneously hypertensive rat (SHR)[1] documented an increased net Ca absorption (1). These changes are associated with enhanced duodenal Ca uptake in situ and with absorptive hypercalciuria (1). The relationship between these transport alterations and hypertension is unclear. Chronic treatment of the established hypertension failed to abolish these changes in Ca metabolism (1), suggesting that they are not consequences of hypertension. Recent experiments suggest that the enhanced Ca absorption can also be seen in 25–28-d-old SHR (2), compatible with the hypothesis that disturbances in Ca metabolism may play a role in genetic hypertension (3–7).

The mechanism for the phenomenon of increased Ca absorption in SHR is unknown. Previous studies at 12 wk of age do not support the role of changes in vitamin D metabolism or sensitivity, since serum 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] levels were not measurably different (8), and the acute response to exogenous 1,25$(OH)_2D_3$ was blunted in the everted gut sac or perfused duodenum of SHR (9). In the former study, serum concentrations of 1,25$(OH)_2D_3$ in prehypertensive SHR were however not evaluated (8). In the latter study, tachyphylaxis to 1,25$(OH)_2D_3$ by the hyperabsorbing SHR could not be excluded. It should be emphasized that these conflicting results on Ca transport were obtained in much older SHR (8, 9). Therefore, the role of altered vitamin D metabolism and/or responsivity in mediating whatever changes in intestinal Ca transport seen in the young SHR is unclear.

Another potential explanation not previously addressed is the alteration in intestinal membrane fluidity. Three lines of evidence suggest this may be the case. First, changes in membrane fluidity (10), membrane structure (11), membrane inositol phospholipid metabolism (12, 13), and alterations in ^{45}Ca uptake, binding, and/or permeability (14–18) have been described in SHR as compared to normotensive Wistar-Kyoto (WKy) controls. Second, in models of phospholipid bilayers, permeability to Ca has been correlated with changes in lipid composition and fluidity (19, 20). Third, studies by several laboratories (21–28) have suggested a role for alterations in brush border membrane lipid structure in modulating intestinal Ca transport. However, the hypothesis of a "liponomic regulation" of Ca transport has recently been questioned (29). To shed light on the mechanism for the abnormalities in Ca absorption in the SHR, we therefore examined the potential role of intestinal brush border membrane fluidity and changes in serum 1,25$(OH)_2D_3$ levels.

Methods

The following four protocols were carried out, all using weanling SHR and WKy rats raised by inbreeding similar rats originally purchased from Charles River Breeding Laboratories (Wilmington, MA). Births were coordinated to yield litters of identical age by housing the dams in communal cages to synchronize their estrus cycle.

Protocol I: blood pressure and serum concentrations of 1,25$(OH)_2D_3$ in 28-d-old SHR

19-d-old pups (24 SHR and 19 WKy) were weaned and acclimatized in individual small metabolic cages specifically designed for 25–55-g animals. Simultaneously, they were equilibrated on a synthetic metabolic diet (ICN Nutrional Biochemicals, Cleveland, OH), nutritionally complete for growing rats (1) and containing per 100 g of food, 220 IU of vitamin D_2, 0.87 g of Ca, 0.15 g of Mg, 0.60 g of P, and 1.1 g of Na.

When weight gain and adequate food intake were ensured, they were

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1. *Abbreviations used in this paper:* 2-AS, DL-2-(9-anthroyl) stearic acid; 12-AS, DL-12-(9-anthroyl) stearic acid; DPH, 1,6 diphenyl-1,3,5 hexatriene; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; SHR, spontaneously hypertensive rat(s); WKy, Wistar-Kyoto (rat).

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studied at 28 d of age after an overnight fast. Using P.E. 10 tubing, carotid cannulae were placed under light ether anesthesia. The animal was allowed to regain full consciousness and rectal temperature between 36.5 and 37.5°C in a restraint cage. After at least 30 min, direct arterial blood pressure was recorded for 5 min on a Hewlett-Packard pressure monitor (Hewlett-Packard Co., Palo Alto, CA). The mean value for each rat over this period of time was used for the group analysis. 0.5–1.5 ml of blood was obtained from the arterial line for measurements of serum $1,25(OH)_2D_3$ levels (30). Serum volume was often <1 ml, so that the sera from two to four animals were appropriately pooled according to their strain and sex, as identified by abdominal laparotomy. 20 more WKy rats and 15 more SHR were identically prepared as above except for the measurement of blood pressure. They were bled on day 29 after an overnight fast to yield additional samples for serum $1,25(OH)_2D_3$ to permit adequate statistical analyses.

Protocol II: fluidity and composition of small intestinal brush border membrane in prehypertensive SHR vs. WKy rat

Weanling rats (28 SHR and 28 WKy controls, aged 23–24 d) were fasted for 18 h with free access to water. The animals were anesthetized with ether and the entire small intestine was rapidly removed and put in ice-cold saline solution. Brush border membranes were then prepared as previously described (31, 32) using the entire small intestine. Because the animals were small, each membrane preparation required pooling the tissue from seven rats. Four such membrane preparations were made for each group. Purity was assessed by the marker enzymes sucrase and alkaline phosphatase; specific activity ratios [(purified membranes)/(original homogenates)] ranged from 15 to 20 for these enzymes as described (31, 32). These preparations showed minimal contamination by microsomal, mitochondrial, or basolateral membranes as assessed by using the enzyme markers NADPH-cytochrome *c* reductase, succinic dehydrogenase, and sodium-potassium–dependent adenosine triphosphatase, respectively, as described (31, 32). Protein was measured by the method of Lowry et al. (33).

Fluorescence polarization studies. Three fluorophors were used: 1,6 diphenyl-1,3,5 hexatriene (DPH), DL-2-(9-anthroyl) stearic acid (2-AS), and DL-12-(9-anthroyl) stearic acid (12-AS). All compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Molecular Probes, Inc. (Junction City, OR). Steady-state fluorescence polarization studies were performed with a Perkin-Elmer 650-40 spectrofluorometer (Perkin-Elmer Corp., Norwalk, CT) adapted for fluorescence polarization. The methods used to load the membranes and liposomes and the quantification of the polarization of fluorescence have been described (31). Liposomes were prepared from extracted lipids as described (31). The content of each fluorophore in the membranes and liposomes was estimated fluorometrically as described by Cogan and Schachter (34). Final molar ratios of probe/lipid ranged from 0.001 to 0.002 and the anisotropy differences noted in these studies could not be ascribed to differences in probe concentrations in the membranes or liposomes. Corrections for light scattering (membrane suspensions minus probe) and for fluorescence in the ambient medium (quantified by pelleting the membranes after each estimation) were made routinely, and the combined corrections were <3% of the total fluorescence intensity observed for DPH-loaded membranes and <5% of that observed for anthroyloxysterate-loaded suspensions. The results were obtained according to the modified Perrin relationship (35–37) $r = r_\infty + (r_o - r_\infty)\,[T_c/(T_c + T_f)]$, where r is the fluorescence anisotropy, r_o is the maximal limiting anisotropy, taken as 0.365 for DPH (38) and 0.285 for the anthroyloxy probes (39), r_∞ is the limiting hindered anisotropy, T_c is the correlation time and T_f is the mean lifetime of the excited state. Possible changes in the excited-state lifetime, T_f, of the various probes were monitored by calculation of the total intensity of fluorescence, $F = I_{11} + 2I_\perp$, where I_{11} and $I_\perp$ are the fluorescence intensities oriented, respectively, parallel and perpendicular to the plane of polarization of the exciting light (32). Changes in the fluorescence anisotropy, r, were not the result of changes in the excited-state lifetime as assessed by F. Values of r_∞ for DPH were calculated from r values as previously described by Van Blitterswijk et al. (37). The static component of membrane fluidity was assessed by an order parameter, S, where $S = (r_\infty/r_o)^{1/2}$ as described previously (35–37). To exclude the possibility of sex differences influencing the results on strain comparison, 24 SHR (12 in each sex) and 24 WKy rats (half male and half female), aged 27 d, were similarly treated to yield brush border membranes (two preparations per sex per strain) for identical studies.

Composition studies. Total lipids were extracted from the membranes by the method of Folch et al. (40). Cholesterol was measured by the methods of Zlatkis et al. (41) and phospholipids by the methods of Ames and Dubin (42). The phospholipid composition of the extracts was further examined by thin-layer chromatography and scanning densitometry according to the procedure of Katz et al. (43). Fatty acids of the total lipid extracts were derivatized as described by Gartner and Vahouny (44). Fatty acid methyl esters were determined on a 6790A gas-liquid chromatograph (Hewlett-Packard Co.) equipped with a flame ionization detector and interfaced with a 3390A integrator (Hewlett-Packard Co.), using authentic fatty acid methyl esters to identify retention times (44).

Protocol III: intestinal Ca absorption in prehypertensive SHR vs. WKY rats

Weanling SHR ($n = 21$) and WKy pups ($n = 18$) were identically prepared as in protocol I. When weight gain and adequate food intake were ensured, balance studies were performed from day 25 through day 28 of age by methods previously described (1).

Protocol IV: blood chemistry in prehypertensive SHR

Pups were weaned and treated identically as in protocol III except that instead of balance studies, these animals were placed under light ether anesthesia in the morning of day 25. Arterial blood was obtained from the abdominal aorta for pH, ionized Ca, total serum Ca, and Mg determinations.

Analysis. Ashing of diet and feces was performed by methods previously published for our laboratories (1). Ca in urine, digested diet, and feces was measured by atomic absorption spectrophotometry as previously described (1). Blood pH and ionized Ca were measured by radiometer blood gas analyzer and Orion Ca sensitive electrode, respectively (1). Serum $1,25(OH)_2D_3$ levels were measured in triplicate by a radioreceptor assay from 1.0-ml aliquots of serum as previously described by us (30).

All results were statistically analyzed by Student's t test. Data are presented as mean±standard error of the mean. A P value of ≤0.05 is considered significant.

Results

Conscious arterial blood pressure in 28-d-old SHR. Systolic blood pressure was comparable between the SHR and WKy for both sexes (Table I). These findings make it valid for us to consider

Table I. Arterial Systolic Blood Pressure in 28-d-old Conscious Rats

	Pressure	
Rat	Male	Female
	torr	*torr*
WKy	107±3 ($n = 7$)	104±3 ($n = 12$)
SHR	106±4 ($n = 12$)	109±3 ($n = 12$)
P value	NS	NS

Values are given as mean±SE.

*Table II. Fluorescence Polarization Studies of Brush Border Membranes and Liposomes of 3.5-wk-old SHR and WKy Rats**

Probe	Preparation	Fluorescence anisotropy, r	Limiting hindered anisotropy, r_∞	Ordered parameter, S
DPH	BBM-WKy	0.254±0.003	0.239±0.006	0.810±0.008
	BBM-SHR	0.240±0.002‡	0.220±0.005‡	0.776±0.008‡
	Liposomes-WKy	0.196±0.002	0.161±0.004	0.665±0.008
	Liposomes-SHR	0.183±0.002‡	0.144±0.004‡	0.628±0.007‡
2-AS	BBM-WKy	0.129±0.002	—	—
	BBM-SHR	0.199±0.001‡	—	—
	Liposomes-WKy	0.101±0.002	—	—
	Liposomes-SHR	0.090±0.002‡	—	—
12-AS	BBM-WKy	0.122±0.003	—	—
	BBM-SHR	0.110±0.001‡	—	—
	Liposomes-WKy	0.092±0.002	—	—
	Liposomes-SHR	0.077±0.002‡	—	—

Abbreviation: BBM, brush border membrane. * Values represent means±SE of eight separate determinations of four different preparations of each membrane or liposome measured at 25°C. Each preparation was derived from seven rats. ‡ $P < 0.05$ or less compared with WKy values of liposomes or membranes.

the subsequent three groups of SHR (aged ≤28 d) as normotensive, similar to their age-matched WKy control.

Brush border membrane fluidity (Table II) and lipid composition (Fig. 1 and Table III). The lipid fluidity of SHR-brush border membranes, as assessed by steady-state fluorescence polarization techniques using all three fluorophores, was found to be significantly higher ($P < 0.05$) than WKy-membranes (Table II). It should be noted that these probes differ in a number of respects (45–48). DPH molecules are rod-shaped (48), localize deep in the lipid bilayer (40), and are aligned relatively parallel to the phospholipid acyl chains (47, 48). The transition moments for absorption and emission of this probe are along its long axis (47). Rotation along this axis will not result in depolarization, whereas rotation about another axis normal to its long axis will cause depolarization (47). The main depolarizing rotations sensed by DPH, therefore, are along its x- and z-axes (47). The anthroyloxy fatty acid probes (2-AS and 12-AS) assume a more spherical shape in bilayers than DPH (47) and localize at various depths in the bilayer, i.e., 2-AS localizes in the bilayer closer to

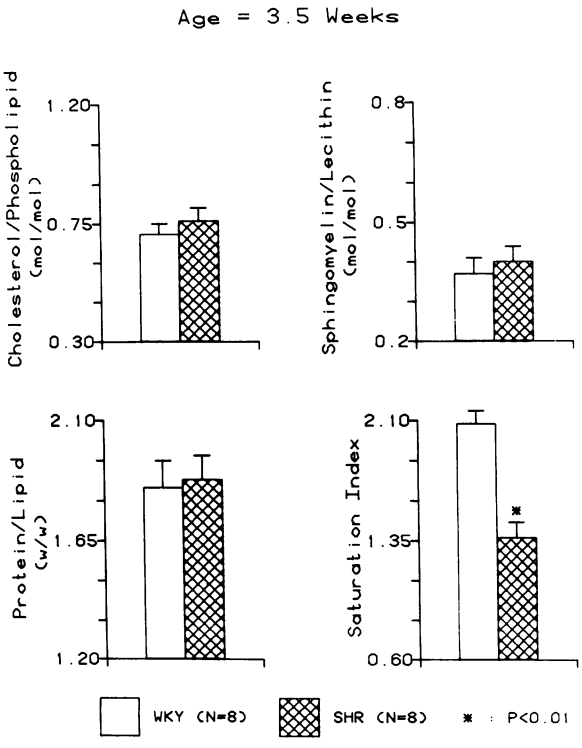

Figure 1. Compositional analysis comparison between SHR and WKy control. Factors known to regulate intestinal brush border membrane lipid fluidity. Two determinations were made on each of the four membrane preparations studied in each group. Each membrane preparation was in turn derived from seven rats of a given strain. Saturation index is derived by the ratio of the number of saturated acyl chains to the sum of each unsaturated acyl chain multiplied by the number of double bonds.

*Table III. Compositional Analysis of Total Fatty Acids of Rat Brush Border Membranes of WKy and SHR Rats**

Fatty acid	WKy-BBM	SHR-BBM
	% by mass	*% by mass*
14:0	1.1±0.2	1.5±0.2
14:1	0.6±0.1	0.7±0.1
16:0	34.0±1.1	31.0±4.3
16:1	1.9±0.2	1.6±0.5
18:0	38.2±0.9	32.2±2.6‡
18:1	13.9±0.5	13.3±2.6
18:2	7.6±1.6	12.2±2.0‡
20:4	3.2±0.9	6.6±2.1

Abbreviation: BBM, brush border membrane.
* Values are means±SE for lipid extracts from four preparations of membranes of each type. Each preparation was derived from seven rats.
‡ $P < 0.005$ or less compared with WKy values.

the aqueous interface than 12-AS (45, 47). Their emission moment is in the plane of the anthracene ring at 30° to their short molecular axis. Thus the main depolarizing rotations of these probes are along their x- and y-axes (47). In biological and artificial membranes, the structural organization of the lipid bilayer appears to limit the extent of rotation of DPH, therefore, r_∞ values for this probe are high and largely determine r (37). Other probes such as 2-AS and 12-AS yield relatively low values of r_∞ in bilayer membranes and their r values reflect mainly T_c, i.e., the speed of rotation (49, 50).

In the present studies, both the static and dynamic components of membrane lipid fluidity, as assessed by r_∞ and S of DPH, and r values of 2-AS and 12-AS, respectively, were found to be higher in brush border membranes prepared from the SHR (Table II). Similar differences in fluidity were seen in liposomes prepared from these membranes (Table II).

Compositional studies (Fig. 1. and Table III). To characterize the compositional changes that might underlie the fluidity differences in these membranes described above, lipid extracts of WKy and SHR brush border membranes were examined. Prior studies in model bilayers and natural membranes have correlated a high lipid fluidity with low molar ratios of cholesterol/phospholipid and sphingomyelin/lecithin (38, 51). A low ratio of protein/lipid (wt/wt) (39) as well as less saturated and shorter acyl chains in phospholipid (52) also appear to be associated with a higher lipid fluidity. As shown in Fig. 1, the cholesterol/phospholipid (mol/mol), sphingomyelin/lecithin (mol/mol) and protein/lipid (wt/wt) ratios were similar in both membranes. The saturation index, however, was higher in WKy brush border membranes than their SHR counterparts. Alterations in the levels of stearic (18:0) and linoleic (18:2) fatty acids were responsible for differences in the latter parameter (Table III).

A possible sex influence on the observed strain difference was excluded by comparing the female to male rats of identical age (Table IV). For both SHR and WKy rats, brush border membrane fluidity was not different between the sexes, using all three fluorescent probes. Furthermore, the increased fluidity in the SHR was again observed (Table III).

Absorption and retention of Ca in prehypertensive SHR (Table V). Body weight was comparable between the female SHR and WKy rats from the beginning (36±1 vs. 37±1 g) through the end of the balance study (45±1 vs. 46±1 g). Likewise, the male SHR and WKy rats did not differ in weight at the beginning (38±1 vs. 39±1 g) or the end of the metabolism study (47±1 vs. 48±1 g). Food intake was similar between the female (9.0±1.4 vs. 8.8±1.4 g) and male (8.9±1.5 vs. 8.6±1.1 g) SHR and WKy rats. Diet Ca was found to be 0.878% g by analysis. Accordingly, Ca intake was also similar (Table V). Fecal Ca elimination was reduced in the SHR compared with sex-matched WKy control, indicating an increase in net Ca absorption for both sexes (Table V). These results not only confirm our previous observation of hyperabsorption in the adult SHR (1), but also extend that to the prehypertensive stage. Urine Ca was lower in the SHR regardless of sex, despite the increased Ca absorption, suggesting inappropriate renal Ca retention. Therefore, overall external Ca balance, or Ca retention, was increased in both the female and male normotensive SHR compared to age- and sex-matched WKy controls. These results not only corroborate our previous findings in the adult hypertensive rat (1), but also establish for the first time that these changes are not the consequences of hypertension.

Serum concentrations of divalent ions and 1,25(OH)$_2$D$_3$ in prehypertensive SHR (Table VI). At the 25th day of age, there was no difference in blood pH, total serum Ca, ionized Ca, or serum Mg between sex-matched SHR and WKy rats. Serum 1,25(OH)$_2$D$_3$ was significantly greater in the eight pooled serum samples of female SHR than in eight pooled samples of WKy rats (340 vs. 160 pg/ml). In the male, serum 1,25(OH)$_2$D$_3$ was also increased in the 7 pooled samples of SHR than in the eight pooled samples of WKy rats (230 vs. 150 pg/ml) (Table VI).

Discussion

We confirmed previous direct arterial measurement (53) that blood pressure was normal in SHR between 26 and 30 d of age (Table I). This is true for both the male and female. Thus, results

*Table IV. Comparison between Sexes: Fluorescence Polarization Studies of Brush Border Membranes from 27-d-old SHR and WKy Rats**

Probe	Preparation	Fluorescence anisotropy, r	Mean limiting hindered anisotropy, r_∞	Mean order parameter, S
DPH	Male-WKy	0.275±0.002	0.267	0.855
	Female-WKy	0.273±0.002	0.264	0.850
	Male-SHR	0.266±0.002‡	0.255	0.836
	Female-SHR	0.266±0.002‡	0.255	0.836
2-AS	Male-WKy	0.114±0.002	—	—
	Female-WKy	0.148±0.003	—	—
	Male-SHR	0.132±0.002‡	—	—
	Female-SHR	0.136±0.003‡	—	—
12-AS	Male-WKy	0.112±0.001	—	—
	Female-WKy	0.110±0.002	—	—
	Male-SHR	0.105±0.001‡	—	—
	Female-SHR	0.107±0.002	—	—

* Values represent mean±SE of four separate determinations of two different preparations of each membrane at 25°C. Each preparation was derived from six rats of a given sex and strain. ‡ $P < 0.05$ or less compared with values of membranes prepared from WKy animals of the same sex.

Table V. Ca Metabolism in Prehypertensive SHR

Sex	Strain	Ca Intake	Fecal Ca	Net Ca absorption		Urine Ca	Retained Ca	
		μmol/d	*μmol/d*	*μmol/d*	*%*	*μmol/d*	*μmol/d*	*%*
Female								
	Wky ($n = 11$)	1,930±33	793±48	1,138±80	58.4±3.2	79.3±7.3	1,059±81	54.3±3.3
	SHR ($n = 11$)	1,987±43	526±47	1,461±62	73.4±2.3	45.6±8.0	1,416±60	71.2±2.3
P value		NS	<0.001	<0.005	<0.001	<0.005	<0.005	<0.001
Male								
	WKy ($n = 7$)	1,900±46	631±71	1,278±63	67.3±3.4	76.1±18.2	1,202±73	63.3±3.3
	SHR ($n = 10$)	1,948±61	420±22	1,528±58	78.4±1.1	32.5±8.3	1,494±46	76.4±0.9
P value		NS	<0.02	<0.02	<0.01	<0.05	<0.005	<0.005

of the present studies are applicable to the prehypertensive phase of SHR.

Serum total and ionized Ca were similar between these young SHR and their age-matched and sex-matched controls. Previous studies yielded conflicting results in older rats, including a reduction in the SHR at the 5th, 13th, and beyond the 16th wk of age (3, 54, 55), but an increase at the 23rd wk of age (1). The age dependency of the hypercalciuria and the feeding-dependency of the hypercalcemia in the SHR have previously been noted by us (1). These might account for the apparent differences between the prehypertensive and hypertensive phases. Differently from our present experiments, previous studies did not measure blood pH, making it difficult to exclude hyperventilation in the more anxious SHR as a possible reason for the reduced serum ionized Ca levels. Indeed, stress alone could produce a lower ionized Ca in the rat, according to one preliminary report (56). Focusing on Ca absorption, our studies were not designed to evaluate ionized Ca, although there are well-known conditions such as PO_4 deprivation (57, 58) and immobilization (59, 60), in which the increased ionized Ca levels do not reflect the reduced Ca balance. Measuring Ca absorption and Ca retention directly, we found increased rates in these 3½-wk-old SHR compared to age- and sex-matched controls (Table IV), as was previously documented in their adult counterparts (1).

At 5 wk of age, duodenal Ca absorption, as measured in vitro by the everted gut sac technique was found to be decreased by one laboratory (8) but increased by another laboratory (9). In vivo studies using the recirculation perfusion techniques in 12-wk-old anesthetized SHR also yielded conflicting results, with reports of both increased (9) and decreased (9) Ca absorption in the proximal small intestine. The reasons for the data discrepancy in these in vitro studies and in anesthetized rats are not apparent. One possible explanation is the inclusion of jejunal cells by one study (8), in which segments of 20 cm distal to the pylorus were used. In this connection, the only other study reporting a reduced absorptive flux in the SHR also employed a segment that contained the jejunum (61). As recently reviewed for regular laboratory rats (62), the jejunum differs markedly from the duodenum in that only net Ca secretion has been consistently observed across the Ussing chamber in the unstimulated

Table VI. Serum pH, Divalent Ions, and 1,25(OH)$_2$D$_3$ Levels

Sex	Strain	Serum	Serum Ca*		Serum Mg*	Serum 1,25(OH)$_2$D$_3$‡
			Total	Ionized		
		*pH**	*mM*	*mM*	*mM*	*pg/ml*
Female						
	WKy	7.39±0.03 ($n = 6$)	2.50±0.10 ($n = 6$)	1.10±0.03 ($n = 6$)	1.03±0.04 ($n = 6$)	160±18 ($n = 8$)
	SHR	7.36±0.05 ($n = 6$)	2.58±0.11 ($n = 6$)	1.11±0.03 ($n = 6$)	1.10±0.07 ($n = 6$)	340±36 ($n = 8$)
P value		NS	NS	NS	NS	<0.001
Male						
	WKy	7.37±0.03 ($n = 6$)	2.61±0.11 ($n = 6$)	1.17±0.03 ($n = 6$)	1.05±0.04 ($n = 6$)	150±16 ($n = 8$)
	SHR	7.34±0.04 ($n = 6$)	2.70±0.10 ($n = 6$)	1.17±0.03 ($n = 6$)	1.12±0.08 ($n = 6$)	230±25 ($n = 7$)
P value		NS	NS	NS	NS	<0.01

* *n* refers to the number of rats measured, with each animal contributing one data point to the mean. ‡ *n* refers to the number of pooled samples of sera, each derived from two to four rats of a given sex and strain.

state. Indeed, our preliminary results in equally old (12 wk) SHR and WKy control demonstrated precisely this functional heterogeneity (63).

In any case, in the conscious adult SHR, balance studies in their natural physiologic state, however, documented an increase in net Ca absorption in most experiments (1), as were the results in the present study in the prehypertensive stage. In situ ligated duodenal gut sac studies also revealed a higher rate of Ca uptake in the conscious SHR (1), not only reinforcing our present and earlier (1) in vivo findings, but also suggesting that the changes in net Ca absorption are mediated at least in part by the small intestine.

The mechanism for the enhanced Ca absorption by the adult SHR was not clear from previous investigations (1). Measurements of serum $1,25(OH)_2D_3$ levels in the hypertensive phase revealed no differences compared to the WKy control at 6 (64), 10 (64), 12 (8), or 14 wk of age (8). In 3.5-wk-old prehypertensive SHR, we presently found increased serum levels of $1,25(OH)_2D_3$, suggesting but not proving a role of this metabolite in the increased Ca absorption at this early age.

In view of the recently reported differences in membrane fluidity, structure, phospholipid metabolism (10–13), and membrane Ca handling (14–18) between the SHR and WKy control, and in view of the postulated role of altered membrane lipid composition in Ca transport (19–28), we evaluated whether small intestinal brush border membrane in the SHR is characterized by changes in fluidity and lipid content. Our data revealed an increase in lipid fluidity of the SHR membrane as measured by several different probes (Table II) and at a time before hypertension was detectable. These observations are further strengthened by finding a lower fatty acyl saturation index in SHR membranes (Fig. 1), which would independently predict such an increase in fluidity in the SHR (52). Parenthetically, studies in blood vessels (14, 15), myocytes (16, 17), and erythrocytes (18) have suggested reduced binding of Ca to plasma membrane in the SHR, which per se ought to increase lipid fluidity. Presently, this formulation must be regarded as entirely speculative. Nonetheless, based on these changes in membrane fluidity and lipid composition, the available information (19–28) would predict increased Ca transport by the SHR, as we have empirically found. It should be stressed that our present studies establish only an association and not necessarily a cause-and-effect relationship.

The mechanism(s) responsible for the difference in fatty acid composition are unclear at this time. While speculative, they may involve difference in fatty acyl chain desaturase activities and/or differences in reacylation–deacylation of the membrane fatty acids. In regard to the first possibility, prior studies with hepatocytes (65) have demonstrated that alterations in certain microsomal desaturase activities led to changes in plasma membrane fatty acid composition and fluidity. Our laboratory has recently demonstrated that such activities are indeed present in the rat intestine (66), alterations of which could conceivably be responsible for the fatty acid compositional differences.

In regard to the second possibility, O'Doherty (27) has shown that $1,25(OH)_2D_3$ increases the activity of the intestinal phosphatidylcholine deacylation–reacylation cycle in the rat. In the present studies, this cycle was not examined but conceivably this could have been increased in SHR membranes secondary to the increased levels of circulating $1,25(OH)_2D_3$ in these animals (Table VI). Further studies, however, will be required to clarify this issue.

It is not possible to differentiate between the role of increased $1,25(OH)_2D_3$ levels and the increased membrane fluidity in the hyperabsorptive phenomenon. Furthermore, it is entirely conceivable that the membrane changes are also secondary to the increased $1,25(OH)_2D_3$ levels, similar to the increased Ca absorption. In this connection, it is interesting to note that the most consistent alteration in fatty acid composition in intestinal and renal brush border membrane induced by $1,25(OH)_2D_3$ administration is the increase in linoleic acid content of the phosphatidylcholine fraction (67–69). A decrease in stearic acid was also produced by this hormone in the phosphatidylethanolamine fraction of rat duodenal mucosa (68). These effects are similar to the changes in fatty acids found in the total brush border membrane lipid of the SHR, which also had higher serum $1,25(OH)_2D_3$. Nevertheless, a liponomic mechanism for regulating Ca transport is supported only by indirect evidence, because simultaneous measurement of Ca transport, membrane fluidity, and lipid structure have rarely been performed on the same preparation.

In the only studies known to us, where all these determinations were made, no effects of subacute administration of cholecalciferol on lipid fluidity or polarity could be detected in the vitamin D-deprived chick intestinal brush border membrane, as measured by one probe (5-nitroxide stearate) (29). Clearly, it is not possible to extrapolate these findings to earlier studies, which used different protocols and examined the effects of the more polar metabolites, $25(OH)D_3$ (20) or $1,25(OH)_2D_3$ (22, 23, 26, 27). A recent study in vitamin D-deficient chicks detected no change in brush border membrane fluidity after acute $1,25(OH)_2D_3$ treatment, as measured by the DPH probe (70). Lipid composition was however not analyzed.

In summary, we have documented increases in Ca absorption and serum $1,25(OH)_2D_3$ levels in the prehypertensive SHR. These changes were associated with increased small intestine bruch border membrane fluidity and altered fatty acid composition. Although the elevated serum $1,25(OH)_2D_3$ concentrations could provide an adequate explanation for the enhanced intestinal Ca transport, additional studies are however necessary to critically evaluate a possible cause-and-effect relationship between these physiologic and chemical alterations. Similarly, although the presence of these changes in the prehypertensive stage of the SHR raises the possibility that they could play a role in the hypertension, more compelling evidence than those summarized (71) depends on future studies specifically evaluating the blood pressure response to correcting these abnormalities in very young SHR.

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