Effects of Weight Reduction on Cellular Cation Metabolism and Vascular Resistance


The early stages of weight loss are associated with a reduction in blood pressure, and the mechanisms mediating this reduction remain unclear. Platelet free calcium levels, \([\text{Ca}^{2+}]_{j}\), have been reported to be elevated in essential hypertension and to decrease with pharmacological treatment of the hypertension. In the current study, 18 obese subjects had measurements of blood pressure, forearm blood flow, forearm vascular resistance, and both basal platelet \([\text{Ca}^{2+}]_{j}\), and \([\text{Ca}^{2+}]_{j}\), responses to vasopressin during 12 weeks on a very low calorie (3,360 kJ, or 800 kcal) diet. Weight reduction was associated with reduction in mean arterial blood pressure at 3–4 weeks. There were associated reductions in forearm vascular resistance and platelet \([\text{Ca}^{2+}]_{j}\), as well as increases in forearm blood flow at 3–4 weeks of the diet. Increased forearm blood flow was correlated with weight loss. Vasopressin-induced platelet \([\text{Ca}^{2+}]_{j}\), responses increased, which correlated with the reduction in mean arterial pressure at 7–8 weeks of weight loss. Assuming that platelet \([\text{Ca}^{2+}]_{j}\), metabolism reflects vascular smooth muscle cell \([\text{Ca}^{2+}]_{j}\), metabolism, the data suggest that blood pressure reduction after weight loss may be related to reduced vascular smooth muscle cell \([\text{Ca}^{2+}]_{j}\). The reason for the increased vasopressin-induced \([\text{Ca}^{2+}]_{j}\), after weight reduction is unclear. (Hypertension 1993;21:308–314)

KEY WORDS • weight loss • vascular resistance • calcium

Weight loss after food restriction is associated with a reduction in blood pressure independent of effects of salt restriction.1–11 This reduction in blood pressure in association with caloric restriction is associated with a reduction in plasma renin activity4–8 and sympathetic nervous system activity.5–8 The renin-angiotensin system and the sympathetic nervous system affect blood pressure by increasing peripheral vascular resistance,12,13 as well as by promoting renal reabsorption of sodium and water and by interfering with the pressure-natriuresis response.14,15 Finally, the enhanced sympathetic nervous system activity seen in obesity16 may increase blood pressure through trophic effects on blood vessels that render them more responsive to vasoconstrictor stimuli.17 Results from several studies have suggested that hypertension associated with obesity is characterized by abnormalities in cell cation (e.g., calcium and sodium) metabolism and that weight loss and associated reductions in blood pressure are accompanied by corrections of these cell cation abnormalities.6,11,18 Vascular smooth muscle contraction is stimulated by increased intracellular free calcium ([Ca$^{2+}$]$_{i}$),19 and hypertensive states are often associated with increased levels of [Ca$^{2+}$]$_{j}$.20–24 Similarly, elevations in [Ca$^{2+}$]$_{j}$ have also been demonstrated in platelets from hypertensive individuals.19–24 Thus, platelets have been used as surrogates for vascular smooth muscle cells, as both cell types have similar Ca$^{2+}$ regulatory systems24–26: both share an $\alpha_{2}$-adrenergic receptor–cyclase system25 and a Ca$^{2+}$-dependent contraction-coupling mechanism.26 Accordingly, the present study was designed to evaluate the effects of weight loss on blood pressure, peripheral vascular resistance, and platelet [Ca$^{2+}$], metabolism in 18 obese subjects.

Methods

Eighteen subjects undergoing a weight-reduction program at Wayne State University were included in this study after we obtained approval from the institutional review committee and informed consent from the subjects, in accordance with our institutional guidelines. Potential subjects (>30% of calculated ideal body weight) were excluded if they had insulin-dependent diabetes mellitus, cardiac disease, or severe hypertension (systolic blood pressure >180 mm Hg, diastolic >114 mm Hg) or were taking either oral hypoglycemic or antihypertensive agents. Clinical characteristics of the study population are given in Table 1. Hemodynamic and biochemical determinations, as described below, were made before subjects began a very low calorie (3,360 kJ, or 800 kcal) liquid diet (VLCD) (Optifast, Delmar, Minn.) and at 3–4 weeks, 7–8 weeks,
and 11–12 weeks after the diet was begun. All subjects were monitored by serial measurements of potassium, carbon dioxide content, and other parameters as previously described.1-8

Measurements of Hemodynamics and Peripheral Vascular Resistance

Blood pressure was measured in the right arm positioned at heart level using a large cuff and a standard mercury column sphygmomanometer. Values of three recordings taken at 1-minute intervals with subjects in the sitting position were reported as mean±SEM. Forearm blood flow (FBF) and forearm vascular resistance (FVR) were measured by electrical impedance plethysmography (IPG-104, RJL Systems, Detroit, Mich.) as previously described.27-29 Briefly, to measure FBF, we placed two detecting electrodes 10 cm apart centered on the right forearm and two signal electrodes approximately 20 cm proximal and distal to the area being measured. FBF was then quantified from the resulting tracings using the back slope projection method of Nyboer.30 FVR was calculated by dividing the mean arterial pressure (MAP) by FBF. FBF determined by this method exhibits an intraindividual coefficient of variation of less than 3% in our laboratory.29

Measurements of Platelet \([Ca^{2+}]_i\) and \([Ca^{2+}]_o\), Responses to Arginine Vasopressin

Platelet \([Ca^{2+}]_i\) was measured from blood samples (=15 mL each) that were collected in EDTA-treated Vacutainer tubes and centrifuged at 200g for 15 minutes at 21°C for the determination of platelet \([Ca^{2+}]_i\) levels as previously described.30 Briefly, platelet-rich plasma was incubated in 3 \(\mu\)mol fura-2-acetoxymethyl ester (fura-2) per liter at 37°C for 30 minutes and centrifuged at 650g for 10 minutes at 21°C; the plasma and extracellular fura-2 were removed by aspiration. The platelet pellet was then suspended in a calcium-free HEPES (10 mM) buffer (pH 7.4) and centrifuged at 650g; the platelets were suspended at a concentration of \(1 \times 10^7\) cells/mL in HEPES (10 mM) containing calcium (1.5 mM). We have previously found that this method of preparing platelets by differential centrifugation yields results indistinguishable from those obtained in platelets purified by gel filtration.30 \([Ca^{2+}]_i\) was measured in a model 1680 dual excitation monochromator spectrofluorometer (SPex Industries, Edison, N.J.) with excitation wavelengths of 340 and 380 nm, an emission wavelength of 505 nm, and entry and exit slits set at 3 nm. The response of \([Ca^{2+}]_i\), to arginine vasopressin (AVP) was determined by measuring the peak response to 9 \(\mu\)mol AVP per liter, after a stable baseline value for \([Ca^{2+}]_i\), had been established before AVP was added. The response to AVP is expressed as the percent increase over the baseline \([Ca^{2+}]_i\). All measurements were made in a water jacketed cuvette at 37°C, with continuous stirring. \([Ca^{2+}]_i\) was calculated from the ratio of fluorescence at 340 nm to that at 380 nm, as described by Grynkiewicz et al.31; the maximal ratio was obtained with 0.7% Triton X-100 and the minimal ratio with 5 mM EGTA-Tris (pH 8.6).31

Statistical Analysis

Statistical time-related changes in hemodynamic and biochemical parameters were evaluated by repeated-measures analysis of variance after it was verified that the data were normally distributed. Multiple regression analysis was used to calculate coefficients of correlation among various parameters. When appropriate, the significance of differences was also assessed using Student’s t test. All values are expressed as mean±SEM (with number of observations in parentheses); values of \(p<0.05\) were considered significant.

Results

Table 1 gives the baseline clinical, hemodynamic, and biochemical data for the 18 obese subjects in this study. The study population included 11 blacks and seven whites, 12 women and six men. The subjects were all more than 30% of ideal body weight (74.3±4.1%). Body mass index for the 18 subjects was 37.2±2.9 kg/m², and collectively they exhibited borderline hypertension as...
Figure 1. Line plots show weight loss over time in study subjects on 3,360-kJ (800-kcal) diet (panel A) and effect of weight loss on mean arterial pressure (panel B). The mean of triplicate measurements was used for calculation of mean arterial pressure. Each data point is the mean±SEM of the study group (n=18 in weeks 0–8; n=10 in weeks 11–12). *p<0.05, **p<0.01.

Results of FVR and FBF measurements are illustrated in Figures 3A and 3B. Similar to our observations of MAP, FVR was also reduced in subjects during the 12-week study (Figure 3A). After 3–4 weeks of the VLCD, FVR decreased by 7.1 mm Hg/(mL/100 mL per minute) (p<0.01, n=18). The reduction in FVR was even more pronounced after 7–8 weeks (week 0, 34±2.0; week 7–8, 22±1.2 mm Hg/(mL/100 mL per minute); p<0.001, n=18). However, although FVR tended to continue to decrease at 11–12 weeks (n=10), it was not significantly different than FVR at 7–8 weeks.

As FVR decreased in subjects during the VLCD (Figure 3A), a concomitant increase in FBF was observed (Figure 3B). After 7–8 weeks, FBF increased by 54±3% (p<0.001). Although FBF tended to continue to increase at week 11–12 (57±4%), it did not reach a level of significance beyond the FBF observed at week 7–8 (week 0, 2.8±0.2; week 7–8, 4.3±0.2; week 11–12, 4.4±0.2 mL/100 mL per minute; p<0.01).

Changes in FBF were negatively correlated with changes in FVR at 7–8 weeks (r=-0.58, p<0.02), as would be anticipated (data not shown). There was also a significant correlation (r=0.58, p<0.03) between weight loss and the change in FBF observed at weeks 7–8 (Figure 4A). However, no significant correlations were observed between changes in either FVR or FBF and any of the other parameters measured during the 12-week VLCD.
Figure 3. Line plots show effect of weight loss over time on forearm vascular resistance (panel A) and peripheral blood flow (forearm blood flow) (panel B) in study subjects on 3,360-kJ (800-kcal) diet. Results were determined from measurements of electrical impedance plethysmography, calculated as described in "Methods." The mean of triplicate measurements was used in data analysis. Each data point is the mean±SEM of the study group (n=18 in weeks 0–8; n=10 in weeks 11–12). *p<0.05, **p<0.01, ***p<0.001.

Results of baseline platelet [Ca^{2+}] and AVP-stimulated [Ca^{2+}] responses over the duration of the VLCD are illustrated in Figures 5A and 5B. After 3–4 weeks of the VLCD, basal platelet [Ca^{2+}], decreased from 38.1±2.8 to 18.9±2.1 nM (n=18, p<0.05) (Figure 5A), whereas AVP-stimulated [Ca^{2+}] actually increased two-fold compared with baseline (n=18, p<0.01) (Figure 5B). Compared with baseline [Ca^{2+}], there was both an additional reduction in platelet [Ca^{2+}], (16.7±1.6 nM, p<0.01) and a further increase in AVP-stimulated [Ca^{2+}], (n=18, p<0.001) after 7–8 weeks of the VLCD. However, there were no additional significant changes in baseline or AVP-stimulated [Ca^{2+}], in the 10 remaining subjects after 11–12 weeks of the VLCD. Neither baseline [Ca^{2+}], nor the changes in baseline [Ca^{2+}], observed during the 12-week VLCD correlated with the observed changes in blood pressure or other hemodynamic parameters. However, there was a correlation between the mean decrease in MAP and the mean increase in AVP-stimulated [Ca^{2+}], responses at 7–8 weeks of weight reduction (r=0.512, n=18, p<0.04) (Figure 4B).

Discussion

These data indicate that the weight loss–induced reduction in blood pressure in obese individuals is accompanied by significant decreases in platelet [Ca^{2+}]. These data are consistent with the observations of Scherrer et al, who recently reported that platelet [Ca^{2+}], decreased in obese subjects after 10 weeks of dieting. Our results indicate that reduction in platelet [Ca^{2+}], occurs within the first month of weight loss and continues to decrease throughout a 12-week VLCD. In the study by Scherrer et al, only those subjects who had a change in body mass index of more than 5% were included in the various parameters analyzed, including the platelet [Ca^{2+}]. Our results indicated that even small reductions in weight (changes in body mass index <5%) are associated with reduced platelet [Ca^{2+}]. The levels of baseline platelet [Ca^{2+}], observed in our study (38.1±2.8 nM) were lower than those we had observed in our previous study with young nonobese individuals. Thus, it is possible that these levels change with age, adiposity, or both. These baseline levels were also lower than those observed by Scherrer et al in obese subjects (165 nM). There are several possible explanations for these differences. In our study, we used the dye indicator fura-2, whereas Scherrer et al used the quin-2 method. Differences in auto-fluorescence, degree of quenching, dye leakage, and reproducibility of results suggest that the fura-2 method is preferable. A number of other factors, such...
as the lag time between collection of blood and dye loading of platelets, increased incubation time in Ca\(^{2+}\)-supplemented media, and platelet activation and titratable dye leakage from platelets, can all lead to factiously “high” [Ca\(^{2+}\)], determinations.\(^{30}\) In general, increased caution and awareness of these factors have resulted in increasingly lower values being reported for platelet [Ca\(^{2+}\)], levels over the past decade.\(^{20,24,30}\)

Our observations, in addition to those of Scherrer et al.,\(^{11}\) suggest that nonpharmacological reduction of blood pressure can decrease platelet [Ca\(^{2+}\)]. There tended to be a correlation \((p=0.06)\) between baseline platelet [Ca\(^{2+}\)], and systolic blood pressure, but no such relation was noted between platelet [Ca\(^{2+}\)], and diastolic blood pressure or MAP. The absence of a direct correlation between the level of diastolic pressure and platelet [Ca\(^{2+}\)], is in agreement with the observations of Lechi et al.\(^{21}\) but not others.\(^{20,23}\) Unlike Scherrer et al.,\(^{11}\) who showed a correlation between the decreases in platelet [Ca\(^{2+}\)], and diastolic blood pressure at 10 weeks of weight-reduction, we observed no such relation between these parameters at 3-4, 7-8, or 11-12 weeks after the weight-reduction regimen was begun. Similarly, we observed no such relation between reductions in platelet [Ca\(^{2+}\)], and reductions in FVR or increases in FBF. The relatively small decrease in blood pressure associated with weight loss in this study may have accounted for the lack of correlation between platelet [Ca\(^{2+}\)], and blood pressure. Perhaps studying more subjects with a greater decrease in blood pressure would uncover such relations. However, this does not explain the positive findings of Scherrer et al.,\(^{11}\) who studied a similar number of subjects \((n=19)\) at only one time point \((10\) weeks) after onset of weight reduction. Again, it should be noted that Scherrer et al divided their study population into responders and nonresponders, whereas the present results reflect the entire population studied.

The mechanism involved in the fall in baseline platelet [Ca\(^{2+}\)], associated with weight reduction and the accompanying decrease in blood pressure is unclear. It has been suggested\(^{11}\) that a fall in circulating catecholamines associated with weight loss\(^{5-11}\) could contribute to a fall in platelet [Ca\(^{2+}\)], by reducing the stimulation of platelet \(\alpha\)-adrenergic receptors that mediate an increase in [Ca\(^{2+}\)].\(^{25,26}\) Platelets also manifest angiotensin II receptors,\(^{35}\) and angiotensin II stimulates platelet [Ca\(^{2+}\)], responses.\(^{36}\) Plasma renin substrate and plasma renin activity in obese subjects have been shown to decrease within the first 2 months of weight reduction with a dietary regimen similar to that used in the current study.\(^{4-8}\) Thus, it is possible that the reduction in platelet [Ca\(^{2+}\)], may result, in part, from decreased angiotensin II-mediated effects on platelet [Ca\(^{2+}\)].\(^{11}\) It has also been suggested that diet-induced reductions in low density lipoprotein cholesterol may contribute to the decrease in platelet [Ca\(^{2+}\)].\(^{11}\) This is plausible, as the lipid content of cellular membranes is an important determinant of cellular Ca\(^{2+}\) homeostasis,\(^{37,38}\) and low density lipoprotein cholesterol, per se, has been demonstrated to increase platelet [Ca\(^{2+}\)].\(^{39,40}\) It is also possible that blood pressure, per se, is a determinant of platelet [Ca\(^{2+}\)], and that the reduction in blood pressure associated with weight reduction causes a decrease in [Ca\(^{2+}\)] through effects on platelet aggregation, endothelial function, or other mechanisms.

Our observation that the AVP-stimulated platelet [Ca\(^{2+}\)], responses actually increased in parallel with the reduction in baseline [Ca\(^{2+}\)], was somewhat surprising. Plasma AVP levels were not measured in these individuals. However, one could speculate that if plasma AVP levels decline with weight loss, the platelet sensitivity to AVP might increase. Thus, we can only speculate on the reciprocal relation between baseline and agonist-stimulated platelet [Ca\(^{2+}\)], responses. Because AVP-induced platelet [Ca\(^{2+}\)], responses are dependent on both mobilization of intracellular Ca\(^{2+}\) stores and transmembrane flux of extracellular Ca\(^{2+}\), it is possible that increasing basal levels of platelet [Ca\(^{2+}\)] inhibits one of these processes. In contrast to vascular smooth muscle cells,\(^{2,24}\) the transmembrane flux of extracellular Ca\(^{2+}\) is an important mechanism in AVP-induced increases in platelet [Ca\(^{2+}\)].\(^{20}\) so we speculate that increasing basal levels of platelet [Ca\(^{2+}\)], may inhibit this process. Increased platelet [Ca\(^{2+}\)], may exhibit “Ca\(^{2+}\) channel blocking” properties. We did not measure circulating divalent cation levels with weight loss in this study. However, because physiological concentrations of extracellular Mg\(^{2+}\) may also be an important determinant for AVP-induced platelet responses, it is possible that changes in serum Mg\(^{2+}\) concentrations may help to
explain this increase in AVP-induced [Ca\(^{2+}\)], rise with weight reduction.\(^{2,24}\) However, the reason for the greater AVP-stimulated [Ca\(^{2+}\)] responses after weight loss remains unclear.

It is interesting that increases in AVP-induced platelet [Ca\(^{2+}\)] responses did correlate with decreases in MAP after 2 months of weight reduction in these obese subjects. This observation suggests that changes in AVP-stimulated platelet [Ca\(^{2+}\)] may be a more sensitive reflection of the relation between changes in platelet Ca\(^{2+}\) metabolism and changes in peripheral vascular resistance. More prospective studies including larger numbers of subjects with greater lowering of blood pressure may be necessary to clarify this issue.

This is the first study to prospectively evaluate the effects of weight reduction in obese subjects on peripheral vascular resistance and peripheral blood flow. Our data indicate that peripheral vascular resistance was markedly reduced, with accompanying increases in blood flow, within 3–4 weeks of the 12-week weight-reduction program. A strong correlation was observed between reductions in body weight and increases in peripheral blood flow, but not vascular resistance on blood pressure. Because vascular resistance is derived from peripheral blood flow and blood pressure measurements, our data suggest that measurement of peripheral blood flow is a more sensitive measure than blood pressure in determining changes in hemodynamics accompanying weight reduction.

Our studies do not rule out the importance of a reduction of cardiac output with weight reduction\(^{44}\) as another factor involved in the lowering of blood pressure in obese individuals undergoing a weight-loss program. Thus, our data suggest that a reduction in peripheral vascular resistance is an important hemodynamic mechanism involved in blood pressure reduction associated with weight reduction in obese individuals.

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