Plasma Fatty Acids Response to Central Volume Expansion in Salt-Sensitive Hypertension

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The purpose of the present study was to investigate the possible regulation of plasma fatty acids by an acute isotonic-isooncotic central volume expansion. We measured the levels of nonesterified fatty acids (NEFA) in plasma from 12 essential hypertensive patients subjected to water immersion (WI). Central hypervolemia by WI over 2 hours caused the levels of most NEFA to increase, concomitantly with a marked natriuretic and kaliuretic response. With respect to baseline values, serum insulin levels did not change during WI, while there was a profound suppression of plasma renin activity (PRA) and plasma aldosterone. In addition, when individual NEFA percent increase was expressed as a function of salt-sensitivity index (calculated as the change in mean arterial pressure [MAP] divided by the change in urinary sodium excretion rate), a greater percent increase in stearic acid ($r = .72$, $P < .009$), palmitic acid ($r = .83$, $P < .001$), and palmitoleic acid ($r = .58$, $P < .048$) was found during WI in those hypertensive subjects showing higher salt-sensitivity index. Thus, by demonstrating that an acute isotonic-isooncotic volume expansion may induce a significant increase of most NEFA plasma levels, we suggest that volume expansion per se could be included among the well-recognized risk factors for cardiovascular morbid events.

THE SALT-SENSITIVE state of hypertension has been recently identified as an independent cardiovascular risk factor in both normotensive[1] and hypertensive subjects.[2,3] Dyslipidemia, with elevated serum nonesterified fatty acids (NEFA), often coexists with insulin resistance and hyperinsulinemia in subjects with cardiovascular disorder,[4,5] and an association between salt sensitivity and insulin concentration has also been suggested in patients with hypertension.[6,7]

Salt-sensitive individuals are also characterized by a modest degree of extracellular fluid volume (ECFV) expansion,[8] and it has been demonstrated that ECFV expansion by isotonic saline loads may increase plasma concentration of most NEFA in hypertensive subjects.[9]

Whether the mechanism by which isotonic saline infusion affects fatty acids plasma levels is related either to NaCl administration or is directly mediated by volume expansion per se has not been previously elucidated.

Water immersion (WI) to the neck induces an isotonic-isooncotic volume expansion without resorting to exogenous fluid infusion; hemodynamic and humoral effects are similar to those obtained during intravenous (IV) short-term isotonic saline administration.[10]

The aim of our study was to investigate the actual role of central blood volume expansion per se, induced by head-out WI, in mediating changes in the plasma concentration of NEFA in hypertensive subjects evaluated for their salt sensitivity.

MATERIALS AND METHODS

Twelve uncomplicated, hypertensive, but otherwise healthy, subjects (9 men, 3 women) ranging in age from 20 to 46 years, body weight, 74 ± 3 kg, were entered into the study after informed consent had been obtained, and the protocol had been approved by our institutional Ethical Committee.

Each hypertensive subject had an outpatient diastolic blood pressure measurement by conventional sphygmomanometry in excess of 95 mm Hg (seated posture), with the arm in the horizontal position, after 5 minutes of quiet sitting, on at least 3 occasions documented for at least 3 months before the study and had never received any antihypertensive treatment. The patients had no major symptoms or signs of target organ damage, nor did they have other major diseases besides hypertension; renal disease was excluded by documenting a normal urinalysis and creatinine clearance. The study was performed under outpatient conditions.

Experimental Protocol

The patients were instructed to follow a diet of approximately 200 to 220 mmol sodium and 60 mmol potassium daily; this closely paralleled their usual sodium and potassium intake. After complying with this diet for 7 days, the subjects were allowed entry into the study. Blood pressure and 24-hour urinary sodium and potassium were all monitored for the last 3 days of the study period.

WI Study

On day 8, after the completion of the high-salt diet period, each hypertensive subject was submitted to acute central volume expansion by WI that was performed as previously described.[11] Briefly, at 8 AM, after an overnight fast and fluid deprivation (10 hours), an antecubital vein was catheterized for blood sampling; the subjects voided, received 200 mL water to drink, then sat quietly outside the immersion tank for 2 hours at a room temperature between 26°C ± 0.4°C and 27°C ± 0.4°C (preimmersion study). The water load was repeated hourly throughout the study. The subjects then stepped into the immersion tank and sat on an adjustable chair with water to the neck at constant temperature (34°C ± 0.5°C) with their arms outside the tank in the horizontal position; the subjects remained in the tank for 2 hours (immersion study) and stepped out of it at hourly intervals to void urine.

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Blood samples were drawn just before and after the WI test for serum sodium, hematocrit, creatinine, plasma renin activity (PRA), plasma aldosterone (PA), insulin, and plasma NEFA.

The hourly urine volumes were measured and the concentration of sodium, potassium, and creatinine determined.

Both resting and WI arterial blood pressure were measured in the seated posture with an automatic monitor Spacelabs (model 90207; Redmond, WA) with the arm in the same position before and during WI. Plasma samples were stored frozen at –20°C until assayed.

Assessment of Salt Sensitivity

According to a method previously described,[12] the 12 hypertensive patients were given a low-salt diet containing 30 mmol sodium per day for 14 days; during the first 7 days, 190 mmol sodium chloride was added to the daily intake. Total caloric intake was estimated to keep body weight constant; throughout the study, compliance was assessed by monitoring urinary sodium excretion for the last 3 days of each study period.

At 7 AM on days 7 and 14, the patients rested in a quiet room; they had fasted overnight and were hydrated by ingesting 300 mL water/h. Systolic and diastolic blood pressures were measured in seated subjects, after a 30-minute resting period, for 2 hours from 8 AM to 10 AM, at 5-minute intervals, using the previously described oscillometric technique. After the completion of low-salt diet period, blood samples were drawn again for plasma NEFA determination.

As suggested by previous experience,[13] a significant 10% or greater decrease in mean arterial pressure (MAP) (the sum of diastolic and one third pulse pressure), calculated as the difference between the average of readings under the high- or low-salt periods, may be identified as a salt-sensitive response, whereas a reduction in MAP $< 10\%$ is destined as a salt-resistant response. Although this salt-sensitivity classification is reproducible, its definition is variable, and it separates patients into 2 distinct groups.[13,14]

Finally, to assess the continuous distribution of salt sensitivity in our subjects, we used the salt-sensitivity index, validated by comparison with standard techniques for the assessment of cardiovascular risk[3] and calculated as the change in MAP divided by the change in urinary sodium excretion rate, thus evaluating the effect that changes in sodium intake have on blood pressure in each individual subject. WI and salt-sensitivity tests were randomly assigned.

Laboratory Procedures

Plasma NEFA were extracted with the Dole solvent system, as described by Parmelee at al.[15] As reported by Goodfriend et al, [9] the extraction procedure does not cleave triglycerides into measurable fatty acids.

Half milliliter of plasma was added to 3.0 mL of a solvent mixture consisting of 40 vol isopropyl alcohol, 10 vol n-heptane, and 1 vol 1 N sulfuric acid. One milliliter distilled water was then added and the solution mixed for 15 seconds; 1.5 mL n-heptane was added and the solution again mixed for 15 seconds. Two separate phases were obtained, the upper organic phase was then removed, placed in the same type of tube, and the solvent removed at 35°C with a stream of nitrogen.

The dried lipid mixture extracted was mixed with 500 μL of a triethylamine solution (10 mg/mL in acetone) and with 500 μL of an α-bromoacetophenone solution (10 mg/mL in acetone).[16] The derivative fatty acids mixture was maintained overnight at room temperature.

Analyses were performed using a Spectra-Physics P 2000 (Thermo Separation Products, Milan, Italy) high-performance liquid chromatograph (HPLC); 5 μL of the derivate fatty acids mixture was used for analysis. Analyses were performed by gradient elution programmed linearly from 85:15 acetonitrile-water over 25 minutes to 90:10 until the end of the run, using a constant flow-rate of 1 mL/min. At the beginning of every run, the column was equilibrated with phases to 85:15 (acetonitrile-water) for a 10-minute interval.

A Supelco (Milan, Italy) 250 × 4.5 mm column packed with 5 μm octadecyl-bonded spherical silica was used for elution in HPLC; a Supelco 20 × 4.5 mm column guard packed with 40 μm octadecyl-bonded spherical silica was also used. Eluate was scanned at 242 nm with a variable-wavelength ultraviolet detector. The solvents were degassed under vacuum and maintained under 6 psi helium.

The integration was performed by the method of the outside standard: 1.5 μL of a single fatty acid standard (Sigma 99% of purity), solved in acetone, injected before each sample of a given patient. Each fatty acid peak integration was obtained by calculating the areas. Integration phases and calculation of fatty acids concentrations were performed using a Spectra-Physics PC 1000 software.

PRA, PA, and insulin were determined by radioimmunoassay. Sodium and potassium levels were measured in serum and urine by internal standard flame photometry; serum and urinary creatinine levels were determined by a Technicon analyzer (Rome, Italy).

Table 1. Urinary and Hormonal Parameters in 12 Hypertensive Subjects Undergoing WI

Variable	CP	WI
$U_{Na}V$ (μmol/min)	185 ± 25	397 ± 31*
U_KV (μmol/min)	63 ± 10	93 ± 8†
Creatinine clearance (mL/s)	2.14 ± 0.10	2.12 ± 0.08
Insulin (pmol/L)	111 ± 43	97 ± 50
PRA (ng/L/s)	0.47 ± 0.08	0.08 ± 0.03‡
Plasma aldosterone (pmol/L)	1109 ± 139	499 ± 55‡

NOTE. Values are the mean ± SE.
Abbreviations: CP, control period; WI, water immersion.
Significant differences from CP are shown:
*$P < .0001$; †$P < .002$; ‡$P < .001$.

Statistics

Values were given as mean ± SE. Statistical evaluation was performed by Student's t test for paired or unpaired values or, where appropriate, by repeated measures analysis of variance; regression analysis was by Pearson's correlation coefficient. Differences were considered to be significant at $P < .05$.

RESULTS

WI Study

Table 1 shows that the urinary sodium excretion ($U_{Na}V$) during the preimmersion period was 185 ± 25 μmol/min in the 12 hypertensive subjects and reached a peak value of 397 ± 31 μmol/min ($P < .0001$) during the second hour of WI. A significant increase in urinary potassium excretion (U_kV) was also found in this group during WI (from 63 ± 10 to 93 ± 8 μmol/min, $P < .002$).

WI induced a significant decrease of PRA (from 0.47 ± 0.08 to 0.08 ± 0.03 ng/L/s, $P < .001$) and PA (from 1,109 ± 139 to 499 ± 55 pmol/L, $P < .001$).

No significant changes in hematocrit, creatinine clearance (from 2.14 ± 0.10 to 2.12 ± 0.08 mL/s), serum insulin (from 111 ± 43 to 97 ± 50 pmol/L), and MAP (from 110 ± 2 to 111 ± 2 mm Hg) were found during WI.

As compared with baseline values, there was a significant increase in the individual plasma NEFA concentration when the 12 hypertensive subjects underwent WI at high-salt intake.

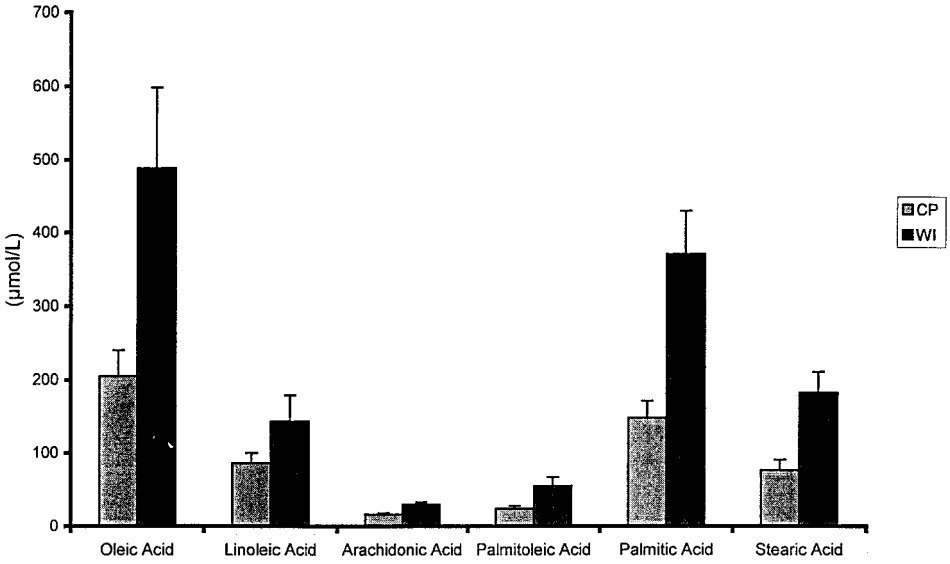

Fig 1. Individual NEFA behavior during WI with respect to control period (CP) in 12 hypertensive subjects.

Among the major plasma NEFA, oleic acid (18:1) increased from 205 ± 35 to 488 ± 110 μmol/L, $P < .009$ (increase of 140%), linoleic acid (18:2 n-6) from 86 ± 14 to 143 ± 36 μmol/L, $P < .04$ (increase of 67%), arachidonic acid (20:4 n-6) from 16 ± 2 to 30 ± 3 μmol/L, $P < .001$ (increase of 80%), palmitoleic acid (16:1) from 24 ± 4 to 55 ± 12 μmol/L, $P < .006$ (increase of 133%), palmitic acid (16:0) from 148 ± 23 to 371 ± 59 μmol/L, $P < .001$ (increase of 150%), and stearic acid (18:0) from 77 ± 14 to 183 ± 28 μmol/L, $P < .001$ (increase of 136%) (Fig 1).

Salt-Sensitivity Test

MAP (from 111 ± 2 to 97 ± 1 mm Hg, $P < .001$) markedly decreased under the low-salt diet in the salt-sensitive subjects, but was only slightly affected in the salt-resistant hypertensives (from 108 ± 2 to 107 ± 2 mm Hg, P = not significant [NS]). In comparison to high-dietary sodium chloride, no significant effect on the individual fatty acids was found in the hypertensive subjects undergoing low-salt intake (data not shown).

When individual NEFA percent increase was expressed as a

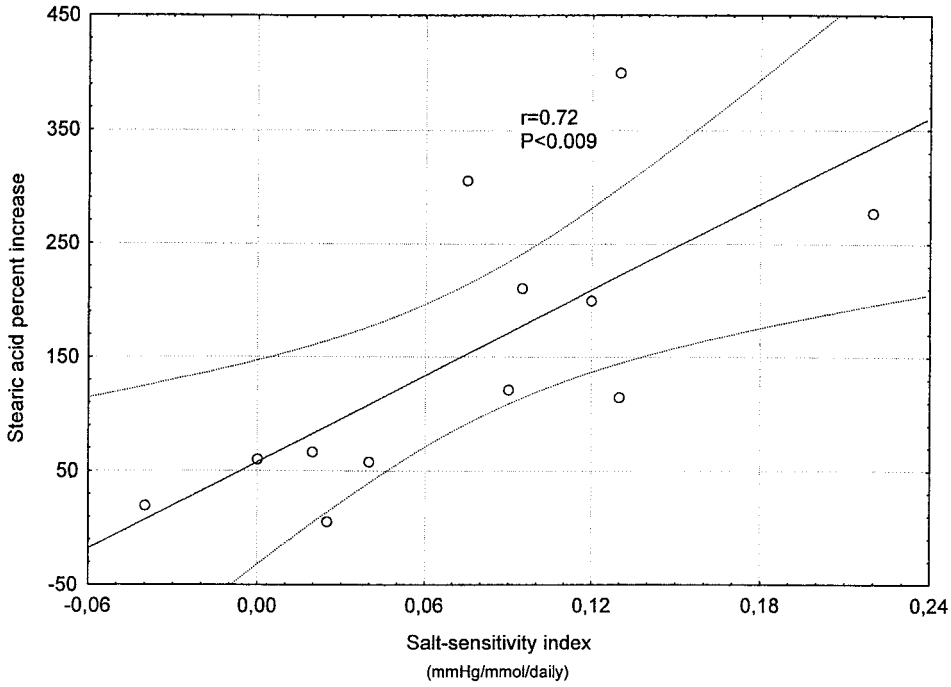

Fig 2. Relationship between percent increase of plasma stearic acid and salt-sensitivity index in 12 hypertensive subjects undergoing WI.

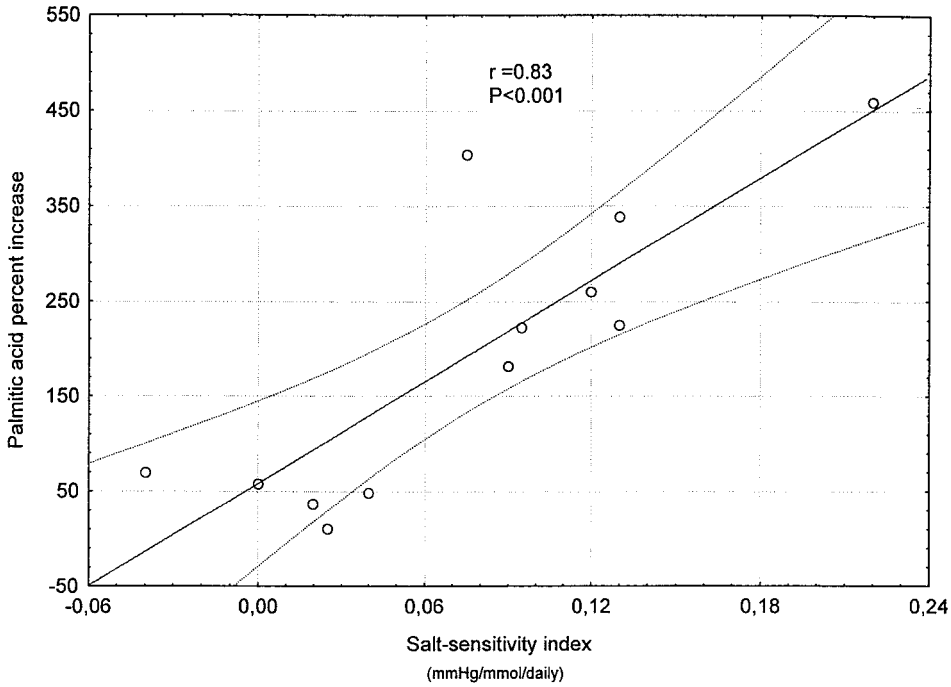

Fig 3. Relationship between percent increase of plasma palmitic acid and salt-sensitivity index in 12 hypertensive subjects undergoing WI.

function of salt sensitivity (quantified by the salt-sensitivity index), a greater percent increase in stearic acid ($r = .72$, $P < .009$) (Fig 2), palmitic acid ($r = .83$, $P < .001$) (Fig 3), and palmitoleic acid ($r = .58$, $P < .048$) (Fig 4) was found during WI in those hypertensives showing a higher salt-sensitivity index.

DISCUSSION

In the experiments described in the present study, human hypertensive subjects responded to an acute central hypervolemia by WI with an increase in urinary sodium excretion and NEFA plasma levels associated with no significant changes in

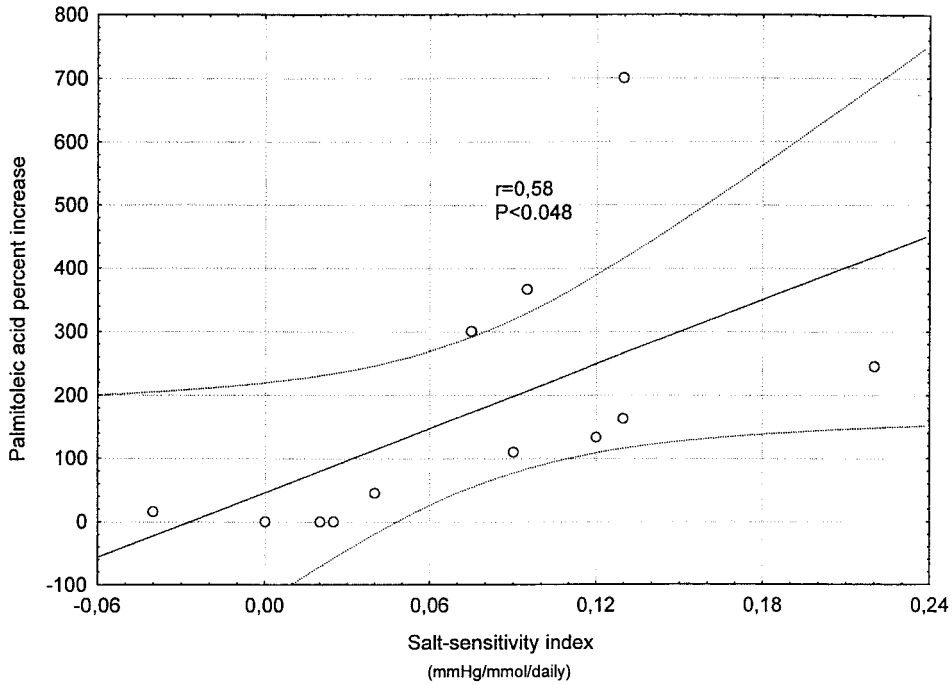

Fig 4. Relationship between percent increase of plasma palmitoleic acid and salt-sensitivity index in 12 hypertensive subjects undergoing WI.

serum insulin and a profound suppression of PRA and PA. This is, to our knowledge, the first report showing that an acute isotonic-isooncotic central volume expansion per se may have a role in modulating plasma NEFA changes in hypertensive subjects.

What is the mechanism by which volume expansion per se might affect NEFA? The best recognized regulators of plasma NEFA are catecholamines and insulin[17]; catecholamines may elevate plasma fatty acids levels by stimulating hormone-sensitive lipases, while insulin inhibits fatty acid formation by lipases and accelerates esterification of fatty acids to form triglycerides. In a recent report,[9] it has been demonstrated that acute volume expansion by IV saline infusion in hypertensive patients caused the average plasma level of norepinephrine to increase along with fatty acid plasma levels. Furthermore, because high-salt diet caused plasma norepinephrine to decrease in the same subjects not classified for salt sensitivity, the investigators concluded that an increase in catecholamines is unlikely to have been a predominant cause in determining fatty acids increase with salt loads. On the other hand, although plasma catecholamine levels were not determined in the present study, it has been previously suggested that a subset of patients with the so-called salt-sensitive essential hypertension may have impaired suppressibility of plasma norepinephrine levels during high-sodium intake.[18]

Acute volume expansion by saline infusion is known to provoke a significant decrease in plasma insulin,[9] which, by an antilypolytic action, lowers the plasma concentration of NEFA. On the other hand, no significant correlation between the 2 variables (serum insulin and NEFA) was observed in hypertensive subjects undergoing IV isotonic saline infusion, and a more recent study[19] was not able to demonstrate a significant suppression of serum insulin during IV saline administration in normal volunteers. In addition, in the present study, no significant changes in serum insulin were found in hypertensive subjects submitted to WI. Thus, serum insulin modifications do not fully explain the increase in NEFA in response to volume expansion.

Despite a concomitant increase in urinary sodium excretion and plasma individual NEFA, no significant correlation between these 2 variables was found during WI in hypertensive subjects; therefore, our data do not support the concept that an increase in plasma fatty acids may constitute a physiologic mechanism contributing to salt and water balance. Although our study also showed that NEFA increased when plasma aldosterone decreased during WI, the present data cannot confirm previous results showing a significant inverse correlation between the 2 variables.[9]

We have been unable to find any published evidence on fluctuations in other hormones affecting NEFA levels including corticotropin, glucagon, adrenal glucocorticoids, and thyroxine, and a previous report[20] denied any effect of atrial natriuretic peptide (ANP), which is consistently released during WI, on plasma NEFA.

Whatever the mechanism mediating the increase in NEFA levels during WI, another finding of our study was that in hypertensive patients, salt-sensitivity index during WI displayed a significant direct correlation with the percent increase of some individual NEFA such as stearic, palmitic, and palmitoleic acid. In fact, our data would demonstrate that when submitted to central volume expansion by WI, the hypertensive subjects with higher salt-sensitivity index may present a greater percent increase in NEFA plasma levels.

The salt sensitivity of essential hypertension is characterized by a modest degree of volume expansion due to a long-standing tendency to sodium retention and, along with other volume-expanded conditions, such as primary aldosteronism,[21] diabetes, and obesity, it is associated with increased risk factors for atherosclerotic cardiovascular disease. In particular, a linkage between salt sensitivity and insulin resistance has been suggested,[22,23] and insulin resistance has been considered as one of the important atherosclerotic cardiovascular risk factors, because the lowering effect exerted by insulin on NEFA is impaired in insulin resistance.

Endothelial dysfunction is a common feature of both salt-sensitivity and insulin-resistant states, and increased free fatty acids can acutely produce endothelial dysfunction in healthy humans.[24] In addition, it has been recently demonstrated that dyslipidemia and indices of a high intake of saturated and monounsaturated fats, as shown in salt-sensitive hypertension and insulin-resistant states, predicted the prevalence of left ventricular hypertrophy (LVH) in a prospective longitudinal cohort study, thereby suggesting that fatty acids may be important in the origin of LVH,[25] an important risk factor for cardiovascular mortality and morbidity.[26]

Finally, 2 epidemiologic studies[27,28] reported that intake of individual saturated fatty acid (stearic, palmitic, lauric, and myristic) showed a high correlation with mortality from coronary heart disease, and dietary intervention studies also reported that stearic acid increased plasma/serum lipoprotein (a) concentrations[29] and activated coagulation factor VII.[30]

In summary, by demonstrating that an acute isotonic-isooncotic central volume expansion may induce a significant increase of most NEFA plasma levels, we suggest that volume expansion per se could be included among the well-recognized risk factors for cardiovascular morbid events.

REFERENCES

1. Weinberger MH, Fineberg NS, Fineberg SE, et al: Salt sensitivity, pulse pressure and death in normal and hypertensive humans. Hypertension 37:429-432, 2001

2. Campese VM: Salt sensitivity in hypertension: Renal and cardiovascular implications. Hypertension 23:531-550, 1994

3. Morimoto A, Uzu T, Fujii T, et al: Sodium sensitivity and cardiovascular events in patients with essential hypertension. Lancet 350:1734-1737, 1997

4. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

5. Lind L, Berne C, Lithell H: Prevalence of insulin resistance in essential hypertension. J Hypertens 13:1457-1462, 1995

6. Sharma AM, Ruland K, Spies KP, et al: Salt sensitivity in young normotensive subjects is associated with a hyperinsulinemic response to oral glucose. J Hypertens 9:329-335, 1991

7. Zavaroni I, Coruzzi P, Bonini L, et al: Association between salt

sensitivity and insulin concentrations in patients with hypertension. Am J Hypertens 8:855-858, 1995

8. Luft FC, Weinberger MH, Fineberg NS, et al: Effects of age on renal sodium homeostasis and its relevance to sodium sensitivity. Am J Med 82:9-15, 1987 (suppl 1B)

9. Goodfriend TL, Ball DL, Weinberger MH, et al: Salt loads raise plasma fatty acids and lower insulin. Hypertension 17:958-964, 1991

10. Epstein M: Renal effects of head-out water immersion in humans: A 15-year update. Physiol Rev 72:563-621, 1992

11. Coruzzi P, Biggi A, Musiari L, et al: Calcium and sodium handling during volume expansion in essential hypertension. Metabolism 42:1331-1335, 1993

12. Sharma AM, Schattenfroh S, Kribben A, et al: Reliability of salt-sensitivity testing in normotensive subjects. Klin Wochenschr 67: 632-634, 1989

13. Weinberger MH, Fineberg NS: Sodium and volume sensitivity of blood pressure. Age and pressure change over time. Hypertension 18:67-71, 1991

14. Coruzzi P, Musiari L, Mossini GL, et al: Water immersion and salt-sensitivity in essential hypertension. Scand J Clin Lab Invest 53:593-599, 1993

15. Parmelee DC, Evenson MA, Deutsch HF: The presence of fatty acids in human α-fetoprotein. J Biol Chem 253:2114-2119, 1978

16. Wood R, Lee T: High-performance liquid chromatography of fatty acids: Quantitative analysis of saturated monoenoic, polyenoic and geometrical isomers. J Chromatogr 254:237-246, 1983

17. Khoo JC, Steinberg P, Thompson B, et al: Hormonal regulation of adipocyte enzymes. The effects of epinephrine and insulin on the control of lipase, phosphorylase kinase, phosphorylase, and glycogen synthase. J Biol Chem 248:3823-3830, 1973

18. Campese VM, Romoff MS, Levitan D, et al: Abnormal relationship between sodium intake and sympathetic nervous system activity in salt-sensitive patients with essential hypertension. Kidney Int 21:371-378, 1982

19. Lind L, Fugmann A, Branth S, et al: The impairment in endothelial function induced by non-esterified fatty acids can be reversed by insulin. Clin Sci 99:169-174, 2000

20. Shenker Y: Atrial natriuretic hormone effect on renal function and aldosterone secretion in sodium depletion. Am J Physiol 225:R867-R873, 1988

21. Nishimura M, Uzu T, Fujii T, et al: Cardiovascular complications in patients with primary aldosteronism. Am J Kidney Dis 33:261-266, 1999

22. Kuroda S, Uzu T, Fujii T, et al: Role of insulin resistance in the genesis of sodium sensitivity in essential hypertension. J Hum Hypertens 13:257-262, 1999

23. Falkner B, Hulman S, Kushner H: Hyperinsulinemia and blood pressure sensitivity to sodium in young blacks. J Am Soc Nephrol 3:940-946, 1992

24. Steinberg HO, Baron A: Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest 100: 1230-1239, 1997

25. Sundstrom JJ, Lind L, Vessby B, et al: Dyslipidemia and an unfavorable fatty acid profile predict left ventricular hypertrophy 20 years later. Circulation 103:836-841, 2001

26. Levy D, Garrison RJ, Savage DD, et al: Prognostic implications of ecocardiographycally determined left ventricular mass in the Framingham Heart Study. N Engl J Med 322:1561-1566, 1990

27. Kromhout D, Menotti A, Bloemberg B, et al: Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease. The seven countries study. Prev Med 24:308-315, 1995

28. Hu FB, Stampfer MJ, Manson JE, et al: Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr 70:1001-1008, 1999

29. Aro A, Jauhiainen M, Partanen R, et al: Stearic acid, trans fatty acids, and dairy fat: Effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein (a), and lipid transfer proteins in healthy subjects. Am J Clin Nutr 65:1419-1426, 1997

30. Mitropoulos KA, Miller GJ, Martin JC, et al: Dietary fat induces changes in factor VII coagulant activity through effects on plasma free stearic acid concentration. Arterioscler Thromb Vasc Biol 14:214-222, 1994