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Elevated cytosolic calcium of adipocytes in chronic renal failure

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Elevated cytosolic calcium of adipocytes in chronic renal failure. Chronic renal failure (CRF) is associated with increased calcium content of, and impaired lipase release from lipid cells. This has been attributed to a rise in the cytosolic calcium ([Ca^{2+}]i) of these cells. However, data on [Ca^{2+}]i of lipid cells in CRF and on the mechanisms responsible for such an abnormality are lacking. To study this issue we examined the [Ca^{2+}]i and ATP content of lipid cells and V_{max} of Na^+-K^+-ATPase and Ca^{2+} ATPase of membrane preparation and Na^+-Ca^{2+} exchange of membrane vesicles of adipocytes from normal rats, 6 week CRF, CRF normocalcemic parathyroidectomized (CRF-PTX) and CRF, and normal rats treated with verpamil (CRF-V, normal-V). [Ca^{2+}]i in adipocytes of CRF rats was higher (199 ± 8.5 nM) and ATP lower (2.9 ± 0.31 nmol/10^6 cells) than in normal (120 ± 4.3 nM; 5.7 ± 0.27 nmol/10^6 cells), CRF-PTX (128 ± 4.7 nM; 5.8 ± 0.39 nmol/10^6 cells), normal-V (121 ± 3.2 nM; 5.3 ± 0.36 nmol/10^6 cells), CRF-V (123 ± 7.4 nM; 5.5 ± 0.30 mnol/10^6 cells). V_{max} Ca^{2+}ATPase and the activity of Na^+-K^+-ATPase and of Na^+-Ca^{2+} exchanger were reduced in CRF rats as compared to the other four groups of rats. The values in normal, CRF-PTX, CRF-V and normal-V rats were not different. These results indicate that: (1) in CRF, adipocytes are overloaded by calcium; (2) this abnormality is mediated by the secondary hyperthyroidism of CRF since PTX of CRF rats or interference with the action of PTH by a calcium channel blocker prevented these changes; and (3) the elevation in [Ca^{2+}]i is due to both increased entry of calcium into adipocytes and a decreased extrusion out of these cells.

Abnormalities in lipid metabolism are commonly encountered in chronic renal failure (CRF). Indeed, CRF is associated with hyperlipidemia [1–3] due in major part to impaired removal of triglycerides from plasma [3, 4]. It has been shown that the post-heparin lipolytic activity is reduced [1, 4] and the removal of intravenous lipid load is impaired [4, 5] in CRF. Akmal et al [6] found that these abnormalities are mediated in major part by the state of secondary hyperparathyroidism of CRF. They demonstrated that parathyroidectomy of CRF rats maintained normocalcemic prevented these derangements.

PTH enhances entry of calcium into adipocytes [7], and chronic excess of PTH in CRF is associated with increased calcium content of epididymal fat pads of rats [6]. This latter abnormality appears to play an important role in the deranged lipid metabolism of CRF, since the prevention of calcium accumulation in adipose tissue by treatment of CRF animals with verapamil corrected the hyperlipidemia and normalized the post-heparin lipolytic activity and the abnormal removal of intravenous lipid loads [6].

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Increased calcium content of other tissues in CRF [8–10] is usually associated with an elevation in the basal levels of [Ca^{2+}]i in the cells of these tissues [11–13], and such a change mediates the dysfunction of cells in CRF [14]. It is possible, therefore, that the basal levels of [Ca^{2+}]i of adipocytes are also elevated in CRF.

The present study examined the basal levels of [Ca^{2+}]i of adipocytes in CRF, evaluated the role of excess PTH in the genesis of such an abnormality and explored the mechanisms that lead to a rise in the basal levels of [Ca^{2+}]i of adipocytes in CRF.

Methods

A total of 212 Sprague-Dawley rats weighing 360 to 450 g were used. These animals are used in the study published by us [11]. However in that publication we reported data on the myocardium while in this paper we examine adipocytes. We do not believe that this fact has an adverse effect on the results of the current study, since it is not inappropriate to also use the same animals for the investigation of metabolism of another organ especially when the number of animals is very large. Such an approach saves time and research funds. The animals were housed in individual cages and fed normal rat chow (Wayne Research Animal Diets, Chicago, IL, USA) throughout the study. The diet contained 1.4% calcium, 0.97% phosphorus, and 4.4 IU of vitamin D/g. Studies were performed in 5 groups of animals: (1) normal rats, (2) rats with CRF of 6 weeks duration, (3) normocalcemic parathyroidectomized CRF rats (CRF-PTX) of 6 weeks duration, (4) CRF rats of 6 weeks duration treated with verapamil (0.1 μg/g body wt) which was given subcutaneously twice a day form day one of CRF, (CRF-V), and (5) normal rats treated with verapamil, as described above for 6 weeks (normal-V).

CRF was produced by 5/6 nephrectomy; the animals underwent right 2/3 nephrectomy through a flank incision and a week later, a left nephrectomy was done. PTX was performed by electrocautery, and the success of the procedure was ascertained by a decrease in plasma levels of calcium of at least 2 mg/dl. The PTX rats were allowed to freely drink water containing 5% calcium gluconate. This procedure is adequate to normalize serum calcium in the PTX rats. Seven days after PTX, the rats were subjected to 5/6 nephrectomy as described above. Two days before sacrifice, the animals were housed in metabolic cages and two consecutive 24-hour urine collections were obtained for the measurement of creatinine clearance. The animals were killed by decapitation on day 42 after the completion of the 5/6 nephrectomy in CRF rats (CRF, CRF-PTX, CRF-V) or after the beginning of the treatment with verapamil in normal rats.

Isolation of adipocytes was done according to the method of Rodbell [15]. After the sacrifice of the animals, the epididymal fat

pads were removed, washed with normal saline and cut into small pieces. The tissue was then placed in 50 ml polypropylene tube containing 8 ml of Krebs-Ringer bicarbonate (KRB) solution (137 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$ 12 mM, $NaHCO_3$, 4 mM NaH_2PO_4, 1.4 mM $CaCl_2$), 5 mg collagenase type 1 (Worthington Biochemical, Freehold, NJ, USA), 3 μmol glucose/ml and 4% BSA, pH 7.4. The mixture was incubated in a shaking water bath (37°C) for one hour and then passed trough mesh nitex screen (Tetko, Elmsford, NY, USA). The filtrate was centrifuged at 400 g for two minutes and the floating adipocytes were picked by a pipette and transferred to KRB medium. The tubes containing the adipocytes were shaken gently each time before centrifugation, and any fat droplets that might have been formed from leakage of the fat cells floated rapidly to the surface. They were then picked up by a transferring pipette. After the final centrifugation, the adipocytes floated to the top of the KRB solution. The pH of the KRB solution throughout the procedure was monitored and kept at 7.4. The intactness of the cells was confirmed by microscopy as described by us previously [7].

[Ca^{2+}]i of adipocytes was measured with Fura2-AM (Sigma Chemical Co., St. Louis, MO, USA). An aliquot of 1 ml of the floating layer of adipocytes was added to 8 ml of KRB solution containing Fura 2 at a concentration of 4 μM. The mixture was incubated in a shaking bath (37°C) for 40 minutes; it was then kept at room temperature for 30 minutes followed by washing three times with Krebs-Hepes buffer (118 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 1.25 mM $NaHCO_3$, 20 mM Na Hepes, 5 mg/ml BSA, and 0.3 mg/dl glucose, pH 7.4) and centrifugation at 400 g for two minutes. Fat droplets that might have been formed were removed as described above. Fluorescence was measured with a Perkin-Elmer fluorometer model LS5B (Perkin-Elmer, Norwalk, CT, USA) at excitation wavelengths of 340 and 380 nm and emission wavelength of 510 nm. The maximal (F_{max}) and the minimal (F_{min}) fluorescence were estimated as previously reported [7, 13]. The cuvette containing the loaded cells was shaken gently and the fluorescence was recorded immediately. The cells were lysed with Triton-X (0.05%) to obtain the F_{max}. This concentration of Triton-X does not produce fluorescence. Next, 40 μl of EGTA in 3 M base buffer (pH 8.5) were added to obtain F_{min}. To determine the effect of autofluorescence due to cuvette medium and adipocytes, the fluorescence was measured with an empty cuvette, after the addition of the medium and after the addition of the cells without Fura 2. The unloaded adipocytes generated small amounts of fluorescence. Correction for autofluorescence of the cuvette, medium, and adipocytes was made by setting the fluorometer on autozero before each measurement. Calculation of [Ca^{2+}]i was made using the Grynkiewicz equation [16]. The dissociation constant for Ca^{2+}-Fura 2 was assumed to be 225 nM [16].

The ATP content of adipocytes was determined by the method of Lundin et al [17] using a firefly luminescence assay with LAD 535 luminometer (Turner Design, Sunnyvale, CA, USA). The details of this assay were described previously by us [12]. Adipocytes were counted under the microscope in the 50 μl aliquot used for the measurement of ATP. The value of ATP was then expressed in 10^6 cells.

Measurement of Na^+-K^+-ATPase and Ca^{2+} ATPase activities were made using plasma membranes prepared from adipocytes according to the method described by Resh [18]. The adipocytes obtained from one to two rat epididymal fat pads were suspended in 35 ml of homogenization buffer (225 mM sucrose, 20 mM Tris-HCl, 1 mM EDTA, pH 7.4) and homogenized in a polytron homogenizer (Kinematic Instrument, Switzerland) 10 times for 5 seconds each at 1200 rpm. The homogenate was centrifuged at 16000 g for 15 minutes. The pellet was saved, resuspended in homogenization buffer and spun once again at 16000 for 15 minutes. The final pellet was resuspended in 2 ml of homogenization buffer and layered over 3 ml of 33.5% (wt/wt) sucrose in 20 mM Tris-HCl, 1 mM EDTA, pH 7.4. Following centrifugation for one hour at 70000 g in an Ti 50 rotar using Beckman LS 5 ultracentrifuge (Beckman Instrument, Palo Alto, CA, USA), the plasma membranes band at the sucrose gradient interface was saved and washed in buffer containing 20 mM Tris-HCl, 1 mM EDTA, pH 7.4 and spun at 50000 g for 30 minutes using the same rotor. The final plasma membrane pellet was resuspended in Hepes-Mg buffer containing 100 mM Hepes-Tris, 5 mM $MgCl_2$, pH 7.4 giving the final protein concentration of 1.5 to 3.0 mg/ml. The preparation was stored at −70°.

For the measurement of Na^+-K^+-ATPase, 50 μl of the plasma membranes preparation were added to 900 μl of medium containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA and 4 mM $MgCl_2$ in the presence and absence of 100 mM NaCl and 10 mM KCl. The medium with NaCl and KCl contained 2 mM ouabain. Both samples were pre-incubated for five minutes at 37°C; thereafter, 50 μl of 80 mM of Tris ATP were added to the mixtures to give a final concentration of 4 mM ATP. The mixtures were incubated in a water bath for 20 minutes at 37°C. The ATP levels were measured by the firefly luciferase luminescence technique using LAD 535 luminometer [17] before and at the end of the 20 minute incubation. The values of Na^+-K^+-ATPase activity were calculated as the difference between the ATP levels consumed by the plasma membranes during the 20 minute incubation in the presence and absence of NaCl and KCl.

The plasma membrane Ca^{2+} ATPase activity was determined by continuous monitoring of ATP consumption using the firefly luciferase luminescence technique [17]. An aliquot of 50 μl of the plasma membranes was added to 900 μl of medium containing 160 mM KCl, 20 mM MOPS, 5 mM $MgCl_2$, 5 mM NaN_3 and 0.2 mM EGTA without calcium and with two concentrations of calcium of 0.162 and 0.181 mM, that were equivalent to 1.0 and 2.0 μM free calcium, respectively. For the determination of free calcium concentration, the association constant of 4.47×10^6 was used for Ca^{2+}-EGTA binding [19]. At the end of five minutes pre-incubation at 37°C, 50 μl of 20 mM of Tris-ATP were added to the mixtures giving a final volume of 1 ml and a concentration of ATP of 1 mM. The mixtures were incubated for 20 minutes at 37°C. The Ca^{2+} ATPase activity was calculated as the difference between the ATP consumption in the presence and absence of calcium. The activity of the enzyme at these two concentrations of calcium were not different; thus, the values obtained represent the V_{max} of the Ca^{2+} ATPase.

For the measurement of Na^+-Ca^{2+} exchange, membrane vesicles were prepared according to the method described by Jarett [20]. The Na^+-Ca^{2+} exchange was estimated as Na^+-dependent ^{45}Ca uptake by a modification of the method described by Bersohn et al [21]. An aliquot of 5 μl of NaCl-loaded vesicles was added to 245 μl of uptake medium containing 160 mM KCl, ^{45}Ca 0.3 μCi (specific activity 10 to 40 mCi/mg calcium, Amersham, Arlington Heights, IL, USA), 10 μM $CaCl_2$, 20 mM MOPS (pH 7.4). After different times of 5, 10, 15, 30, 60, and 90 seconds, 30

Table 1. Body weight, biochemical parameters and serum PTH levels in all groups of rats at the time of sacrifice

	N	Body weight *g*	Plasma *mg/dl* Creatinine	Plasma *mg/dl* Calcium	Plasma *mg/dl* Phosphorus	Serum PTH *pg/ml*	Creatinine clearance *μl/min/100 g*
Normal	36	397 ± 9.2	0.32 ± 0.01	9.0 ± 0.1	6.6 ± 0.2	17 ± 1.9	536 ± 16
Studies at 6 weeks							
CRF	34	397 ± 7.0	1.30 ± 0.01[a]	8.7 ± 0.2	6.8 ± 0.3	54 ± 6.6[a]	165 ± 6[a]
CRF-PTX	31	401 ± 6.6	1.01 ± 0.03[a]	8.6 ± 0.2	6.8 ± 0.2	11 ± 4.3	162 ± 11[a]
CRF-V	32	408 ± 8.0	1.02 ± 0.01[a]	9.0 ± 0.2	6.7 ± 0.3	49 ± 4.7[a]	159 ± 10[a]
Normal-V	32	387 ± 5.0	0.33 ± 0.01	8.9 ± 0.2	6.9 ± 0.5	20 ± 4.6	546 ± 13

Data are presented as mean ± SE. These animals are part of the animals reported in *Kidney Int* 45:1113–1119, 1994 for the study of cardiac myocytes. Therefore, the biochemical data are similar to those reported earlier. However, in the current study we examined the metabolism of another tissue (adipocytes).

[a] $P < 0.01$ vs. other groups

μl of 10 mM EGTA (pH 7.4) were added, followed by 1 ml of ice-cold 1 mM EGTA-KCl-MOPS to stop the reaction. An aliquot of 1 ml was then taken from the mixture and passed through millipore membrane filter (0.45 μm, Millipore Corp., Bedford, MA, USA). The membranes were subsequently washed twice in 2 ml of ice-cold 1 mM EGTA-KCl-MOPS. The membranes were then placed in scintillation vials containing 10 ml of scintillation fluid (Ecolite, ICN Biochemicals, Irvine, CA, USA) and counted with Beckman LS 7000 scintillation counter. K^+-loaded vesicles were treated in a similar manner. The Na^+-Ca^{2+} exchange was calculated as the difference between the uptake by NaCl-loaded vesicles and KCl-loaded vesicles.

The measurement of calcium in the plasma was made by Perkin-Elmer atomic absorption spectrophotometer, model 503, and those of plasma creatinine and phosphorus and urine creatinine by an autoanalyzer (Technicon Instrument Crop., Tarrytown, NY, USA). The serum levels of PTH were determined by an INS-PTH immunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). This assay recognizes the aminoterminal fragment of PTH. The lowest detectable level is 3 pg/ml; the interassay variations is 7.3% and the intrassay variations 4%. Protein content of the sarcolemmal vesicles was measured by the method of Bradford [22].

Statistical analysis was done by one-way analysis of variance (ANOVA) and significant differences with Bonferroni-Dunn test for multiple comparison between the groups. Data are presented as mean ± 1 SE.

Results

Table 1 provides the effects of the various experimental procedures on body weight, plasma concentration of creatinine, calcium and phosphorus, and serum levels of PTH and creatinine clearance. There were no significant differences in body weight and the plasma concentrations of calcium and phosphorus among the five groups of rats. The plasma concentrations of creatinine were significantly ($P < 0.01$) higher and the creatinine clearances were significantly ($P < 0.01$) lower in CRF, CRF-PTX and CRF-V rats than in normal and normal-V animals. There were no significant differences between these parameters among CRF, CRF-PTX and CRF-V rats. The serum levels of PTH were significantly ($P < 0.01$) higher in CRF and CRF-V rats than in normal, normal-V and CRF-PTX animals.

Figure 1 depicts the data on the basal levels of $[Ca^{2+}]i$ in all groups of animals. The $[Ca^{2+}]i$ in adipocytes from CRF animals (199 ± 8.5 nM) was significantly ($P < 0.01$) higher than that in adipocytes from normal rats (120 ± 4.3 nM). PTX of CRF rats or

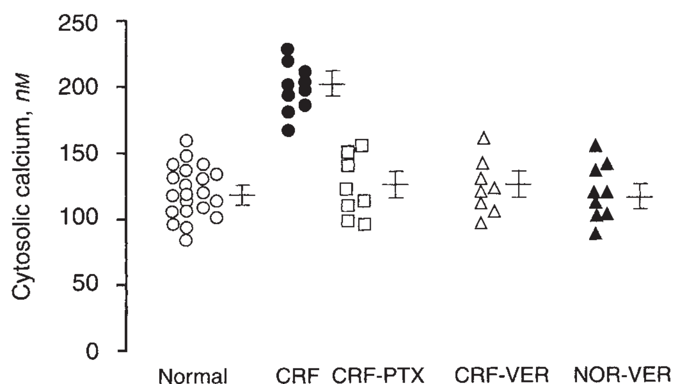

Fig. 1. *Basal levels of cytosolic calcium in adipocytes of normal, CRF, CRF-PTX, CRF-V and normal-V rats.* Each datum point represents one rat and brackets denote ± 1 SE.

their treatment with verapamil prevented the rise in $[Ca^{2+}]i$ of adipocytes. Indeed, the basal levels of $[Ca^{2+}]i$ in adipocytes from CRF-PTX rats (128 ± 4.7 nM) and in CRF-V (123 ± 7.4 nM) were not different from normal. The treatment of normal rats with verapamil did not alter the $[Ca^{2+}]i$ of their adipocytes (121 ± 3.2 nM).

The ATP content of adipocytes after six weeks of CRF (2.92 ± 0.31 nmol/10^6 cells) was significantly ($P < 0.01$) lower than that observed in normal rats (5.7 ± 0.27 nmol/10^6 cells; Fig. 2). The values of ATP content in the CRF-PTX (5.84 ± 0.39 nmol/10^6 cells), CRF-V (5.30 ± 0.36 nmol/10^6 cells) and normal-V (5.50 ± 0.3 nmol/10^6 cells) rats were not different from those in adipocytes of normal rats.

The values of the V_{max} of Ca^{2+} ATPase are depicted in Figure 3. The activity of this enzyme in the adipocytes from CRF rats (1.96 ± 0.11 μmol Pi/mg protein/hr) was significantly ($P < 0.01$) lower than that in normal animals (2.82 ± 0.16 μmol Pi/mg protein/hr). PTX of CRF rats and their treatment with verapamil prevented the reduction of the V_{max} of the Ca^{2+} ATPase with the values being 3.08 ± 0.21 and 2.95 ± 0.18 μmol Pi/mg protein/hr, respectively. The values in normal-V rats (3.15 ± 0.27 μmol Pi/mg protein/hr) were not different from normal.

Similarly, the activity of Na^+-K^+-ATPase of adipocytes from CRF rats (10.6 ± 0.97 μmol Pi/mg protein/hr) was significantly ($P < 0.01$) lower than that in normal animals (15.7 ± 1.0 μmol Pi/mg protein/hr; Fig. 4). Again the values were normalized by PTX of CRF rats (15.7 ± 1.1 μmol Pi/mg protein/hr) or by their treatment

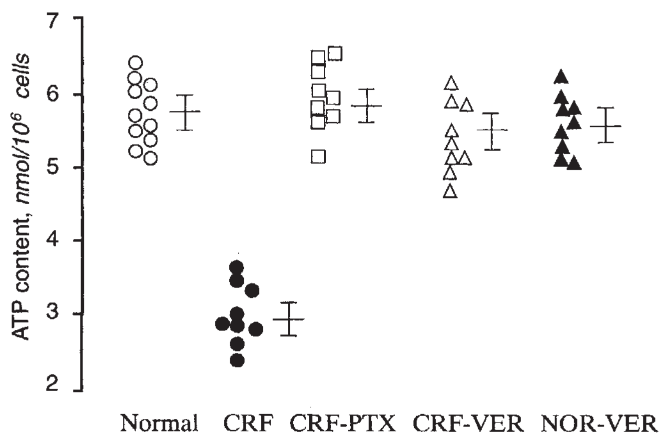

Fig. 2. *ATP content of adipocytes from normal, CRF, CRF-PTX, CRF-V and normal-V rats.* Each datum point represents one rat and brackets denote ± 1 SE.

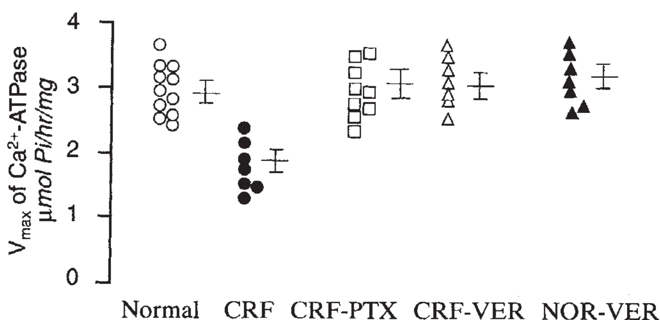

Fig. 3. *The V_{max} of Ca^{2+} ATPase of adipocytes from normal, CRF, CRF-PTX, CRF-V and normal-V rats.* Each datum point represents one rat and brackets denote ± 1 SE.

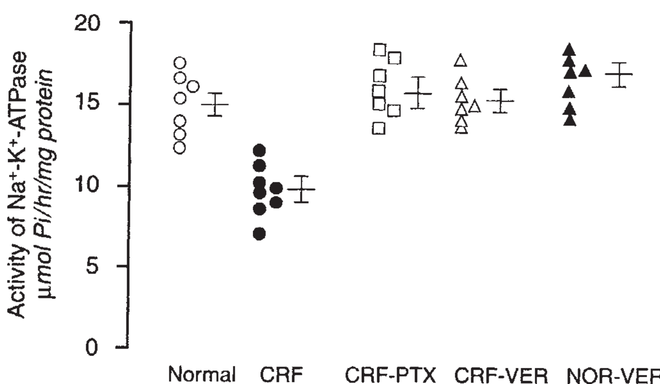

Fig. 4. *The activity of Na^+-K^+-ATPase of adipocytes from normal, CRF, CRF-PTX, CRF-V and normal-V rats.* Each datum point represents one rat and brackets denote ± 1 SE.

with verapamil (14.9 ± 0.92 μmol Pi/mg protein/hr) and values were not different from normal ones.

The Na^+-dependent ^{45}Ca uptakes by adipocytes membrane vesicles in all groups of animals studied are shown in Figures 5 and 6. Only CRF animals displayed significantly lower levels of uptake ($P < 0.01$) during 30 to 120 minutes of the uptake study. The values of the Na^+-Ca^{2+} exchange are shown in Table 2. These values in CRF animals were significantly ($P < 0.01$) lower than in all other groups of rats. The values of Na^+-Ca^{2+} exchange among the normal, CRF-PTX, CRF-V and normal-V were not different.

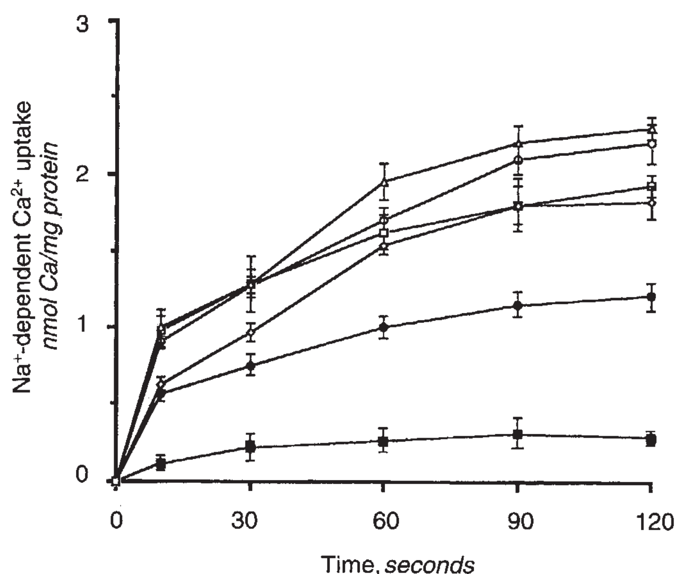

Fig. 5. *Time course of Na^+-dependent ^{45}Ca uptake by membrane vesicles of adipocytes from 10 normal (○), 12 CRF (●), 7 CRF-PTX (□), 9 CRF-V (◇), and 9 normal-V (△) rats.* Each datum point represents the means of -studies. and brackets denote ± 1 SE. The uptake by KCl loaded vesicles (■) is also shown.

Discussion

The data of the present study show that the basal levels of $[Ca^{2+}]i$ of adipocytes from rats with CRF are markedly and significantly higher than values observed in adipocytes of normal animals. Two observations in our study indicate that this derangement is due to the state of secondary hyperparathyroidism of CRF. First, the values of $[Ca^{2+}]i$ in adipocytes of CRF-PTX animals maintained normocalcemic are normal. Second, the treatment of CRF-rats with verapamil, an agent that interferes with the PTH-induced calcium entry into adipocytes [7], also prevented the rise in their $[Ca^{2+}]i$.

Our findings in adipocytes of CRF-rats are similar to previous observations in other cells. Indeed, CRF is associated with a rise in basal levels of $[Ca^{2+}]i$ in pancreatic islets [12], polymorphonuclear leukocytes [23], brain synaptosomes [13], β cells [24], T cells [25], thymocytes [26], platelets [27] and cardiac myocytes [11]. In all these cells, the elevation in their $[Ca^{2+}]i$ was also due to the sustained elevation in the blood levels of PTH. It is evident that the state of secondary hyperparathyroidism of CRF induces a generalized increase of $[Ca^{2+}]i$ of cells and such an abnormality contributes in major part to the organ dysfunction in uremia [14].

Hypertriglyceridemia and fat intolerance exist in CRF humans [1–3, 5] and animals [4, 6]. This abnormality is in major part due to reduced peripheral clearance of triglycerides secondary to impaired lipolytic activity [4, 6]. Akmal et al [6] showed that PTX or verapamil therapy of CRF rats prevented the derangements in their triglyceride metabolism. It is, therefore, reasonable to propose that the elevation in the basal levels of $[Ca^{2+}]i$ of adipocytes interferes with the synthesis and/or release of their lipoprotein lipase and, thus, contribute to the reduced peripheral clearance of triglycerides.

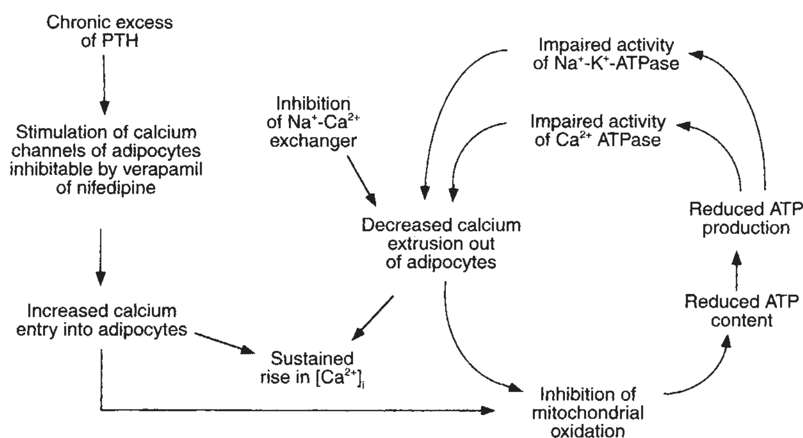

Fig. 6. *A schematic presentation of the sequence of events that lead to the elevation in [Ca^{2+}]i of adipocytes.* All steps have been tested in the present study or in a previous study [7] from our laboratory. It should be noted that treatment of CRF animals with verapamil, by blocking the continued PTH-mediated entry of calcium into the adipocytes, removes the mechanism that leads to inhibition of mitochondrial oxidation. Consequently, the ATP production returns to normal. This figure does not imply that the reduction in ATP affects the Na^+-Ca^{2+} exchanger, and the mechanism for the inhibition of this exchanger is discussed in the text.

Table 2. Na^+-Ca^{2+} exchange by plasma vesicles of adipocytes from normal, CRF, CRF-PTX, CRF-V, and normal-V rats

		Na^+-Ca^{2+} exchange *nmol Ca/mg/protein*	
	N	30 seconds	90 seconds
Normal	10	1.07 ± 0.05	1.74 ± 0.11
CRF	12	0.56 ± 0.05[a]	0.86 ± 0.04[a]
CRF-PTX	7	1.09 ± 0.09	1.58 ± 0.11
CRF-V	9	0.79 ± 0.05	1.63 ± 0.09
Normal-V	9	1.18 ± 0.10	1.80 ± 0.11

Data are presented as mean ± 1 SE.
[a] $P < 0.01$ vs. other groups

PTH increases calcium entry into adipocytes in a dose dependent manner [7], and this action of the hormone could be responsible for the elevation in [Ca^{2+}]i of adipocytes when the cells are exposed to chronic excess of PTH as in CRF. However, augmented entry of calcium into cells is not adequate to cause a sustained rise in their [Ca^{2+}]i since cells are endowed with powerful pumps that permits the extrusion of excess calcium out to the cells [28]. Three processes participate in pumping calcium out of cells. These are Ca^{2+} ATPase, Na^+-Ca^{2+} exchange and Na^+-K^+-ATPase. Our data show that activity of all these pumps is reduced in CRF. Thus, it appears that both increased influx into and decreased efflux out of the adipocytes are responsible for the rise in basal levels of their [Ca^{2+}]i.

Chronic and sustained entry of calcium into cells is associated with inhibition of mitochondrial oxidation, and hence reduced ATP production [29–31]. Indeed, our data show the ATP content of adipocytes from CRF rats is significantly lower than that of adipocytes from normal rats. Both Ca^{2+} ATPase and Na^+-K^+ requires ATP [28, 32] and the fall in ATP content of adipocytes is, at least partly, responsible for the decreased activity of this enzyme.

It could be argued that the affinity of Na^+-K^+-ATPase for ATP is in the micromolar range, and therefore, the decrease in ATP content observed in our study is not adequate to adversely affect the activity of the enzyme. It should be mentioned, however, that Niki et al [32] reported that Na^+-K^+-ATPase activity in HIT-TIS β cells was progressively inhibited as ATP concentration was lowered from 3.0 to 1.5 mM. It is possible that compartmentalization of ATP may exist within adipocytes with the concentration of ATP in the submembrane pool is considerably lower than in other cytosolic pools of ATP. Under such circumstances a decrease in ATP concentration in the vicinity of cell membrane could affect the activity of Na^+-K^+-ATPase and Ca^{2+} ATPase.

One should also consider that other consequences of CRF or alterations in cell membrane, such as derangements in their phospholipids content, may also contribute to the impaired activity of these enzymes. Indeed, phospholipids of cell membrane are reduced in CRF [33].

The changes in the activity of Na^+-K^+-ATPase and Ca^{2+} ATPase could be due to a reduction in the number of enzyme units per cell, in the activity of each enzyme unit or both. Our data do not differentiate between these alternatives. However, the data of Greiber et al [34] in adipocytes of CRF rats is consistent with the inhibition of the activity of Na^+-K^+-ATPase. They found that in adipocytes of CRF rats, the intracellular sodium is increased, ouabain-binding is reduced but immunoreactive α and β subunits of Na^+-K^+-ATPase are not depressed.

It is apparent from the above discussion that the initial event leading to the rise in [Ca^{2+}]i is the PTH-induced entry of calcium in the adipocytes. This action would then trigger other cellular events that lead to a decrease in calcium extrusion. This sequence of events is presented schematically in Figure 6. This formulation is supported by the findings that both PTX of CRF rats which abolishes the state of secondary hyperparathyroidism or their treatment with verapamil, which blocks the PTH-mediated entry of calcium into cells, prevented the reduction in ATP content of adipocytes and the impairment in the activity of the pumps responsible for the calcium extrusion out of these cells. Thus, neither PTH *per se* (CRF-V rats) nor CRF *per se* (CRF-PTX rats) generate the abnormalities in ATP and in the activity of Ca^{2+} ATPase, Na^+-K^+-ATPase and Na^+-Ca^{2+} exchange.

The factors that may modulate the Na^+-Ca^{2+} exchange of adipocytes are not well understood. It is possible that CRF or a metabolic consequence of CRF is associated with inhibition of the activity of the Na^+-H^+ antiport, an event that would result in a decreased entry of sodium into adipocytes and hence a decrease in Na^+-Ca^{2+} exchange. Indeed, the activity of Na^+-H^+ antiport of thymocytes from CRF animals is impaired [35] and PTH inhibits the Na^+-H^+ activity of renal cells [36] and hepatocytes [37]. Fraser and Sarnacki [38] showed that the inosital 1,4,5-triphosphate (IP_3) inhibited Na^+-Ca^{2+} exchange in brain synaptosomes. If PTH stimulates IP_3 production in adipocytes, one may expect that the generated IP_3 would lead to inhibition of the Na^+-Ca^{2+} exchanger. Whatever the mechanisms underlying the impairment

in Na^+-Ca^{2+} exchange in CRF, it must be mentioned that a similar abnormality was reported in cardiac myocytes [11] and in hepatocytes [39] from rats with CRF.

The results of the present study may have important clinical implications. The prevention or reversal of the elevation in [Ca^{2+}]i adipocytes in CRF may be associated with improvement in the synthesis and/or release of lipases with consequent amelioration of the hyperlipidemia of CRF. Indeed, Akmal et al [6] showed that PTX of CRF rats or their treatment with verapamil was followed by reversal of the increased calcium content of liver and epidiymal fat, normalization of the post-heparin lipolytic activity in plasma, hepatic lipase activity in liver homogenate, removal of intravenous lipid load and consequently the hyperlipidemia. Thus, our data and those of Akmal et al [6] provide the scientific rational for the examination of the effects of therapy with calcium channel blocker on the hyperlipidemia of patients with CRF.

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References

1. Chan MK, Varghese Z, Moorhead JF: Lipid abnormalities in uremia, dialysis and transplantation. *Kidney Int* 19:625–637, 1981
2. Appel G: Lipid abnormalities in renal disease. *Kidney Int* 39:169–183, 1991
3. Attman P-O, Samuelsson O, Alaupovic P: Lipoprotein metabolism and renal failure. *Am J Kid Dis* 21:573–592, 1993
4. Akmal M, Kasim SE, Soliman AR, Massry SG: Excess parathyroid hormone adversely affects lipid metabolism in chronic renal failure. *Kidney Int* 37:854–858, 1990
5. Russel GI, Davies JG, Walls J: Evaluation of the intravenous fat tolerance test in chronic renal disease. *Clin Nephrol* 13:282–286, 1980
6. Akmal M, Perkins S, Kasim SE, Oh H-Y, Smogorzewski M, Massry SG: Verapamil prevents chronic renal failure induced abnormalities in lipid metabolism. *Am J Kid Dis* 22:158–163, 1993
7. Ni Z, Smogorzewski M, Massry SG: Effects of PTH on [Ca^{2+}]i of rats adipocytes. *Endocrinology* 135:1837–1844, 1994
8. El-Belbessi S, Brautbar N, Anderson K, Campese VM, Massry SG: Effect of chronic renal failure on heart: Role of secondary hyperparathyroidism. *Am J Nephrol* 6:369–375, 1986
9. Fadda GZ, Akmal M, Premdas FH, Lipson LG, Massry SG: Insulin release from pancreatic islets: Effects of CRF and excess PTH. *Kidney Int* 33:1066–1072, 1988
10. Smogorzewski M, Campese VM, Massry SG: Abnormal norepinephrine uptake and release in brain synaptosomes in chronic renal failure. *Kidney Int* 36:458–465, 1989
11. Zhang Y-B, Smogorzewski M, Massry SG: Altered cytosolic calcium homeostasis in rat cardiac myocytes in CRF. *Kidney Int* 45:1113–1119, 1994
12. Fadda GZ, Hajjar SM, Perna AF, Zhou X-Y, Lipson LG, Massry SG: On the mechanism of impaired insulin secretion in chronic renal failure. *J Clin Invest* 87:255–261, 1991
13. Smogorzewski M, Koureta P, Fadda GZ, Perna AF, Massry SG: Chronic parathyroid hormone excess in vivo increases resting levels of cytosolic calcium in brain synatposomes: Studies in the presence and absence of chronic renal failure. *JASN* 1:1162–1168, 1991
14. Massry SG, Smogorzewski M: Mechanisms through which parathyroid hormone mediates its deleterious effects on organ function in uremia. *Semin Nephrol* 14:219–231, 1994
15. Rodbell M: Metabolism of isolated fat cells. *J Biol Chem* 239:375–380, 1964
16. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1984
17. Lundin A, Hasenson M, Persson J, Pousette A: Estimation of biomass in growing cell line by adenosine triphosphate assay. *Meth Enzymol* 133:27–43, 1986
18. Resh MD: Quantitation and characterization of the (Na^+,K^+)-adenosine triphosphatase in the rat adipocytes plasma membrane. *J Biol Chem* 257:11946–11952, 1982
19. Pierce GN, Dhallo NS: Cardiac myofibrillar ATPase activity in diabetic rats. *J Med Cell Cardiol* 13:1063–1069, 1981
20. Jarett L: Subcellular fractionation of adipocytes. *Meth Enzymol* 174:60–71, 1987
21. Bersohn MM, Vemuri R, Schuil DW, Weiss RS, Philipson KD: Effect of temperature on sodium-calcium exchange in sarcolemma from mammalian and amphibian hearts. *Biochim Biophys Acta* 1062: 19–23, 1991
22. Bradford M: A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–259, 1976
23. Alexiewicz JM, Smogorzewski M, Fadda GZ, Massry SG: Impaired phagocytosis in dialysis patients: Studies on mechanisms. *Am J Nephrol* 11:102–111, 1992
24. Gaciong Z, Alexiewicz JM, Linker-Israeli M, Shulman IA, Pitts TO, Massry SG: Inhibition of immunoglobulin production by parathyroid hormone: Implications in chronic renal failure. *Kidney Int* 40:96–106, 1991
25. Alexiewicz JM, Gaciong Z, Klinger M, Linker-Israeli M, Pitts TO, Massry SG: Evidence of impaired T cell function in hemodialysis patients: Potential role for secondary hyperparathyroidism. *Am J Nephrol* 10:495–501, 1990
26. Stojceva-Taneva O, Smogorzewski M, Fadda GZ, Massry SG: Elevated basal levels of cytosolic calcium of the myocytes in chronic renal failure. *Am J Nephrol* 13:155–159, 1993
27. Moosa A, Greaves M, Brown CB, MacNeill S: Elevated platelet free calcium in uremia. *Br J Haematol* 74:300–305, 1990
28. Carafoli E: Calcium pumps of the plasma membrane. *Physiol Rev* 71:129–153, 1991
29. Baczynski R, Massry SG, Kohan R, Saglikes Y, Brautbar N: Effect of parathyroid hormone on myocardial energy metabolism in the rat. *Kidney Int* 27:618–725, 1985
30. Denton RM, McCormack JG: Ca^{2+} transport by mammilian mitochondria and its role in hormone action. *Am J Physiol* 299:E543–E554, 1985
31. Trump BE, Berezski IF: The role of ion deregulation in toxic cell injury. *Adv Modern Env Tox* 14:27–50, 1987
32. Niki I, Ashcroft SJ: The dependence on intracellular ATP concentration of ATP-sensitive K-channels and of Na-K-ATPase in intact HIT-T15 beta cells. *FEBS Lett* 257:361–364, 1989
33. Islam A, Smogorzewski M, Massry SG: Effect of Chronic failure and paratyroid hormone on phospholipid content of brain synaptosomes. *Am J Physiol* 256:F705–F710, 1989
34. Greiber S, England BK, Price SR, Medford RM, Ebb RG, Mitch WE: Na pump defects in chronic uremia cannot be attributed to changes in Na-K-APTase mRNA or protein. *Am J Physiol* 266 (Renal Fluid Electrol Physiol 35):F536–F542, 1994
35. Greiber S, O'Neill WC, Mitch WE: Impaired ion transport in uremia: Changes in proton and cation transport in rat thymocytes. (abstract) *JASN* 1:629, 1990
36. Sasaki S, Marumo F: Mechanisms of inhibition of proximal acidification by PTH. *Am J Physiol* 260:F833–F383, 1991
37. Michnowska M, Smogorzewski M, Klin M, Massry SG: PTH and hepatocytes pHi and Na^+-H^+ antiport. (abstract) *JASN* 5:885, 1994
38. Fraser CL, Sarnacki P: Inositol 1,4,5-triphosphate may regulate rat brain Ca^{++} by inhibiting membrane bound Na^+-Ca^{++} exchanger. *J Clin Invest* 86:2169–2173, 1990
39. Klin M, Smogorzewski M, Massry SG: Elevated cytosolic calcium of hepatocytes in chronic renal failure (CRF). (abstract) *JASN* 5:886, 1994