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Comparaison des réponses hémodynamiques d'un repas modérément salé et d'un repas riche en sel: une hypothèse pour expliquer l'hypertrophie ventriculaire gauche des régimes hypersodés

THESE

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TABLE OF CONTENTS

ABSTRACT

Many clinical studies have shown a close correlation between a chronic high salt diet and the development of left ventricular hypertrophy. This association has been classically attributed to the long-term hypertensive effects of a high salt diet. However, epidemiological studies have also shown that left ventricular hypertrophy may occur independently of changes in arterial pressure.

Since salt ingestion during a high salt diet is not distributed evenly over a 24-hr period, but occurs essentially during meal periods, we speculate that each acute salt load could lead to greater acute increases in blood pressure, heart filling pressure, stroke volume and cardiac output, putting an additional work load on the heart, promoting in the long run cardiac hypertrophy.

To test whether a high salt meal leads to hemodynamic changes that may favor cardiac hypertrophy, we compared in the same healthy young individual the response to a moderately salted meal (45 mmol) and to a high-salt meal (165 mmol sodium), given in a random order on separate days, on various cardiovascular parameters that were continuously monitored before and up to 140 minutes after the meal. Our results show that the post-prandial increases in stroke volume, and cardiac work were more pronounced after a high-salt meal than after a low-salt meal.

We speculate that repetitive salt loads associated with a high salt diet may lead to repetitive hemodynamic loads. Since plasma sodium concentration, which is increased after a salty meal, is also capable to stimulate myocyte growth, it is possible that the combination of post-prandial hypernatremic peaks and of cardiac loads may be responsible, when repeated many times over period of months, of the cardiac hypertrophy often seen with a high salt diet.

RÉSUMÉ

De nombreuses études cliniques ont révélé une corrélation étroite entre un régime alimentaire riche en sel et le développement d'une hypertrophie ventriculaire gauche. Cette association a été classiquement attribuée aux effets hypertensifs à long terme d'une alimentation riche en sel. Toutefois, les études épidémiologiques ont également démontré que l'hypertrophie ventriculaire gauche peut survenir indépendamment de changements de pression artérielle.

L'ingestion de sel n'étant pas distribuée de manière homogène durant la journée mais ayant lieu principalement durant les repas, nous émettons l'hypothèse que chaque repas riche en sel induit une augmentation aiguë de la pression artérielle, des pressions de remplissage cardiaque, du volume d'éjection systolique et du débit cardiaque. L'augmentation résultante du travail cardiaque pourrait ainsi à la longue entraîner une hypertrophie cardiaque.

Pour tester si un repas riche en sel conduit à des modifications hémodynamiques favorisant l'hypertrophie cardiaque, nous avons comparé chez la même personne jeune et en bonne santé la réponse hémodynamique à un repas modérément salé (45 mmol) à celle d'un repas riche en sel (165 mmol de sodium). Les repas ont été pris de manière randomisée à 7 jours d'intervalle. Divers paramètres hémodynamiques ont été mesurés en continu avant et jusqu'à 140 minutes après chaque repas. Nos résultats montrent que les augmentations post-prandiales du volume d'éjection systolique et du travail cardiaque ont été plus prononcées après un repas à haute teneur en sel par rapport à un repas modérément salé.

Nous spéculons que des apports chroniques en sel induisent des charges hémodynamiques répétées. Étant donné que la concentration plasmatique de sodium, qui est augmentée après un repas salé, est également capable de stimuler la croissance des myocytes cardiaques, il est possible que la combinaison sur des mois ou des années de pics hypernatrémiques post-prandiaux et de charges cardiaques soit responsable de l'hypertrophie cardiaque souvent observée avec une alimentation riche en sel.

1. INTRODUCTION

Many clinical studies have shown a close correlation between a high salt diet and the development left ventricular hypertrophy (LVH) (2). Much of the increase in cardiac hypertrophy has been classically attributed to the long-term hypertensive effects of a high-salt diet. We will thus review the evidence that a sustained high-salt intake may lead to chronic arterial hypertension.

However, epidemiological studies have also shown that LVH may occur independently of changes in blood pressure (BP) (2) and thus that high salt diet may directly affect cardiac function (7). In fact, clinical studies that have studied the relationship between salt diet and cardiovascular dysfunction usually focus on resting levels of blood pressure and other cardiovascular parameters (i.e. away from a postprandial state), or on a 24-hour blood pressure average. However, since salt ingestion during a high salt diet is not distributed evenly over a 24-hr period, but occurs essentially during meal periods, we speculate that acute and repetitive salt loads may submit the heart to an additional hemodynamic load after salty meals although the resting blood pressure may be normal. We will thus review the evidence but also test in controlled experiments in healthy subjects whether a high-salt meal may lead acutely to an increased work load of the heart.

Finally, salty meals are also accompanied by a post-prandial hypernatremic peak (27). Since sodium concentration is capable to stimulate myocyte growth directly (44), it is possible that the combination of hypernatremic peaks and added load on the heart by a high salt-meal may in the long run (i.e. when repeated many times over months) promote cardiac hypertrophy and mortality independently of changes in basal blood pressure.

1.1. The association between salt intake and left ventricular hypertrophy

Ventricular hypertrophy is considered to be an adaptive mechanism to normalize ventricular wall stress in response to a volume/pressure overload (43; 114). According to the Framingham Heart study, left ventricular hypertrophy is associated with a relative risk of cardiovascular disease of 1.49 in men, 1.57 in women for each increment of 50g /meter in left ventricular mass (66). Multiple mechanisms may be involved in the development of ventricular hypertrophy seen with high salt intake: salt-induced elevation of blood pressure (29), increased preload in the absence of hypertension (92), augmented sympathetic activity (80) and upregulation of adrenergic receptors

(102). Elevated blood-pressure is one of the most powerful determinants of left ventricular hypertrophy. Indeed, many clinical studies have demonstrated the association of hypertension and left ventricular hypertrophy, and its regression under antihypertensive treatments (19). Since epidemiological studies show an association between dietary salt intake and arterial hypertension, there should be an indirect relation between salt intake and ventricular hypertrophy.

However, there is controversy whether salt intake necessarily leads to hypertension since many studies have reported large variability between individuals regarding the effect of salt on blood pressure. Furthermore, the degree of left ventricular hypertrophy in patients with mildly elevated arterial pressure is not uniform and may range from normal ventricular mass to severe hypertrophy (38). In fact, epidemiologic studies have shown that an increase in left ventricular mass index can also be seen in a general normotensive population (33). Altogether, these facts suggest that factors other than hypertension may contribute to ventricular hypertrophy. Several groups have confirmed a relationship between dietary salt intake and left ventricular hypertrophy that is independent of blood pressure, so that high salt diet may directly affect cardiac and vascular structures (94).

1.2. Evidences and controversies for an association between salt intake and arterial hypertension

1.2.1. Evidences on the relationship between salt intake and hypertension

Epidemiological evidence on the relation between dietary salt and blood pressure varies between the clear-cut absence of hypertension in population consuming < 3g per day to the high prevalence of hypertension in populations absorbing > 20g per day (73). Between these two extremes, the relation between salt and blood pressure is less easy to establish since the range of salt intake in a population is often narrower and, more importantly, there are large fluctuations in day-to-day salt-intake in the same individual, which makes a single 24-h salt excretion a relatively poor estimator of the true average sodium intake (68). Interestingly, there is no increase in arterial pressure with ageing in the up to 40 non acculturated tribes that have been studied and who consume less than 3 g salt per day (26). In contrast, as shown in Figure 1 from Meneton et al. (73), the systolic pressure rises progressively with age in western populations eating 6 to 18g salt per day. This difference is not explained by the level of acculturation since high salt intake also led to increase in blood pressure in non acculturated populations (84).

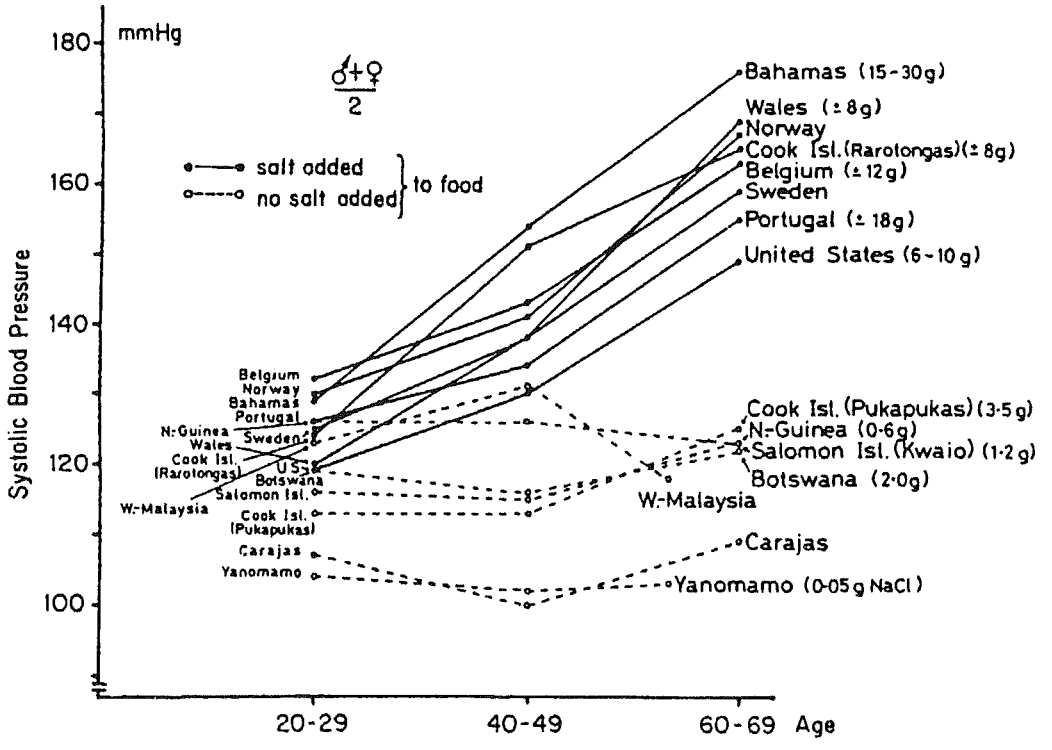


Figure 1. Systolic blood pressure change with age in various populations according to their habitual daily intake. *Adapted from Meneton et al. 2005*

Migratory studies provide further evidences for a relation between habitual salt intake and blood pressure. Kenyan farmers increased their blood pressure after a few months when they migrate to an urban community where they underwent a marked increase in daily salt intake. There was no increase in blood pressure in the control group who did not migrate. (88). Regional differences in salt intake are accompanied by parallel changes in the prevalence of hypertension. Such evidences were obtained among the Salomon Islanders: 1 percent of the population had hypertension in tribes living away from the coast and consuming < 2g salt per day. The prevalence of hypertension increased to 3%, respectively 8%, in tribes eating 3-8 g salt/day, respectively 9-15 g salt /day.

The INTERSALT study, an international epidemiological study started in 1981, examined the relationship of salt intake and hypertension in 10'079 men and women aged from 20 to 59 years drawn from 52 centers around the world. Initial calculations found a significant relationship between salt excretion and blood pressure in individuals, but not across centers. Yet, when including salt excretion data of all centers, salt excretion was related to the slope of the rise of blood pressure with age. Subsequently, further statistical analyses correcting for day-to-day variations in

individual salt excretion confirmed a significant positive correlation emerged between sodium excretion and systolic blood pressure.

In further support of the link between salt intake and blood pressure, many studies have demonstrated the hypotensive effect of lowering salt intake in hypertensive patients. A meta-analysis performed by He and MacGregor (49) concluded that a modest reduction in salt intake for at least 4 weeks had a significant blood pressure lowering effect in normotensive and hypertensive individuals. They even found a correlation between the magnitude of salt reduction and the reduction in blood pressure and believed that reducing salt intake could reduce strokes, heart attacks, and heart failure. Various meta-analyses about the effects of a salt reduction have been performed (18; 42; 49; 74), demonstrating a more pronounced reduction of systolic and diastolic pressure in hypertensive subjects compared to normotensive ones.

Conversely, raising salt intake over long period may increase blood pressure. Such as study would be difficult to realize in humans for ethical reasons, but many studies in animals have demonstrated that increasing salt intake leads to chronic hypertension. For example, in a classical study by Meneely et al.(72), young adult rats fed different amounts of salt intake in their diet ranging from 0.01 to 9.8% over 12 months showed an increase in systolic blood pressure that was proportional to the amount of salt in the diet (Figure 2).

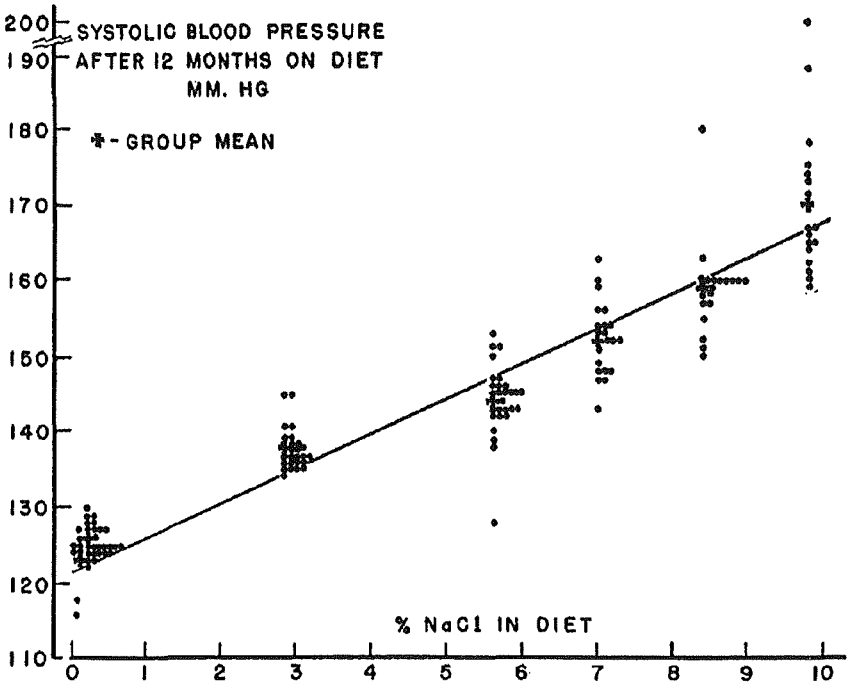


Figure 2. Dose-dependency of the effects of excess dietary salt on tail systolic blood pressure in rats (male outbred rats of the Sprague Dawley) exposed to varying salt intakes (0.01 to 9.8% NaCl in diet) for 12 months. Reproduced from *Meenely et al., The Journal of Experimental Medicine, 98: 71 – 80, 1953*

A similar study in pigs fed either 0.5 or 3% salt for 8 months showed higher blood pressure values in swine with the high salt intake (15). Baboons exposed for one year to 4% added salt to their diet showed a significant increased in blood pressure (12). It was argued that humans may response differently than animals, but a study in chimpanzees, our closest relatives from a genetic viewpoint, also showed a progressive increase in blood pressure over a 20-month period when salt intake was raised from the naturally low value for chimpanzees to the human dietetic range (25). (Figure 3)

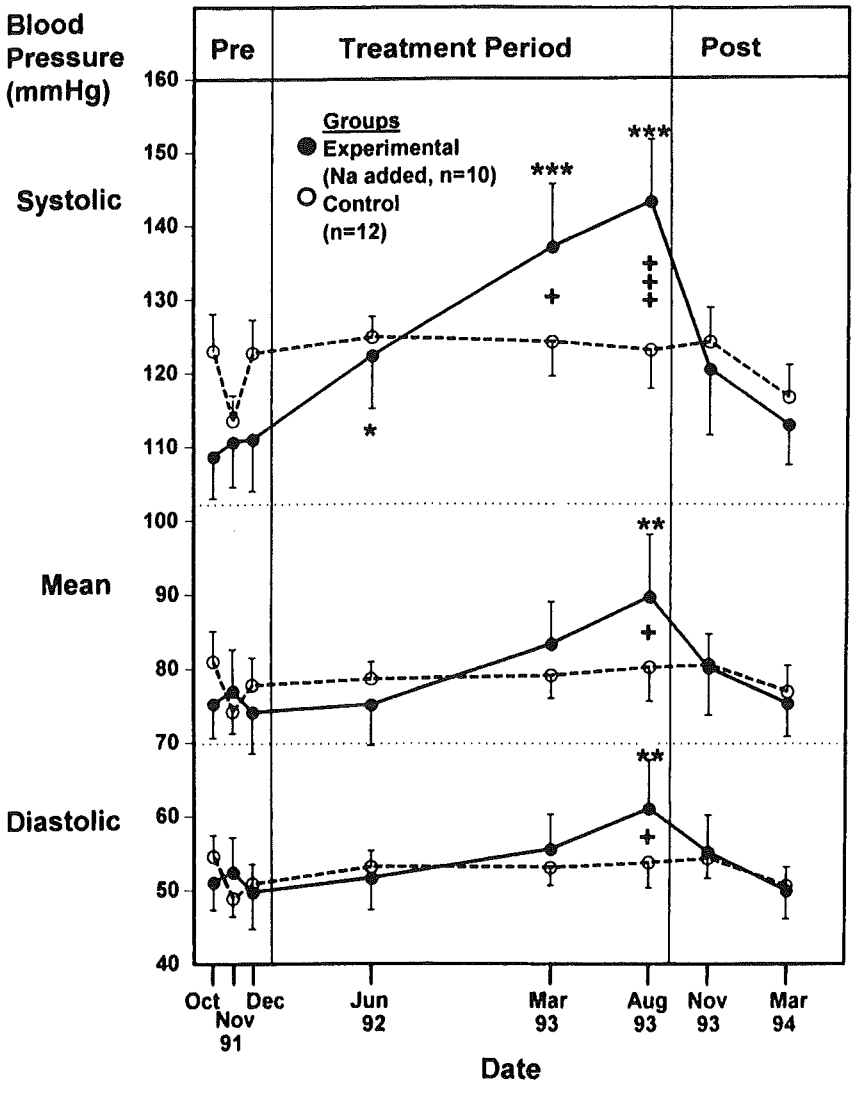


Figure 3. Salt-induced increases in blood pressure over a 20 month period in chimpanzees. The diet of the experimental group (filled symbols) was supplemented with ~256 mmol sodium per day. Control animals maintained on a regular fruit diet (open symbols) exhibited no change in blood pressures. *From Denton et al. Nat Med 10:1009-1016, 1996*

The final strong support of the link between salt intake and hypertension comes from the genetic studies. In the last decades, over twenty genes have been identified to be associated with essentially hypertension or responsible for rare monogenic diseases that are characterized by low or high blood pressures. Intriguingly, most of the genes encode proteins that are involved with renal sodium handling. As shown in **Table 1**, mutations that raise renal sodium reabsorption, such as in the gain of function of the Epithelium Sodium Channel (ENaC), increase blood pressure, whereas mutations that diminish tubular sodium reabsorption (Bartter's or Gitelman's syndrome), decrease blood pressure.

Table 1A. Mutations with increased sodium reabsorption increase blood pressure.

Genetic defect	Tubular sodium reabsorption	Blood pressure
Mutation of WNK 1 and 4 **(Gordon's syndrome)**	Increased	Increased
Mutation in β- and γ- subunits of ENaC **(Liddle's Syndrom)**	Increased	Increased
Deficiency in 11β-HSD2 **(apparent mineralocorticoid excess)**	Increased	Increased
17 α-hydroxylase or 11β-hydroxylase deficiency **(congenital adrenal hyper plasia)**	Increased	Increased
Chimera gene CYP11B2 **(Glucocorticoid remediable aldosteronism)**	Increased	Increased

They include syndromes characterized by mineralocorticoid-independent excessive sodium uptake in the distal convoluted tubulus (Gordon syndrome: mutation in 2 isoforms of with no lysine serine-threonine kinase WNK 1 and 4) and the collecting duct (Liddle syndrome: gain-of-function mutation in the in β- and γ- subunits of the amiloride-sensitive epithelium Na-channel (ENaC)) and various syndromes characterized by excess mineralocorticoid action in the later parts of the nephron, such as apparent mineralocorticoid excess (deficiency in 11β-hydroxysteroid dehydrogenase type II (11β-HSD2) with resulting diminished metabolization of the excess cortisol to cortisone. This results in an increased renal concentration of cortisol which is a powerful agonist of the aldosterone receptor), congenital adrenal hyperplasia due to 17 α-hydroxylase or 11β-hydroxylase deficiency (which lead to an overproduction of the potent activator of the mineralocorticoid receptor deoxycorticosterone) and the glucocorticoid remediable aldosteronism (chimera gene CYP11B2 of the aldosterone synthase placing it under the control of ACTH dependent promoter).

Table 1B. **Mutations that lead to salt wasting decrease blood pressure**, particularly when sodium intake is low.

Genetic defect	Tubular sodium reabsorption	Blood pressure
Mutation in NKCC2, ROMK1 or CIC-Kb **(Bartter's syndrome I, II or III)**	Decreased	Decreased
Mutation in Na-Cl cotransport **(Gitelman's syndrome)**	Decreased	Decreased
Loss of function mutation of mineralocorticoid receptor **(dominant pseudohypoaldosteronism type I)**	Decreased	Decreased

These include syndromes with deficient transport proteins in the thick ascending limb of Henle's loop (Bartter syndromes type I , II and III : respectively due to a defect in the Na-K-2Cl-contransport (NKCC2), the apical K-channel (ROMK1) and the basolateral Cl-channel (CIC-Kb)), in the distal convoluted tubulus (Gitelman syndrome: loss of function mutation of the apical thiazide-sensitive Na-Cl-cotransport). Other salt-wasting syndromes are related to mineralocorticoid deficiency in renal tubular cells due to loss-of-function mutation in the mineralocorticoid receptor (dominant pseudohypoaldosteronism type 1).

1.2.2. Controversies on the relationship between salt intake and hypertension

The controversy on the relationship between salt intake and hypertension arises because some studies have found that **short-term** alterations in sodium intake may lead to paradoxical changes in blood pressure, with an increase in blood pressure under salt restriction and a fall in blood pressure under high salt intake. In an anecdotic observation, two young healthy volunteers fed 500 mmol /day over 48h exhibited a fall in blood-pressure (6). Sodium restriction may also increase blood pressure in certain individuals, particularly in younger individuals. For example, in 46 non obese essential hypertensive patients of all ages (range 25 to 80 years), the switch from one week of low (20 mmol sodium/day) to one week of high (300 mmol sodium/day) salt intake was characterized in a small number of individual (17%) by a decrease in BP of at least 5 mmHg during the high salt diet. Those "counter-regulators" (i.e. an increase in BP with salt-restriction) were found in young and middle-age people (< 55 years of age) but not among older people (83). In a similar study in normotensive subjects, an increase in blood-pressure under salt restriction was

more likely to occur in younger individuals and in subjects without a familial history of hypertension (82). Interestingly, the rise in plasma renin activity during salt restriction was most pronounced in counterregulating subjects, leading to believe that an exaggerated renin release during sodium restriction may be responsible for the hypertensive effect of salt restriction. In rats, unilateral nephrectomy of sodium-restricted male Sprague-Dawley rats produced a sustained elevation in systolic blood pressure that was reversed by sodium repletion (97). However, the development of hypertension with sodium-depletion could be completely prevented if the rats were treated with an inhibitor of the renin-angiotensin system, further supporting the role of renin in the hypertension of salt-depletion. Finally, it should be stated that all those studies are short-term studies and there is no experimental evidence for a **long-term** hypotensive effect of high salt intake.

The observation that salt is a major determinant of the rise in blood pressure with age has also been questioned. In a 20-year study conducted in 144 Italian nuns and 138 controls living in the vicinity of the convent, it was found that, despite similar urinary sodium excretion over years in the two groups, blood pressure increased with age only the control group (103). The results suggest that the hypertensive effects of salt may be influenced by some environmental factors such as the level of stress.

1.2.3. Large individual variability in the blood pressure response to salt

One of the difficulties in studying the hypertensive effect of salt is that there are enormous individual variations in blood pressure response to salt. Submitting humans to a high salt intake will raise blood-pressure in some individuals, defined as salt-sensitive, whereas it will not induce changes in pressure in other individuals, defined as salt-resistant or salt-insensitive. Many different protocols have been used clinically to classify salt sensitivity, such as the response to one week of high salt intake or conversely of low salt intake, or the acute response to a saline infusion or to salt-depletion with diuretics. Studies also define arbitrarily the cutoff value between salt "responders" and "nonresponders", a 10 mmHg change in BP being often used. An additional difficulty is that salt responsiveness is not always perfectly reproducible when subjects are tested twice within the same year (111). The variety of protocols makes comparisons between studies quite difficult. In 15 untreated hypertensive patients undergoing a sodium restriction (5 g/day) during 1 week and then submitted to a daily salt-intake of 20 g during a further week, six patients had an average increase of arterial pressure from 104.1 ± 5.9 mmHg to 118.0 ± 8.8 mmHg, whereas no changes (from 109.0 ± 8.7 mmHg to 107.0 ± 10.3 mmHg) were observed in the other nine subjects (76). Using as criterion of salt-sensitivity a fall in blood pressure of more than 10 mmHg, going from a state of acute salt loading with a saline infusion to a state of acute sodium restriction with a one-day sodium-poor diet and furosemide administration, Weinberger et al. found in a larger population (230 hypertensive subjects and 430 normotensive volunteers) that 65% of normotensive under 30 of age and 50% hypertensive under 30 of age were reproducibly salt-resistant (measured twice within 12 months) whereas the remaining subjects were either salt-sensitive or indeterminate, switching from one category to the other when measured twice within 12 months (111). Salt-sensitivity was clearly related to age. Among people older than 50, only 23% of normotensive and 15% of hypertensive remained clearly salt-resistant when tested twice within 12 months.

The importance of the protocol used to test salt-sensitivity is exemplified by the study of de la Sierra et al. (21). Twenty-nine essential hypertensives underwent two different procedures separated by 1 month: a dietary test consisting of a 2-week period of low (20 mmol/day) and high (260 mmol/day) salt intakes, and an intravenous test consisting of a 2 litre saline load over a 4-h period, followed by 1 day of low (20 mmol) salt intake and furosemide (120 mg in 3 separate doses) administration. The results show a poor coefficient of agreement between oral and intravenous tests, with a large misclassification of salt-sensitivity when using a saline infusion and furosemide administration protocol. The authors advocate that the diagnosis of salt-sensitive hypertension should be based on the BP response to changes in dietary salt intake.

The individual variability in the BP response to salt, well described in the above short-term studies, has also been observed during long-term exposure to a high salt intake. In a colony of 26 chimpanzees maintained in small groups for 3 years, half of them had salt added to their diet progressively during 20 months within the human dietetic ranges whereas the other half was maintained on their natural low-sodium diet. Exposure to salt caused a significant rise in systolic, mean and diastolic blood pressure over time. However, the BP response to salt was very variable, some chimpanzees (about 60%) having a large blood pressure rise, whereas others (about 40%) showed only a small or no rise at all. (25).

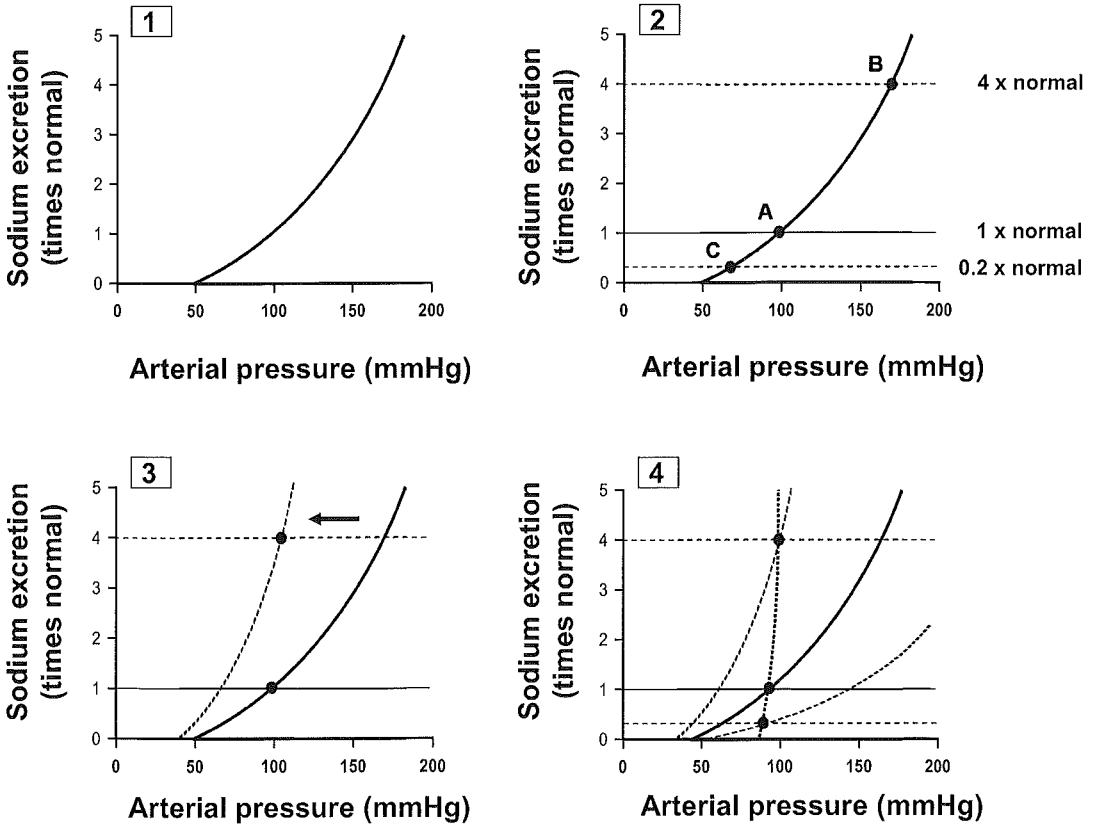
Salt sensitivity is common in specific patient populations including elderly people, obese people and patients with glucose intolerance. The familial aggregation and the higher prevalence of salt-sensitivity in certain ethnic groups (Africans, African-Americans) support the notion that it could be a heritable trait. The influence of a positive familial history of hypertension is not clear; some studies show a greater risk to develop a transient elevation of blood pressure after a few days of increased salt intake(37), others show no impact (46).

1.2.4. Possible mechanisms of salt-sensitivity

The mechanisms of salt sensitivity are still incompletely understood and are the subject of intensive research. Various hypotheses and mechanisms have been proposed.

A. Pathological alteration of the pressure natriuresis curve

Changes in renal perfusion pressure, regardless whether the kidney is studied in vivo or in vitro, lead to profound changes in sodium excretion (Figure 4-1), a phenomenon known as **pressure-natriuresis**. The intrarenal mechanisms for this phenomenon are complex and probably related to physical factors associated with a lack of medullary blood flow autoregulation and subsequent increase in renal interstitial fluid pressure whenever blood pressure is increasing (41).



Figures 4.1 to 4.4. The concept of acute pressure-natriuresis and how adjustments of this relationship facilitate sodium balance during sustained changes in salt intake. **(1)** The basic pressure-natriuresis-curve (PNC). **(2)** Three levels of salt intake are depicted (normal, 0.2x normal and 4x normal). Equilibrium is reached at the intersection between the PNC and the corresponding level of salt excretion that matches salt intake. **(3)** Left-shift of the PNC during a high-salt intake. **(4)** Joining the intersection points reveals an almost vertical chronic pressure-natriuresis relationship (see dotted line), i.e. the chronic renal function curve. The modulation of the PNC during alterations in salt intakes allows thus the body to achieve sodium balance with minimal changes in arterial pressure.

The pressure-natriuresis curve (PNC) is at the center of blood volume and BP control. If the body gains too much fluid (e.g. acute volume load), BP increases. This leads to increased excretion of salt and water via the pressure-natriuresis mechanism, bringing blood volume and BP back towards normal. Conversely, if one looses fluids (e.g. hemorrhage), BP decreases and the kidneys retain salt and water, which helps to bring blood volume and BP back to normal. Equilibrium is thus reached at the intersection point of the PNC with the corresponding salt intake level, as shown on Figure 4-2.

The PNC is not immovable. In fact, it becomes steeper and is shifted to the left during high salt intake (Figure 4-3). This allows the body to achieve sodium balance with minimal increases in blood pressure. Conversely, during low salt intake, the PNC becomes flatter and is shifted to the right. Joining the equilibrium points at the various salt intakes reveals now a very steep "chronic" relationship with little changes in BP (Figure 4-4). That is, the chronic relationship between salt intake and blood pressure has become relatively salt-*in*sensitive. Various neurohormonal mechanisms (angiotensin, aldosterone, atrial natriuretic peptide, sympathetic nerves) contribute to the adjustment of the PNC. However, when these mechanisms are impaired, salt-sensitivity develops. Major causes of salt-sensitivity are:

a) Inadequate suppression of the renin-angiotensin system (RAS) during high salt intake

The inhibition of the systemic RAS during high-salt intake plays a major role in preventing large increases in extracellular volume and blood-pressure. It normally shifts the PNC to the left by reducing tubular reabsorption of sodium, thereby facilitating urinary sodium excretion with minimal increases in blood-pressure. Some studies have found that the suppression of plasma renin activity and aldosterone by high-salt intake was significantly smaller in salt-sensitive patients than in salt-resistant patients (22; 55). So salt-sensitivity may be an inability to suppress renin. This occurs particularly when renin levels are low to start with, under a normal sodium intake. There is therefore little room to further suppress renin. This may explain the higher prevalence of salt sensitivity in older subject (who show decreased renin levels possibly due to the observed decrease in glomerular number and size associated with ageing) and in subjects with low-renin essential hypertension.

The importance of RAS in salt-sensitivity is further supported by the fact, that genetic polymorphisms of some genes encoding various components of the RAS are significantly associated to salt-sensitive hypertension. For example, hypertensive patients exhibiting the angiotensin-converting-enzyme (ACE) insertion/deletion polymorphism or the 11-beta-hydroxysteroid-dehydrogenase type 2 (11βHSD2) G534a genetic polymorphism showed a larger BP response to high-salt intake (87).

b) Enhanced tubular reabsorption of sodium. Chiolerio et al. (13) hypothesized that salt sensitivity is probably the result of a primary defect in proximal tubular reabsorption of sodium. By switching hypertensive patients from a low salt (70mmol/day) diet to a high salt diet , they observed that salt-resistant patients decreased their proximal sodium reabsorption (as estimated by lithium clearance techniques) whereas salt-sensitive patients were "characterized by an inadequate proximal

sodium retention on low salt and an inability to excrete the excess salt on a high sodium diet". Subjects showing the least reduction in proximal tubular sodium reabsorption had also a more pronounced raise in blood pressure. The exact mechanism of the increased sodium reabsorption in salt-sensitive subjects are not clear, but could involve many factors known to affect sodium reabsorption (RAS, renal nerve sympathetic activity, inadequate release of ANP, renal oxidative stress) as well as putative factors. In all cases, a common link seems to be an insufficient left shift of the PNC during high salt intake.

B. Endothelial dysfunction. The vascular endothelium plays an important role in the local regulation of vascular tone and therefore influences vascular resistances and arterial blood-pressure. It produces various vasoactive substances, most importanly nitric oxide (NO). NO is usually released from the vascular endothelium after stimulation of the vascular endothelial NO-synthase (eNOS) through physical stimuli such as shear stress. NO activates the guanylate-cyclase which forms cGMP. The increased cGMP level induces vascular smooth muscle relaxation with consecutive vasodilation. Inhibition of the NO-synthase results in vasoconstriction and increased arterial blood pressure. The effect of salt on the synthesis of NO remains controversial. Some investigators found a suppression of NO production or of the effect of NO on peripheral resistance vessels by salt (9), whereas others postulated an increased, compensatory NO-production to facilitate sodium excretion and maintenance of normal blood-pressure (65). Clinical data suggest that salt-sensitive patients are more likely to exhibit an endothelial dysfunction and may be therefore unable to upregulate the production of NO in response to salt-intake.(5). Further, the endothelium-dependent vasodilatation by acetylcholine is lower in salt-sensitive versus salt-resistant hypertensive patients and this regardless of the sodium loading (76).

Interestingly, vascular endothelial cells also express the sodium-selective ion channel (ENaC) in response to aldosterone (40). This could act as a functional link between plasma sodium and endothelial function, giving a possible mechanism how sodium might modulate endothelial NO-synthesis. Sodium influx in the endothelial cells via the ENaC is accompanied by water influx and therefore influences their volume and stiffness. Since a high endothelial deformability is a prerequisite for a normal shear-stress induced NO release from the endothelium, small changes in plasma sodium may alter endothelial deformability and reduce endothelial NO-release. In endothelial cell cultures, Oberleithner et al. (81) observed an increase in endothelial cell stiffness as the extracellular sodium concentration in the culture medium was stepwise increased from 120 to 160 mmol/L. Superfusing endothelial cells with pooled human plasma samples (with a sodium concentration of 137 mmol/L) to which sodium was added to obtain physiologic increases in

plasma sodium to 142 or 147 mmol/L also resulted in a dose-dependent increase in endothelial stiffness. The sodium-induced endothelial stiffening was attenuated by the absence of aldosterone in the culture medium and could be prevented by amiloride pretreatment. Finally, the authors were able to demonstrate a reduction in endothelial deformability by scan forces with concomitant reduction in NO release from endothelial cells exposed to increased sodium concentrations.

C. Enhanced CNS response to salt. A high salt intake increases sodium concentration in the cerebrospinal fluid (CSF) of salt-sensitive rats, such as Dahl S-rats and SHR (57) but not in salt-resistant rats such as Dahl R rats (79). Various studies suggest that Dahl S rats have a genetic abnormality of the choroid plexus altering ion transport, which contributes to the increased CSF-Na^+ in response to high salt intake. Measurements of the cerebrospinal Na radioactivity after intravenous injection of radio-actively marked sodium revealed that the blood-brain barrier is 5 to 8 times more permeable to sodium in DS than DR rats (98).

In turn, the higher CSF sodium could increase sympathetic outflow (56) that could increase BP. Huang found an enhanced neuronal responsiveness to CSF-Na in salt-sensitive rats. In conscious Dahl S and Dahl R rats, intracerebroventricularly infused Na increased in a dose-related manner blood pressure, heart rate and renal sympathetic nerve activity. The magnitudes of the responses were significantly larger in Dahl S than in Dahl R rats and are explained by a more marked sympathetic hyperactivity in Dahl S rats (58). Whether abnormality of central sodium transport also play a role in salt-sensitive humans is not certain, but an alteration of the choroid plexus has been hypothesized (57). Interestingly, salt-resistant subjects show a significant reduction of muscle sympathetic nerve activity, plasma concentration and urinary excretion of norepinephrine with a higher salt intake whereas salt-sensitive subjects show no changes of these parameters (75). The authors concluded that the expected sympathetic inhibition with high salt intake failed in salt-sensitive subjects, leading to an increase in blood pressure.

The cerebral renin-angiotensin-system, a central RAS independent from the systemic RAS, may be the link between higher CSF-sodium and increased sympathetic outflow. Comparing the effects of a 4-week high-salt diet on Dahl R and Dahl S rats, Zhao et al. found that ACE mRNA levels in Dahl S rats were almost three-fold higher in the hypothalamus and_two-fold higher in the pons than in Dahl R rats. There was also an increased ACE activity in the hypothalamus and pons in Dahl S but not in Dahl R rats (115).Centrally generated angiotensin II could thus play a role in regulation of arterial pressure since centrally administered angiotensin II is known to increase sympathetic outflow (89).

1.3. LVH may occur independently of changes in arterial pressure

1.3.1. Experimental and epidemiological evidence

Various experiments on **animals** provide evidence for a pressure-independent relationship between dietary sodium intake and left ventricular hypertrophy. Three-month-old Wistar-Kyoto (WKY) rats given 1% NaCl drinking water during 7 months showed a significant increase in left ventricular weight compared to rats given tap water, without any change in blood-pressure (measured by tail-cuff) (63). Further studies in WKY rats comparing a 10-week administration of 4 levels of salt intake (0.01%, 0.44%, 1.44% and 4%) showed that a high sodium intake (4%) induced a significant increase in total and left ventricular mass index without any changes in arterial pressure (measured directly with indwelling catheters in the conscious animal) or in regional and systemic hemodynamics (36). In another study in Wistar rats, substituting 1% saline to the ad lib drinking water caused a concentric LV hypertrophy without an increase in blood pressure (measured with an indwelling catheter in the conscious rat). The hypertrophy was already seen after 3 weeks of saline (but not after 10 days) and was more pronounced after 6 weeks of saline (34). Echocardiographic studies on mice also report a salt-dependent increase in the thickness of the interventricular septum in the absence of arterial pressure changes (30). Three groups of Swiss mice were submitted, for 8 weeks, to different salt diets (0.6, 2 and 4% NaCl). At 8 weeks, the septal thickness was 19% greater in 2%-salt mice and 27% greater in 4%-salt mice when compared to the mice maintained under a moderate salt intake (0.6%).

The pressure-independent relationship is further supported by alteration in arterial structure and function with varying salt intake. A long-term high-sodium diet on WKY rats and SHR performed by Partovian et al.(86).demonstrate that arterial pressure has not an exclusive role in structural alteration of arteries: the intra-arterial blood pressure increased significantly in WKY rats, but induced minor effects on wall-structure of the aorta. In contrast, a significant increase in the aortic wall thickness and medial cross sectional area developed in SHR without alteration in blood pressure. In stroke-prone spontaneously hypertensive rats, an increased sodium intake is associated with a more pronounced increase in wall thickness of cerebral and renal arteries than when low-salt diet is consumed (104). This results from a greater collagen content and abnormal cross linking, and is reversed by lowering sodium intake without any change in blood-pressure.

A limitation of above studies is that BP was measured by tail cuff or for a short period (about 30-min) with an indwelling catheter. Since left ventricular hypertrophy results from a cumulative load to the heart throughout a 24-hour period, it is not possible to exclude a correlation between BP and LVH based simply on an acute measurement. Only measurements taken around the clock, such as 24-hr BP telemetry, will reflect the overall hemodynamic load of BP on the heart (106)

In **humans**, a pressure-independent effect of salt intake on ventricular hypertrophy has also been described. Schmieder et al. (94) reported an independent relationship between 24-hour urinary sodium excretion (an estimator of dietary sodium intake) and left ventricular mass index hypertensive patients. A high level of 24-hour urinary sodium excretion was associated with increased relative wall thickness independent of the level of blood pressure. Stepwise multiple regression analysis confirmed that 24-hour sodium excretion was the strongest predictor of relative ventricular wall thickness. They also demonstrated that high salt intake might aggravate and, conversely, dietary salt restriction might prevent or mitigate the development of left ventricular hypertrophy in patients with mild to moderate essential hypertension.

Even in normotensive subjects there is a significant correlation between salt intake and left ventricular mass. For example, a study in 50 normal subjects aged 25-61 yr and without a family history of hypertension, confirmed the existence of an independent relationship between 24-h urinary sodium excretion and left ventricular mass index. For the authors, the positive correlation between urinary sodium excretion and left ventricular diastolic internal dimension in normotensive subjects suggests that sodium affects ventricular mass through a change in preload with consecutive change in cardiac chambers volume.(28) Kupari and Co observed the same significant correlation between the LV mass and sodium intake in a population of 91 subjects aged 36 to 37 years and living in Helsinki. (64) Finally, the TOHMS study compared the effect of a reduction in sodium intake combined with a placebo or with five various antihypertensive monotherapies (diuretics, β-blocker, α-antagonist, calcium antagonist, angiotensin-converting enzyme inhibitor) in 844 mild hypertensive subjects with documented left ventricular hypertrophy (67). They demonstrated that a reduction in salt intake alone is equally effective in reducing left ventricular mass as salt reduction combined with an antihypertensive medication, despite a larger decrease in BP with antihypertensive therapy.

1.3.2. Salt-induced LVH: a direct effect of sodium?

A possible **direct effect of salt** on heart to promote cardiac cell growth has been shown by Gu et al. (44) in isolated ventricular myoblaste cell cultures incubated at various sodium concentrations (ranging from 146 mmol/L to 182 mmol/L). Increasing the sodium concentration in the medium by as little as 6 mmol/L above a normal control value of 146 mmol/L induced substantial hypertrophy. Additional studies from the same laboratory showed that an augmentation of 2 mmol/L above normal caused the cellular protein content of cultured dog coronary artery smooth muscle cells to increase by 85% (44). Therefore, we have to ask the question whether high dietary salt-intake is able to induce an increase in extracellular sodium concentration, which could lead to pressure-independent ventricular hypertrophy.

It is well known that the plasma sodium concentration can increase in numerous pathological situations such as primary hyperaldosteronism, Cushing disease, high-salt diet in patient with renal insufficiency. However, animal and human studies that have measured plasma sodium under chronic varying salt intakes show no significant changes or only small changes in natremia. In dogs, changing salt intake from a very low intake (5 mmol/day) to normal intake of about 75 mmol/day caused only a small, but significant, increase in plasma sodium concentration (about 2 mmol/L), but further increases in salt intake to ~250 mmol/day and ~500 mmol/day caused no additional changes (48). In humans, only extreme changes in salt intake from a high (350 mmol/day for 5 days) to a very low (10-20 mmol/day for 5 days) sodium intake resulted in about 3 mmol/L decrease in plasma sodium concentration (51). A longer-term (4 weeks) modest reduction in salt intake from about 170 mmol/day to about 100 mmol/day in 118 patients with untreated essential hypertension was accompanied by a small fall in plasma sodium of 0.4 mmol/L. This reduction in plasma sodium was weakly, but significantly correlated to the fall in systolic blood pressure (51).

On the other side, clinical human studies with varying daily salt-intake *over short time periods (24 hours)* confirmed that plasma sodium is increasing respectively decreasing parallely to the changes in consumed salt amount. A progressive increase over 5 days of the daily salt-intake from 10 mmol to 250 mmol in 6 young, normotensive individuals was accompanied by a rise in plasmatic sodium of **3 mmol/L**, from 138.5 mmol/l to 141.5 mmol/l, as shown in Figure 5 (51).

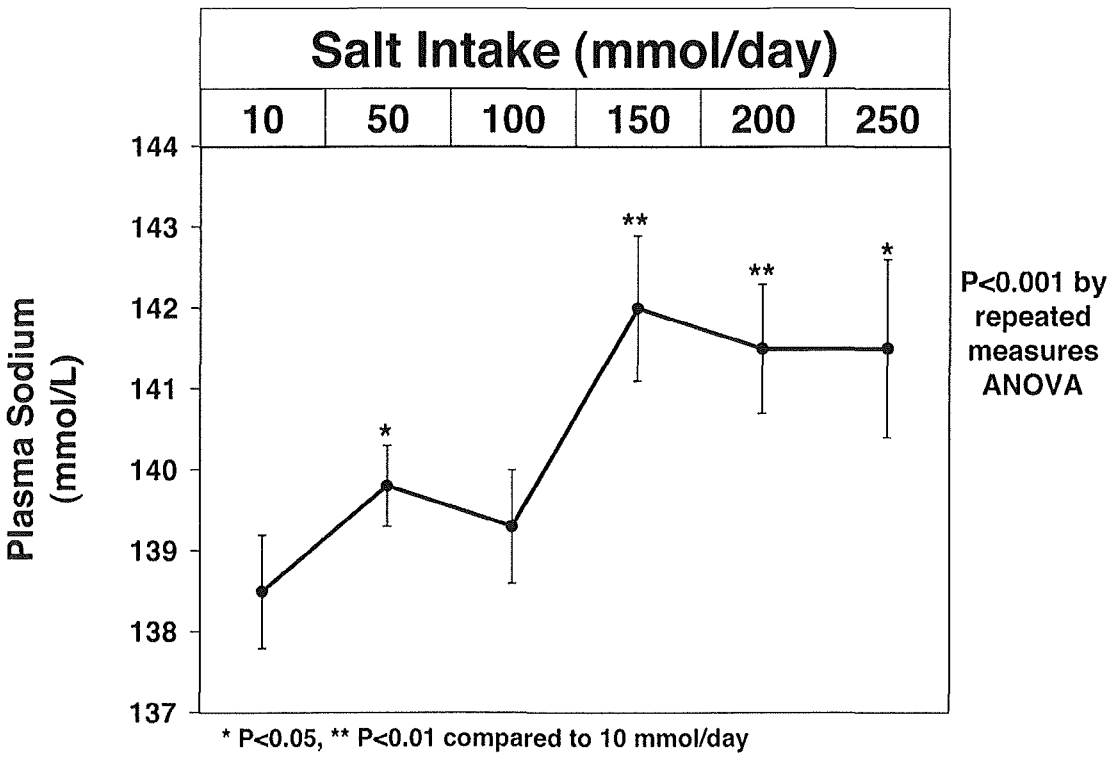


Figure 5. Changes in plasma sodium with progressive increases in salt intake, *from He et al. Hypertension 45 :98-102, 2005*

Changes in plasma sodium are also observed after an acute salty meal (27). Analyzing serum sodium concentration after ingestion of a low-salt breakfast (4.6mmol NaCl) and a high-salt breakfast (104.6 mmol NaCl, i.e. the same breakfast supplemented with 100 mmol of sodium diluted in water) in 12 healthy male subjects, a significant increase in serum sodium was seen during the first four post-prandial hours of the high-salt meal whereas no changes were detectable after the low-salt meal (**Figure 6**). Similar fluctuations in plasma sodium concentration have been observed in animals after a high-salt meal. In dogs, the 4-hour postprandial plasma sodium concentration is linearly related to the magnitude of dietary_sodium intake. The urinary sodium excretion correlated with both the dietary sodium intake and the postprandial hypernatremic peak (95).

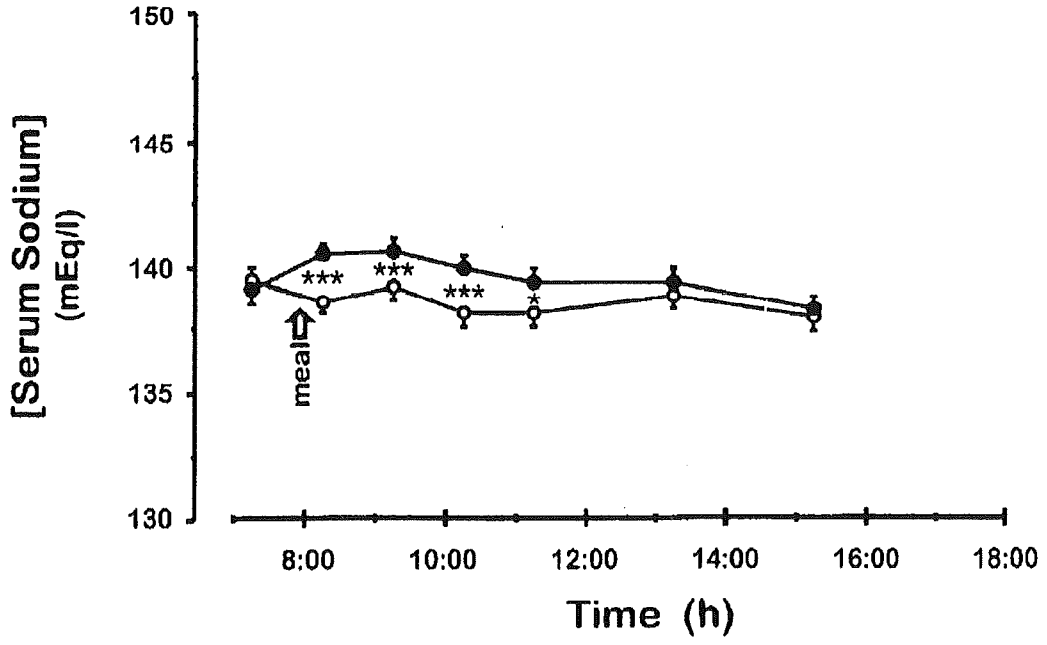


Figure 6. Effects of a high-salt meal (closed symbol) and low-salt meal (open symbol) in 11 healthy male subjects, from *Drummer et al. Am J Physiol 270:F301-F310, 1996*

1.3.3. Salt-induced LVH: can it be explained by repetitive salt loads?

Three possible mechanisms can be postulated to explain how a high-salt meal could put an acute load on the heart and even stimulate cardiac growth directly. If repetitions of small post-prandial loads on the heart occur frequently, they may in the long run lead to significant increases in cardiac mass.

1.3.3.1. Hemodynamic load of an acute salty meal

After a salty meal, the rise in plasma sodium consecutive leads to an increase in extracellular osmolality, thus generating fluid movements from the intracellular to the extracellular compartment. Additionally, the raised plasma osmolality stimulates the secretion of vasopressin so that more water is retained by the kidneys. All of these tend to increase the intravascular volume, which raises heart filling pressures, leading to an increase in stroke volume and cardiac output (Guyton, textbook). In turn, this will increase cardiac work (product of stroke volume and systolic blood pressure) even if an increase blood pressure may not be seen after a high salt meal (27). Interestingly, if the subject drinks the same quantity of water after a low- or a high-salt meal as in

the study of Drummer et al. (27), plasma sodium tends to decrease after the low-salt meal (see Figure 6), which tends to suppress endogenous ADH and increase urinary water excretion, thereby attenuating any meal-induced volume load. In contrast, those mechanisms are not present after the high-salt meal, further contributing to the volume load of a high-salt meal.

Many studies about post-exercise rehydration provide further evidence that sodium ingestion favors extracellular refilling. In a study performed by Maughan et al., six male volunteers were dehydrated by an average of 1.9 % of body mass by intermittent exercise in a warm, humid environment, and consecutively rehydrated post-exercise by drinks with various sodium concentrations (2, 26, 52 and 100 mmol/l). Ninety minutes after rehydration, the increase in plasma volume was significantly greater after the ingestion of drinks containing 52 mmol/l or 100 mmol/l NaCl, respectively, than after ingestion of drinks containing only 2 mmol/l NaCl. (71).

1.3.3.2. Sympathetic hyperactivity after a salty meal

A salty meal induces a sympathetic stimulation in two ways. First, the stepwise proximal gastric distension occurring during the ingestion of any meal increases muscle sympathetic nerve activity (also called gastrovascular reflex) (105). Second, various studies provide evidences that sodium concentration in CSF is increasing with the daily amount of salt consumed and modulates the sympathetic activity. In hypertensive patients increasing their daily salt-intake from 1-3 g/day to 16-18 g/day, the sodium concentration in the CSF increases in parallel from 145.3 to 147.7 mmol/L (62). Increased CSF Na^+ depolarizes relevant excitatory neurons in the brain through amiloride-sensitive Na^+ channels leading to sympathoexcitation. The intracerebroventricular administration of sodium-rich artificial CSF (0.2 M, 0.3 M or 0.45 M Na^+) in Dahl S, Dahl R and Wistar rats provoked a concentration-related increase in MAP, heart rate and RSNA (58). All together, these facts suggest that a high-salt meal will be accompanied by a greater increase of the sodium concentration in CSF and this will induce a more pronounced stimulation of the sympathetic system than obtained after a low-salt meal.

The influence of salt on sympathetic is also supported by the fact that sodium is able to reduce the turnover of norepinephrine and to increase the vascular reactivity to norepinephrine. In rabbits maintained for 3 weeks on high- (86 mEg/day), normal- (14 mEq/day) and low-sodium (0.2 mEq/day) diet, a high sodium intake decreased the turn-over of norepinephrine in the hypothalamus, midbrain, pons and medulla (102). Mancini et al. demonstrated that the pressor response to norepinephrine in 12 hypertensive subjects was significantly lower after a low-salt diet

so that a twofold dosis of norepinephrine was necessary to achieve the same rise in blood pressure. This suggests that salt may increase organ responsiveness to adrenergic stimulation (69).

1.3.3.3. Direct effects of sodium on myocardial growth

The direct effect of salt on heart to promote cardiac cell growth has been shown by Gu et al. (44) in isolated ventricular myoblaste cell cultures. Increasing the sodium concentration in the culture medium by as little as 6 mmol/L above a normal control value of 146 mmol/L induced an increase in the rate of protein synthesis and a decrease in the degradation of protein, an effect that was reversible by reinstitution of normal sodium concentration.

Local cardiac aldosterone release could also play a role in pressure independent, salt-induced ventricular hypertrophy. Aldosterone can be synthesized in extraadrenal tissues such as blood vessels, brain, and heart. Surprisingly, and in contrast to effects on adrenal aldosterone release, a high sodium intake increases cardiac aldosterone synthesis. In WKY rats a salt diet during 8 weeks was accompanied by a ventricular hypertrophy and increased cardiac synthesis of aldosterone and expression of AT1 receptor. This could contribute to cardiac hypertrophy independently of the systemic renin-angiotensin-aldosterone system and of arterial blood pressure (101). Further, sodium possibly alters the expression of growth factors since studies showed that sodium promotes fibrosis in the left ventricle via an overexpression of TGF-β1 in SHRs and WKY rats (113). In isolated ventricular adult rat cardiomyocytes. small increases in osmolarity to 315 ± 5 mosmol/l and 370 ± 8 mosmol/l by hypertonic NaCl solution resulted in dose-dependent transcriptional activation of the immediate-early genes egr-1 (4-and 5-fold) and c-fos (3-and 4-fold), respectively (112).

A growth stimulating effect of sodium seems to be clear since high salt intake with consecutive hypertrophy of other organs than heart has also been described. In young pigs fed on various salt diet (0.02%, 0.11% and 0.18%), pigs fed with 0.02% gained weight slower and less efficiently than pigs fed on higher salt levels (53).

1.4. The purpose of this study

We tested the hypothesis that a single high-salt meal leads to hemodynamic changes that could promote cardiac hypertrophy. We compared in the same healthy young individual the response to a moderately salted meal (about 45 mmol) and to a high-salt meal (same meal added with 120 mmol sodium), given in a random order, on various cardiovascular parameters that were continuously monitored before and up to 140 minute after the meal. In particularly, we tested whether a high-salt meal increases blood pressure, cardiac output and cardiac work more than a low-salt meal. We speculate that if hemodynamical loads are more prone to occur after a high-salt meal than after a low-salt meal, repetition of these loads along with post-prandial hyperenatremic peaks in a person eating too much salt regularly, over a period of months or years, may favor cardiac hypertrophy independently of a chronic effect of salt of the average level of blood pressure..

2. Methods

2.1 Subjects

We studied ten young male white volunteers, aged 20-29 years (22.9 ± 0.8, mean ± SEM). Their height was 180 ± 2 cm and their weight was 73.4 ± 2.7 kg (body mass index 22.6 ± 0.5 kg/m^2). The values for each subject are listed in Table 2. None of the subjects had any diseases or were taking any medication affecting the cardiovascular or autonomic systems. A medical examination confirmed in each subject the absence of arterial hypertension and cardiac problems. All volunteers were paid for their participation. Written informed consent was obtained from each subject. All procedures were carried out in accordance with institutional guidelines and complied with the Declaration of Helsinki.

Table 2. ***Subject characteristics***

Subjects	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m^2)
Subject 1	20	71	176	22.9
Subject 2	23	62	169	21.7
Subject 3	24	64	169	22.4
Subject 4	21	75	176	24.2
Subject 5	21	75	182	22.6
Subject 6	22	65	180	20.1
Subject 7	21	72	186	20.8
Subject 8	29	80	185	23.4
Subject 9	24	80	185	23.4
Subject 10	24	90	190	24.9
Mean	**22.9**	**73.4**	**179.8**	**22.6**
SD	**2.60**	**8.6**	**7.2**	**1.5**
SEM	**0.82**	**2.7**	**2.3**	**0.5**

2.2 Procedure

Each volunteer selected for the study came to our Institute on three separate days. On the first day, the volunteer received a document describing the study. The procedure of the study was explained in details and any questions were answered. Thereafter, the volunteer saw the room in which the experience was to be performed and was connected to the monitoring device (Task Force Monitor, see below) with all the required electrodes and cuffs as for a real experiment (test run of about 15 minutes). This procedure allowed each volunteer to get used to the machine and to understand the purpose of the recording equipment. At the end of this first session, each participant was requested to avoid taking any heavy exercise for at least 24h before the experiment, to avoid consumption of alcohol and coffee since the evening preceding the experiment, and was instructed to take a standardized breakfast the morning of the two experimental days. For each of those two days, the breakfast was to be taken at the same time in the morning and consisted of one or two cereal Kellogs Minipacks (provided by us), a fixed amount of milk and sugar, to ensure that the volunteer would come to the lab after the same breakfast and in similar conditions.

On the day of the experiment (day 2 or day 3), subjects came at 10:30 in the morning to the Institute after having had the standardized breakfast in the morning at home. They were asked to empty their bladder and then to sit in a comfortable armchair. They were connected to the recording equipment and the experience began at ~11 A.M. with the recording of baseline measurements. After one hour of recording, the participant received of the two test meals along with 300 ml water, which had to be fully taken in the following 20 minutes. Each meal consisted of either a moderately salted meal (**~45 mmol**) or the same meal with an **added 120 mmol of sodium chloride**. The two meals were given 7 days apart and the sequence of the meals was randomized for each volunteer (via coin throw) with the subject not knowing which meal he would receive first. Four of out ten ate subjects the low salt meal first. During the 20-min meal, the cuffs (finger and arm) for blood pressure were disconnected for avoid any discomfort while eating. At the end of the meal, the various cardiovascular parameters were recorded for an additional 140-160 minutes. During the pre- and postprandial periods, the subjects were allowed to read a book or to listen to soft music. In summary, the experiment lasted about 4 hours, one hour of baseline recording (preprandial period), the meal period (20 min) and at least 140 minutes of postprandial recording. None of the subjects mentioned a need to urinate during the experiment, which was ended at ~15:00 in the afternoon.

2.3 Meal composition

The meals were cooked on the evening preceding the experiment and stored in the fridge during the night. The meal was rewarmed in the microwave 10 minutes before the subject would eat it. It consisted of a low salt soup, chicken with cream and mustard, mashed potatoes and spinach (or beans for one subject who did not like spinach). It was accompanied by 300 ml of tap water. The composition of the low salt and high salt meal was exactly the same except that 30 ml of water was removed during the food preparation of the high salt meal and replaced by 30 ml of a 4 molar saline solution (10 ml in the soup, 10 ml in the potatoes and 10 ml in the spinach/beans). This corresponded to 120 mmol of NaCl added to the high salt meal. The energetic content of one meal was 739 kcal (3089 kJ.Computed from the nutrition labels on the food package). The precise composition of the meal is listed below:

- The low-salt soup consisted of 10.2 g of powder (Gemüse-Bouillon, natriumarm, Morga, magasin diététique) diluted in 300 ml of pure water. 100 g of powder contains: 22 g of protein, 40g of carbohydrates, < 0.5 g of lipids. Energetic value: 248 kcal for 100 g, or 25 kcal (106 kJ). Salt content: 26.7 mmol.
- 150 grams of frozen chicken produced in Denmark and sold by Migros. The chicken was cooked without butter or oil. 100g contains: 23 g of protein, < 0.5 g of carbohydrates, 2 g of lipids. Energetic value of 100g: 112 kcal, or 168 kcal (702 kJ). Salt content: 4.6 mmol.
- 317 grams of spinash (Blattspinat, Farmer's best, Migros) cooked with 1 dl of pure water. 100g contains: 3 g of protein, 3 g of carbohydrates, 0.5 g of lipids. Energetic value of 100g: 29 kcal, or 92 kcal (384 kJ). Salt content: 1.0 mmol.
- 155 grams of beans (Bohnen fein, Farmer's best, Migros) cooked in 1.5 of water. Bean replaced spinash in one subject only. 100g contains: 2 g of protein, 5 g of carbohydrates, 0.5 g of lipids.
- 67.6 grams of mashed potatoes (Karstoffelstock Mifloc, Migros) cooked in 2.5 dl of pure water. 100g contains: 2 g of protein, 14 g of carbohydrates, 2.5 g of lipids. Energetic value of 100g: 89 kcal, or 61 kcal (253 kJ). Salt content: 3.0 mmol.
- 150 ml of cream (Halbrahm, Valflora, UHT, Migros). The cream was added together with mustard to the chicken as last one was cooked.100 ml contains: 2.5 g of protein, 3.5 g of carbohydrates, 2.5 g of lipids. Energetic value of 100 ml : 251 kcal, or 376 kcal (1574 kJ). Salt content: 2.6 mmol.

- 11.9 grams of mustard (Moutarde du Roi Rotisseur, Migros). The mustard was added together with the cream to the chicken as last one was cooked. 100g contains: 7 g of protein, 10 g of carbohydrates, 8 g of lipids. Energetic value of 100g: 141 kcal, or 17 kcal (70 kJ). Salt content: 7.5 mmol.
- 30 ml of a 4 molar saline solution: 233.76 g of NaCl diluted in 1 liter of distilled water.

All together, the meal provided an energetic value of 739 kcal (3089 kJ). The salt content was ~45 mmol for the low salt meal and 165 mmol for the high salt meal.

2.4 Data acquisition by the Task Force Monitor 3040 i

All cardiovascular data presented in this study were acquired with the Task Force Monitor, a non invasive cardiovascular monitoring system produced by CNSystems, The brain and Heart Company in Graz, Austria. It is a computer-supported system that records the electrocardiogram (ECG), blood pressure and stroke volume continuously with "beat-to-beat" measurements capabilities. The following variables were monitored:

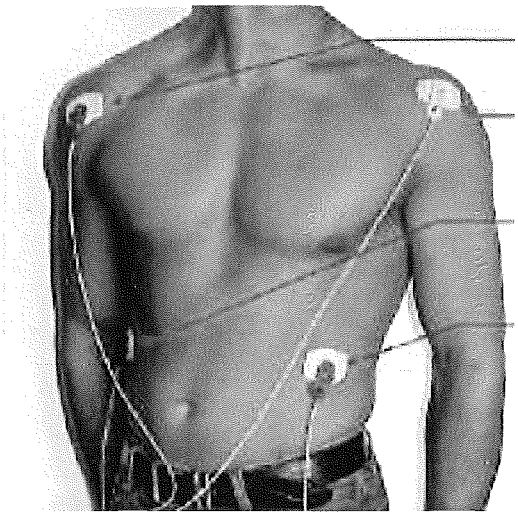


Figure 7. Placement of ECG electrodes

- **Heart rate**. A continuous **6-channel ECG** in the frontal plane with the 3 Einthoven derivations (I, II, III) and the 3 Goldberger derivations (aVR, aVL, aVF) was obtained using 4 electrodes placed on both shoulders and on both sides of the inferior abdomen as shown in the adjacent picture (Figure 7). The beat interval (RR interval, RRI) was derived from the ECG signal and used to compute the mean heart rate for each time period analyzed as 60'000/mean RRI.

- **Brachial blood pressure.** Blood pressure (oscillometric method) was recorded automatically every 10 minutes from an arm-cuff placed on the non dominant arm and set at heart level, to obtain systolic, mean and diastolic blood pressures.

- **Continuous beat-to-beat pulsatile finger blood pressure**. A non-invasive continuous measurement of blood pressure by **finger plethysmography** was obtained from the "Flying-V" finger cuff and the "Task Force Vascular Unloading Monitor". The system consists of two finger-cuffs placed around the third and fourth fingers which can be alternatively selected for the measurement of blood pressure (Figure 8). The cuffs were placed on the dominant hand, lying on a table approximatively at heart level. The absolute pressure values obtained from the continuous blood pressure device were automatically calibrated and corrected with the oscillometric blood pressure device. As much as possible we tried to record the signal on the same finger before the meal and during the 90-min postprandial period. However, in some subjects changes of finger were sometimes required because of a decreased quality of the signal. As the finger was changed, no pressure values were available for 2 or 3 minutes, time required to identify the plethysmographic signal and to recalibrate from two oscillometric measurements of the blood-pressure arm-cuff..

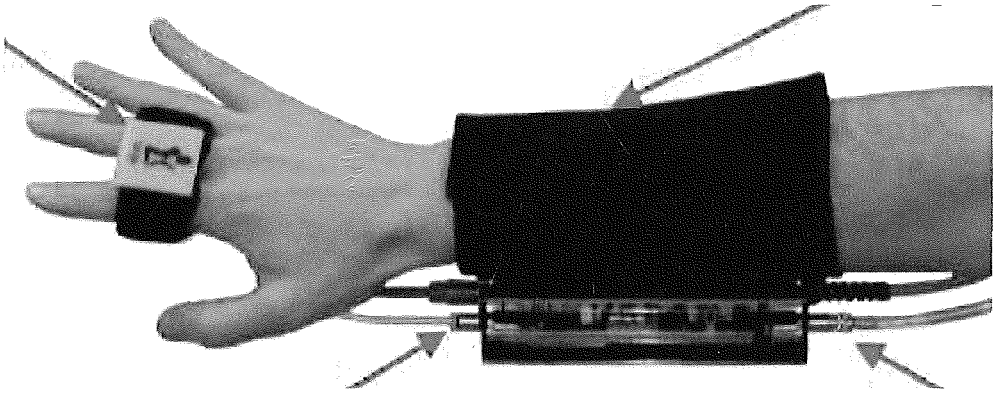


Figure 8. Finger plethysmographic equipment

- **Stroke volume.** A non-invasive continuous beat-to-beat measurement of stroke volume by **impedance cardiography (ICG)** was obtained using band electrodes, one placed on the back of the neck and two parallel electrodes placed on the lateral sides of the thorax at the level of the xiphoid process, as shown in Figure 9.

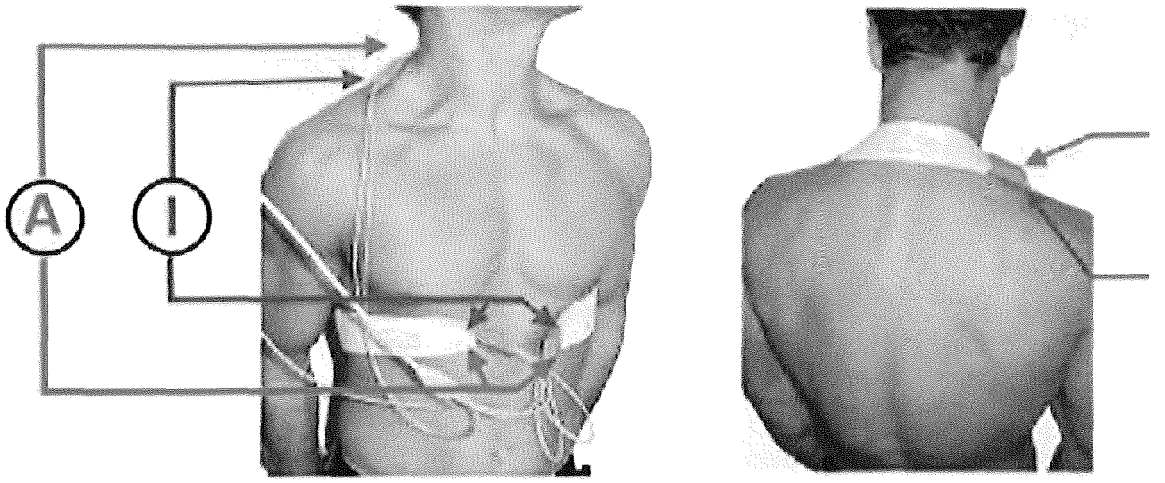


Figure 9. Placement of cardiac impedance electrodes.

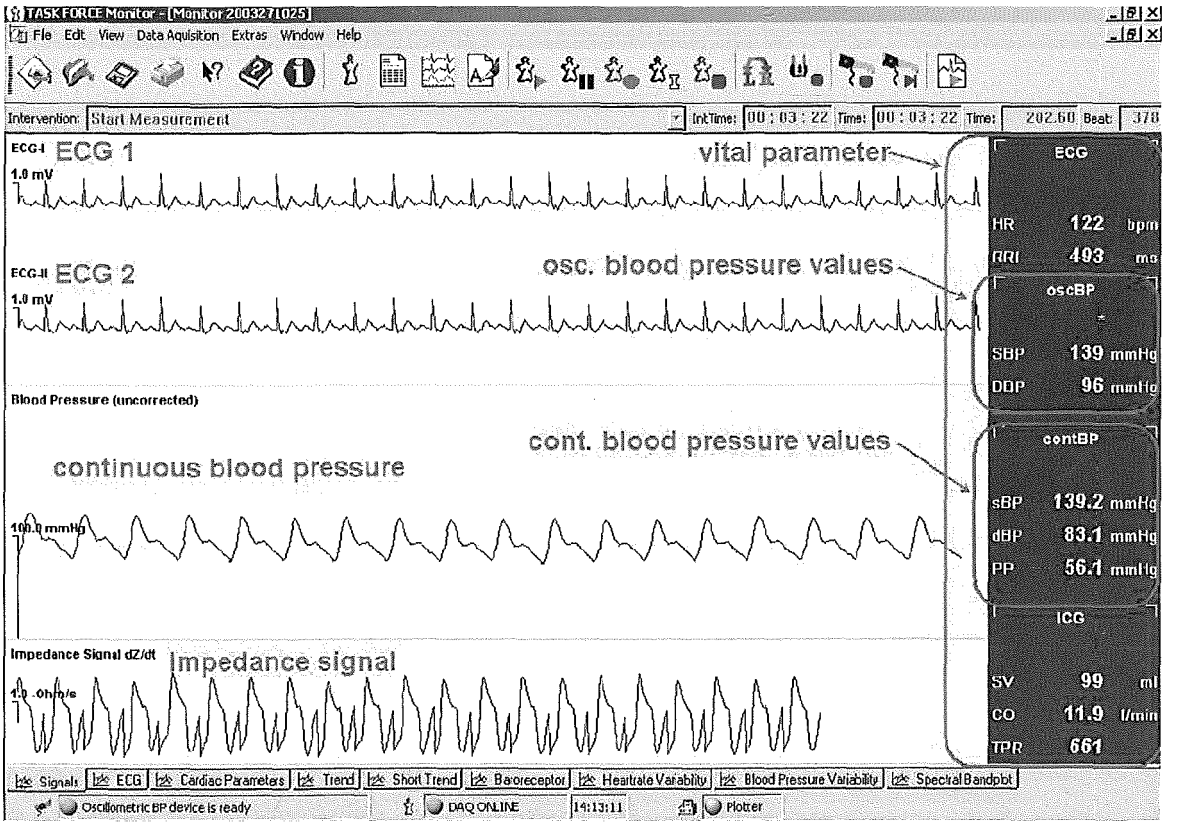


Figure 10. Computer screen output showing the hemodynamic signals.

The electrocardiographic (ECG), blood pressure plethysmographic and impedance (ICG) signals are collected continuously at a sampling rate of 1'000 Hz, displayed on the screen as shown in Figure 10, calculated on line for real time numerical display of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), stroke volume (SV) and cardiac output (CO) as shown on

the right side of the Figure. The signals also stored on disk as raw data for later display and analysis.

- **Cardiac output.** Cardiac output (CO) was computed as the product of mean stroke volume and mean heart rate for each time period analyzed.
- **Total peripheral resistance**. Total peripheral resistance (TPR) was computed as the ratio of mean arterial pressure and cardiac output for each time period analyzed.
- **Stroke work and cardiac power output.** Stroke work was estimated as the product of systolic blood pressure (from the beat-to-beat blood pressure signal) and stroke volume for each individual heart beat. To estimate cardiac work not just of a single heart beat, but over time, that is by incorporating heart rate, cardiac power output was also computed as the product of mean arterial pressure and cardiac output (W' = MAP x CO).
- **Cardiovagal baroreflex sensitivity.** The sensitivity of the baroreflex control of heart rate was determined from spontaneous fluctuations in systolic blood pressure and cardiac interval using the sequence technique (4) which has been shown to give similar results to the gold-standard phenylephrine method (85). Briefly, the Task Force Monitor identifies sequences where systolic blood pressure spontaneously increased or decreased by at least 1 mmHg per beat over at least three consecutive heart beats and, at the same time, cardiac interval changed by at least 4 ms per beat in the same direction. Linear regression was applied to the values of systolic pressure and the subsequent cardiac interval, and the baroreceptor sensitivity was taken as the average regression slope for all sequences with a sufficiently high r^2 value ($\geq$0.85). .

2.5 Analysis of data and statistics

Exclusion of artifacts

During the recording, occasional artifacts in the plethysmographic continuous blood pressure signal, in the ECG signal or the ICG signal were encountered, usually caused by speaking or small movements of the volunteers. Because the TFM software requires the definition of intervention marks at the time of recording with automatic computation of all data averages between one intervention mark and the next one, it was thus not possible to use the TFM software to exclude post-hoc short artifactual recording periods that would bring aberrant values to the true data. Instead, all recorded data were saved on a beat-to-beat basis to an Excel data. Visual replay of the recorded signals with the TFM system allows us to define precisely the beats of each artifactual period which could thus be removed manually from the Excel datasheet.

Analysis of selected data

For graphical display, the 1-hr pre-meal period was divided in three periods of 20 min, and the postprandial period (at least 140 min in all subjects) was divided in seven periods of 20 minutes. For each period, the individual beats of the various cardiovascular parameters (from the cleaned Excel datasheet) were averaged. To avoid computational errors, the average heart rate of a period was computed from the average RR value (HR = 60'000/average RR), and not from the average of all values of individual beat-HR. Similarly, the average CO of a period was computed as the product of the average HR and the average SV for that period, and not from the average of all values of individual beat-CO. TPR of a period was computed as the quotient of the average mean BP value and the average CO value for that period.

For the graphs we used the program Sigmaplot. For each cardiovascular parameter we took the average of the 10 subjects over the same period.

Statistical Analysis

The statistics were performed with the statistical program Instat (version 3.01, GraphPad Software, San Diego California USA). For each subject, the first 20-min of the pre-meal period (used for acclimatizing to the recording system) were excluded from data analysis. The control period consisted thus of the average of the last 40 min of the pre-meal period.

First, to analyze the effects of each meal *per se* on the various cardiovascular parameters (analysis within group), the seven 20-min postprandial periods were compared to the control period, using Analysis of variance for repeated measurements and Dunnett's multiple comparison analysis. Using a simple paired t-test, we also compared the 40-min control period to the first 120-min of the postprandial period.

Second, to compare specifically the low-salt meal to the high-salt meal (analysis between groups), we computed for each 20-min postprandial period the change from the baseline control period. The obtained changes from the low-salt meal were compared to those of the high-salt meal with Bonferroni adjustments for multiple comparisons.

All values are mean ± standard error of mean (SEM). The level of statistical significance was set as $P<0.05$.

3. Results

3.1 Basal cardiovascular values

The baseline values (control period consisting of the 40 minutes recorded preceding the meal) for each cardiovascular parameters as shown in Tables 3.1 (low-salt meal) and 3.2. (high-salt meal).

Table 3.1 Basal hemodynamic values for the low-salt meal.
Variables are sBP (systolic BP, mmHg), dBP (diastolic BP, mmHg), mBP (mean BP, mmHg), PP (Pulse Pressure, mmHg), HR (Heart Rate, beats per min), SV (stroke Volume, mL), CO (Cardiac Output, L/min), TPR (Total Peripheral Resistance, mmHg • min / L), sBPx SV (systolic BP times Stroke Volume, mmHg • mL)

Parameter	sBP	dBP	mBP	PP	HR	SV	CO	TPR	sBP*SV
Subject 1	114.50	62.16	80.18	52.33	63.11	95.95	6.06	13.24	10985.85
Subject 2	119.07	75.36	89.08	43.71	47.56	90.18	4.29	20.77	10737.18
Subject 3	111.57	67.52	79.27	44.05	53.31	91.70	4.89	16.22	10230.40
Subject 4	113.73	69.47	84.15	44.26	67.77	89.48	6.06	13.88	10177.26
Subject 5	112.66	75.05	86.94	37.61	69.01	83.55	5.77	15.08	9412.28
Subject 6	100.80	66.82	77.05	33.97	74.79	71.45	5.34	14.42	7201.44
Subject 7	109.19	66.67	81.51	42.52	61.11	80.59	4.93	16.55	8799.84
Subject 8	119.78	81.16	94.17	38.62	75.63	73.07	5.53	17.04	8752.12
Subject 9	103.55	62.81	76.16	40.74	73.05	88.14	6.44	11.83	9126.53
Subject 10	117.25	68.88	83.12	48.36	64.37	114.85	7.39	11.24	13465.87
Average	**112.21**	**69.59**	**83.16**	**42.62**	**64.97**	**87.89**	**5.67**	**15.03**	**9888.88**
SD	**6.26**	**5.95**	**5.62**	**5.30**	**9.17**	**12.37**	**0.89**	**2.79**	**1680.82**
SEM	**1.98**	**1.88**	**1.78**	**1.68**	**2.90**	**3.91**	**0.28**	**0.88**	**531.52**

Table 3.2 Basal hemodynamic values for the high-salt meal

Parameter	sBP	dBP	mBP	PP	HR	SV	CO	TPR	sBP*SV
Subject 1	107.44	59.62	73.00	47.82	64.43	95.88	6.18	11.82	10301.91
Subject 2	115.35	78.22	91.56	37.13	48.37	88.46	4.28	21.40	10203.61
Subject 3	111.12	68.70	80.64	42.42	54.01	89.08	4.81	16.76	9898.15
Subject 4	119.97	78.79	92.21	41.17	72.22	77.81	5.62	16.41	9334.66
Subject 5	116.92	74.71	87.30	42.21	68.16	80.40	5.48	15.93	9399.90
Subject 6	95.62	60.90	71.79	34.72	74.06	74.50	5.52	13.01	7124.29
Subject 7	102.42	61.53	75.05	40.89	57.23	83.63	4.79	15.68	8564.86
Subject 8	118.90	78.09	91.34	40.81	77.01	78.39	6.04	15.13	9320.93
Subject 9	110.46	67.56	85.23	42.90	81.34	85.64	6.97	12.24	9459.28
Subject 10	102.78	61.15	73.63	41.62	62.09	116.3	7.22	10.20	11952.45
Average	**110.10**	**68.93**	**82.18**	**41.17**	**65.89**	**87.01**	**5.69**	**14.86**	**9556.01**
SD	**8.02**	**7.95**	**8.34**	**3.47**	**10.61**	**12.09**	**0.94**	**3.20**	**1241.39**
SEM	**2.54**	**2.51**	**2.64**	**1.10**	**3.36**	**3.82**	**0.30**	**1.01**	**392.56**

Table 4. Statistical comparison of basal values between low- and high-salt meals.

Hemodynamic Parameters	**Low salt meal**	**High salt meal**	**Paired t-test P-value**
sBP (mmHg)	112.2 ± 1.98	110.1 ± 2.54	P = 0.3461, ns
dBP (mmHg)	69.6 ± 1.88	68.9 ± 2.51	P = 0.7002, ns
mBP (mmHg)	83.2 ± 1.78	82.2 ± 2.64	P = 0.6348, ns
PP (mmHg)	42.6 ± 1.68	41.2 ± 1.10	P = 0.2658, ns
HR (beats per min)	64.9 ± 2.9	65.9 ± 3.36	P = 0.4200, ns
SV (mL)	87.9 ± 3.91	87.0 ± 3.82	P = 0.5705, ns
CO (L/min)	5.7 ± 0.28	5.7 ± 0.30	P = 0.8436, ns
TPR (mmHg • min /L)	15.0 ± 0.88	14.9 ± 1.0	P = 0.7078, ns
sBPx SV (mmHg • mL)	9888.9 ± 531.5	9556.0 ± 392.6	P = 0.1138, ns

As shown in Table 4, the baseline values for all cardiovascular variables reported were not statistically different between the low-salt and the high salt meals (paired t-test).

For each subject, the baseline values of major cardiovascular variables were remarkably stable between the low-salt meal day and the high-salt meal day, as shown in Figure 11.

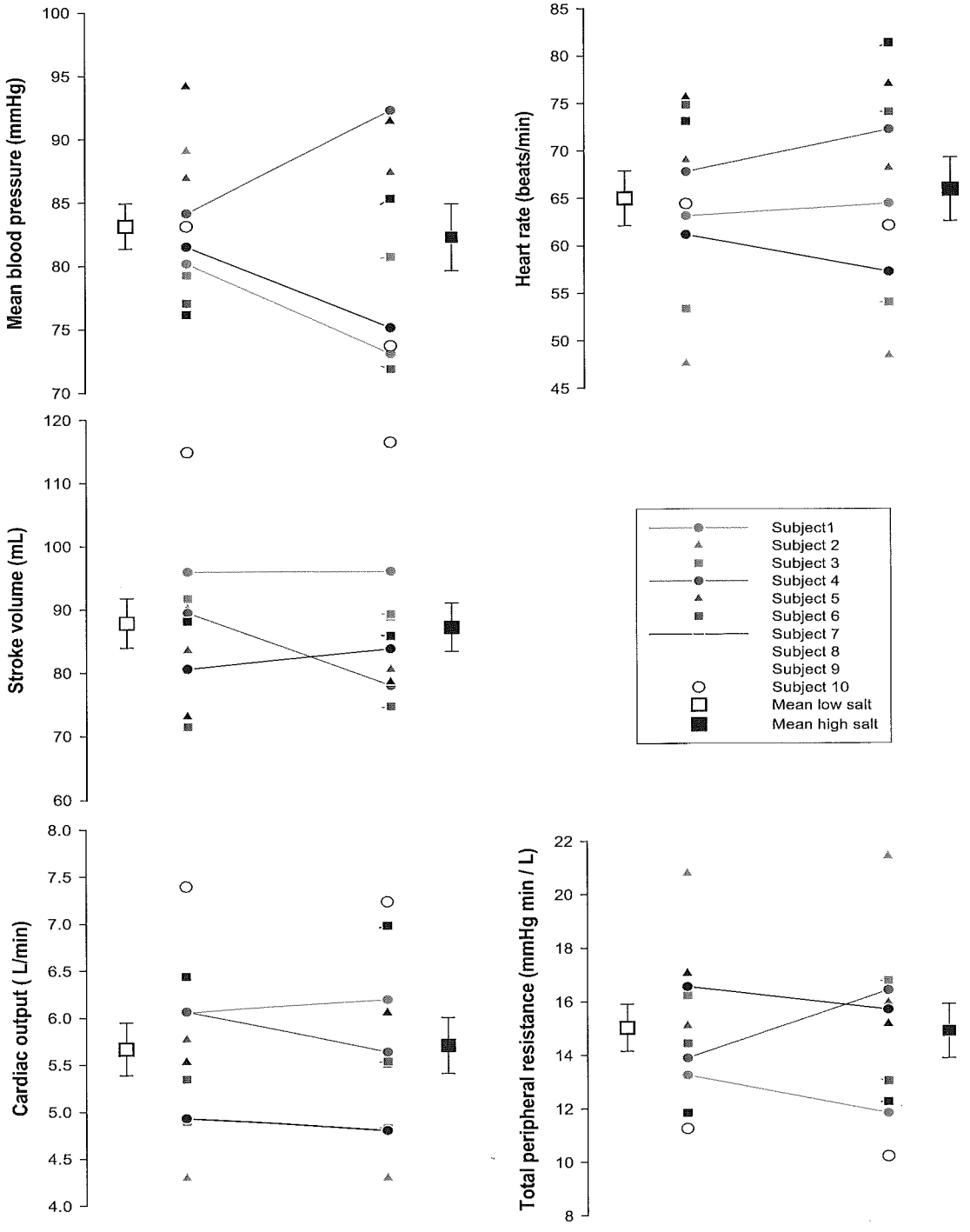


Figure 11. Baseline values for all ten individual subjects before the low salt (left side of each graph) and high salt (right side of each graph) meals for mean blood pressure, heart rate, stroke volume, cardiac output and total peripheral resistance

3.2 Cardiovascular response to the meal

The values of **systolic, mean and diastolic blood pressures** (from the continuous plethysmographic signal) during the control period and in response to the low-salt or the high-salt meal are shown on Figure 12. Diastolic and mean blood pressure values tended to decrease after both meals, with a significant fall at 20-minutes for the low-salt meal (diastolic pressure -4.9 ± 1.7 mmHg, mean arterial pressure -4.9 ± 1.6 mmHg) and at 60-minutes for the high-salt meal (diastolic pressure -3.3 ± 1.6 mmHg, mean arterial pressure -3.5 ± 1.5 mmHg), with a slower return to baseline values after the high-salt meal. For both meals there were no significant changes in systolic BP, although in the high salt meal SBP tended to increase in the period 20-40 min (+2.8 ± 1.5 mmHg, not significant) whereas in the low salt SBP tended initially to decrease during the first 20 min (-3.2 ± 2.3 mmHg, not significant). None of the changes were sustained. BP responses showed was very variable among subjects. In one subject, there was no change in blood-pressure, four subjects showed an increase, five subjects even a small decrease in blood pressure after the high-salt meal.

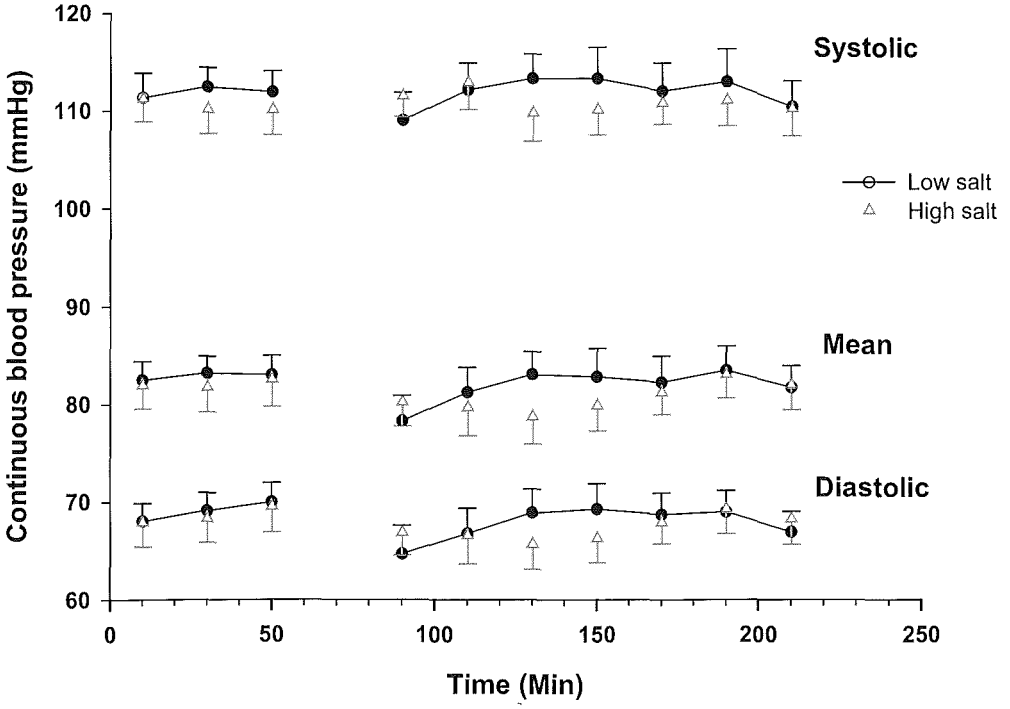


Figure 12. Systolic, mean and diastolic blood pressure measured continuously by finger plethysmography for both low and high salt protocols. Baseline period is displayed for three periods of 20 minutes, but only the last two periods are used for computation of baseline values. The meal is given at +60 minutes and recording is started at the end of the meal, 20 minutes later.

The continuous plethysmographic pressure signal was calibrated every 10 minutes by oscillometry with a brachial cuff. The values of **systolic, mean and diastolic blood pressures** from the oscillometric measurements are displayed in Figure 13.

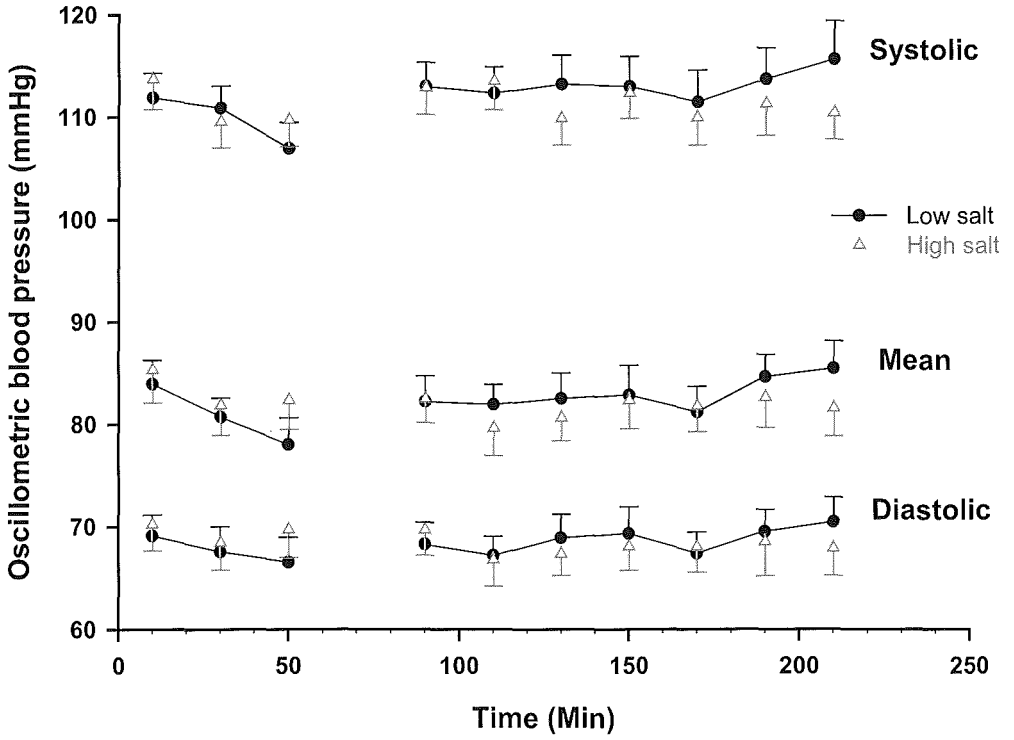


Figure 13. Systolic, mean and diastolic blood pressure measured by oscillometry with a brachial cuff. Blood pressure was measured every 10 minutes and values were used to calibrate values obtained by finger plethysmography.

Pulse pressure (Figure 14) increased significantly after the high-salt meal by 3.5 ± 1.1 mmHg (at 20-min) and 5.1 ± 1.4 mmHg (at 40-min) from a baseline value of 42.2 ± 1.1 mmHg, and then returned slowly towards baseline value. The pulse pressure changes after the low-salt meal were not significant. However, pulse pressure tended to be greater in the subgroup (n = 4) that showed an increase in the blood pressure after the high-salt meal. There were no significant differences between the low and the high salt meal.

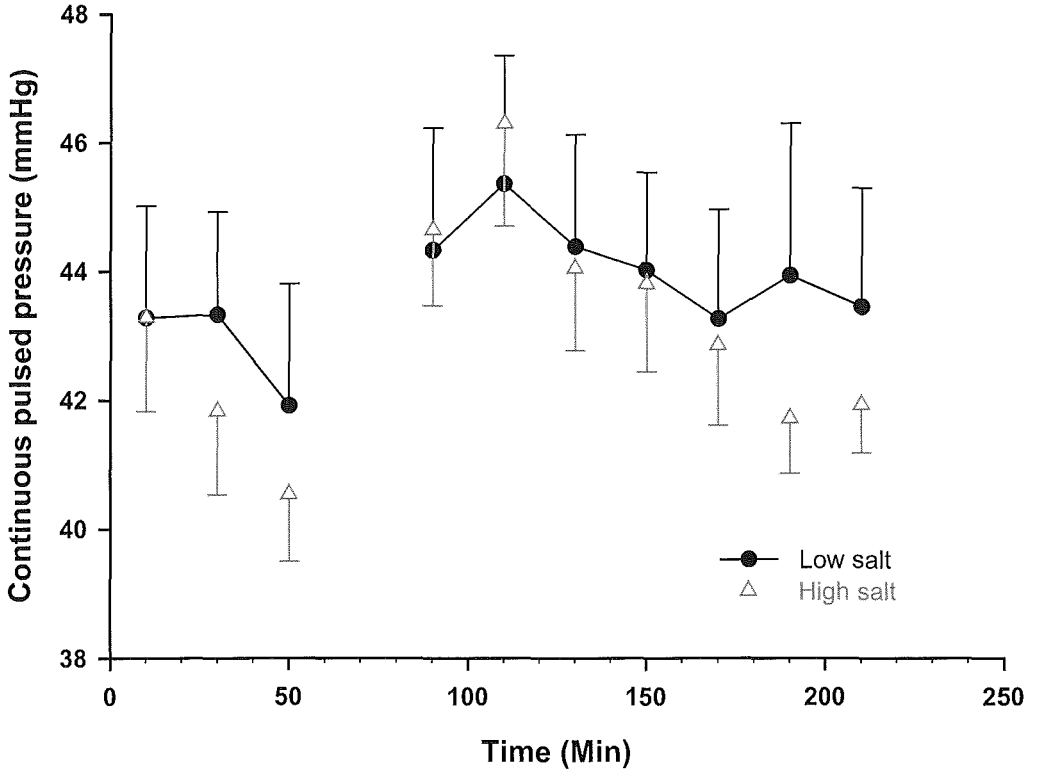


Figure 14. Pulse pressure measured continuously by finger plethysmography for both low and high salt protocols.

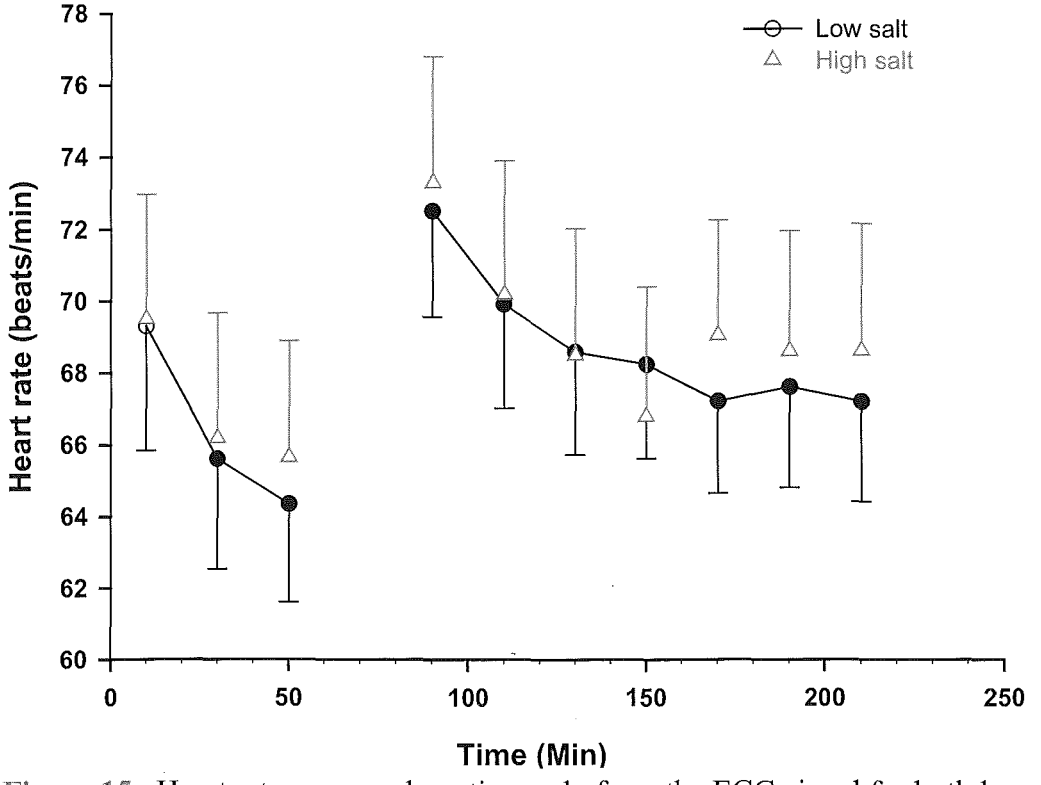


Figure 15. Heart rate measured continuously from the ECG signal for both low and high salt protocols.

The values of **heart rate** are shown in Figure 15. After both meals there was an increase in the heart rate, from 65.0 ± 2.9 to 72.5 ± 2.9 beats per min (20-min postprandial) in the low-salt meal, and from 65.9 ± 3.4 to 73.3 ± 3.5 beats per min (20-min postprandial) in the high-salt meal. In both meals the response peaked in the first 20 minutes and then slowly returned toward basal values. We could not observe a statistical difference between the 2 meals.

In both meals, we found a consistent and significant postprandial increase in **stroke volume** (SV) for all 10 subjects, from 87.9 ± 3.9 to a peak value of 97.5 ± 5.7 mL (+9.6 ± 2.5 mL) after the low-salt meal, and from 87.0 ± 3.8 to a peak value of 100.2 ± 4.9 mL (+13.2 ± 1.9 mL) after the high-salt meal. In both meals, the peak value of SV was reached during the second 20-period (from 20 to 40 minutes), as shown in Figure 16. SV remained significantly elevated throughout the postprandial period for the high-salt meal, and up to 80 minutes for the low salt meal. When averaging the first 2 hours postprandial values, the increase in SV during the high-salt meal (+10.5 ± 2.0 mL) was significantly higher ($p < 0.01$) than during the low-salt meal (+6.5 ± 1.6 mL).

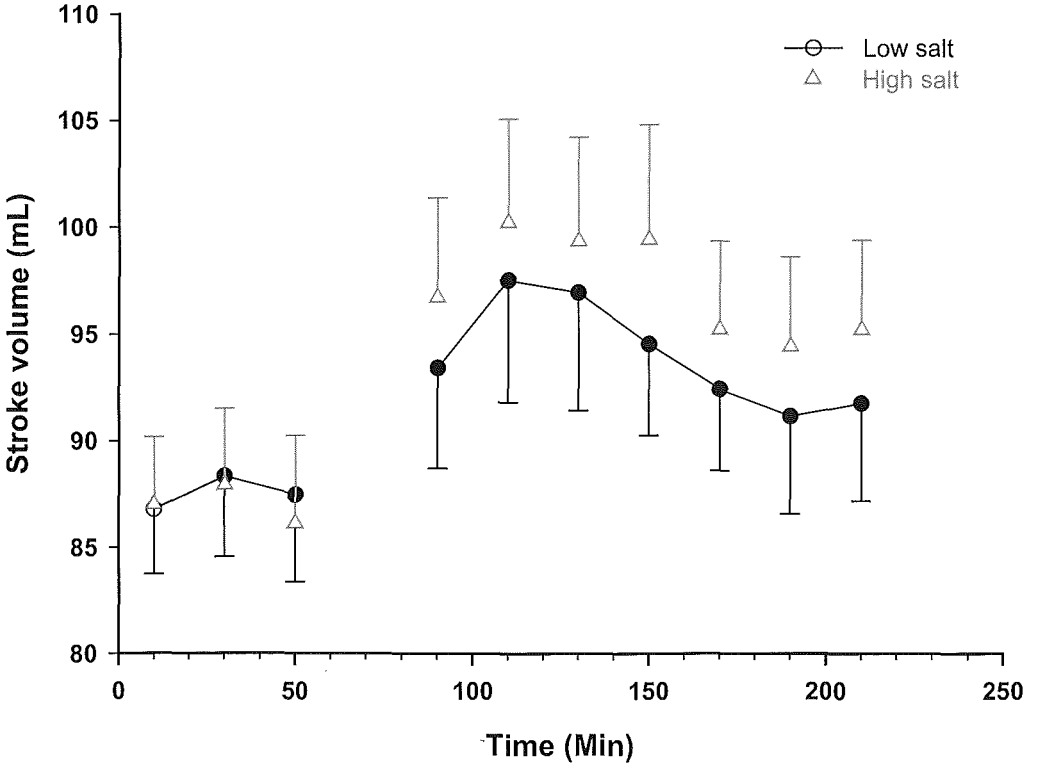


Figure 16. Stroke volume measured continuously by impedance cardiography for both low and high salt protocols.

Cardiac output (CO) increased significantly in both meals, from 5.67 ± 0.28 L/min to a peak value 6.76 ± 0.37 (+ 1.09 ± 0.1 L/min) after the low salt meal, from 5.69 ± 0.30 L/min to a peak value of 7.06 ± 0.42 (+1.37 ± 0.16 L/min) after the high salt meal, as shown in Figure 17. In both meals, the increase in CO was significant for all postprandial periods. However, when averaging the first 2 hours postprandial values, the increase in CO tended to be higher (p = 0.08) during the high-salt meal (+1.03 ± 0.14 L/min) than during the low-salt meal (+0.79 ± 0.10 L/min).

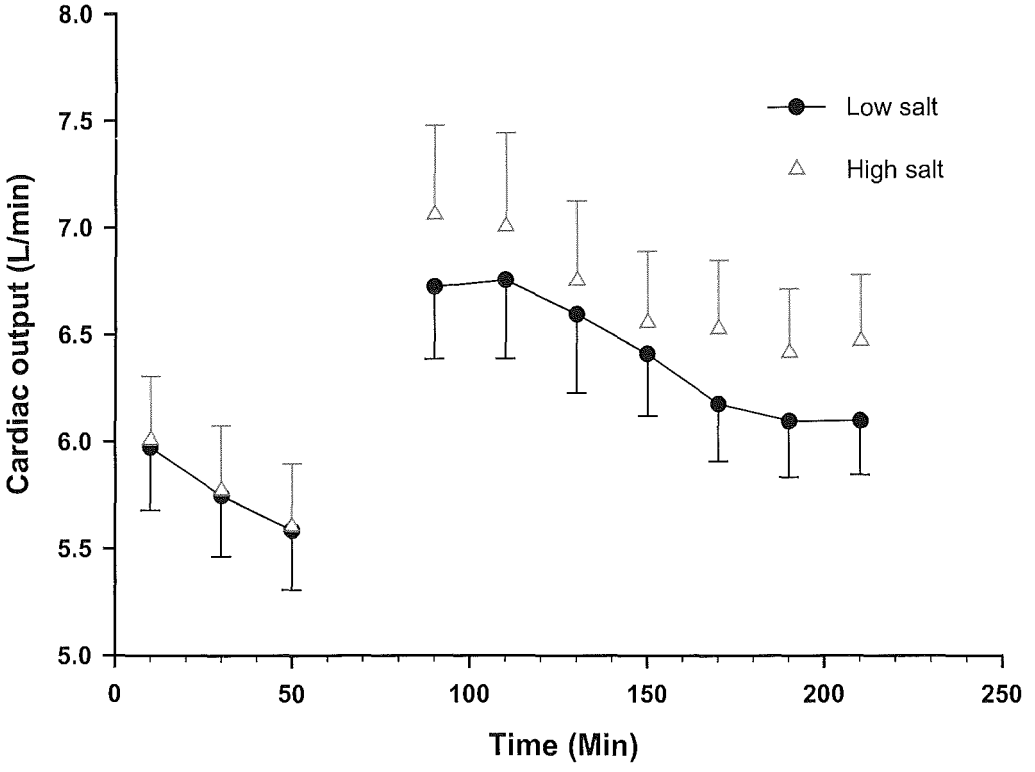


Figure 17. Cardiac output for both low and high salt protocols.

Total peripheral resistance (TPR) decreased significantly after both meals throughout the postprandial period (Figure 18), but tended to remain lower during the high-salt meal. When analyzing individual subjects, the TPR-response to the meals showed great variability with some subjects showing a more pronounced fall in TPR with the high-salt meal whereas others show a greater decrease in TPR with the low salt meal.

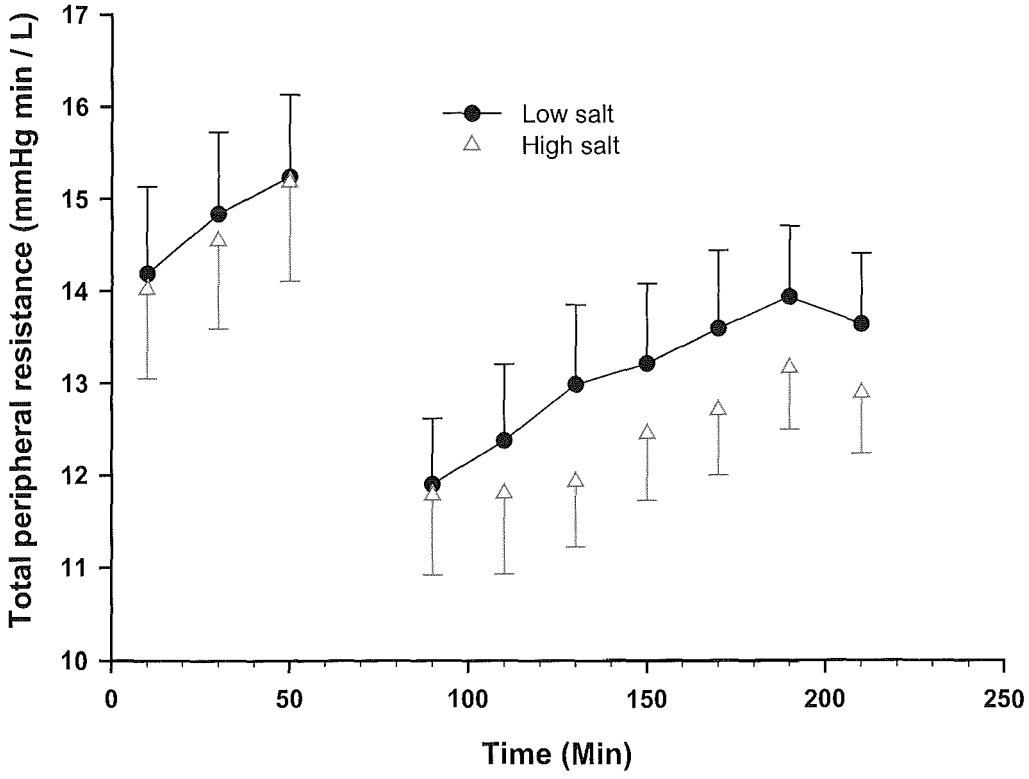


Figure 18. Total peripheral resistance for both low and high salt protocols.

We calculated **stroke work** as the product of plethysmographic systolic blood-pressure and stroke volume. Stroke work increased significantly from 9'889 ± 531 to a peak value of 11'007 ± 724 mmHg x mL in the low-salt meal, and from 9'556 ± 393 to a peak value of 11'299 ± 574 in the high-salt meal (Figure 19). However, the increase in stroke work was only transient in the low-salt meal (periods 40- et 80-min) whereas it remained significant throughout the postprandial period during the high-salt meal. When averaging the first 2 hours postprandial values, the increase in stroke work was significantly higher ($p < 0.01$) during the high-salt meal (+1263 ± 237) than during the low-salt meal (+725 ± 202).

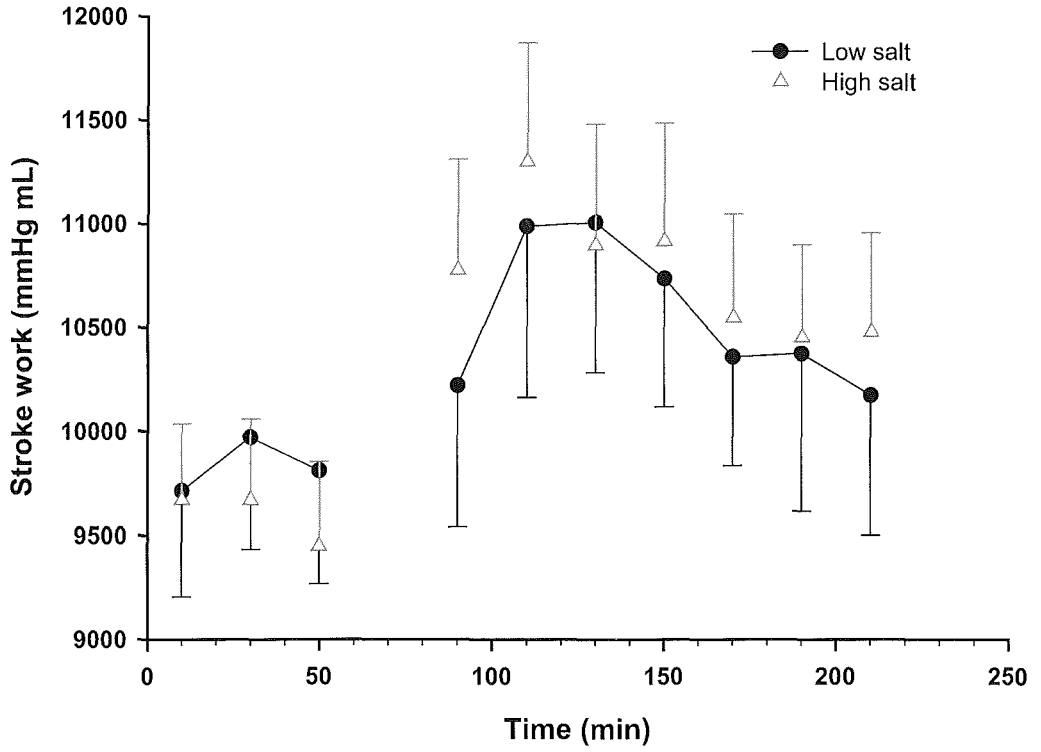


Figure 19. Stroke work for both low and high salt protocols.

Cardiac power output, calculated as the product of mean arterial pressure and cardiac output, represents the mean rate of energy input that the systemic vasculature receives from the heart at the level of the aortic root. Both meals elicited a significant postprandial increase in cardiac power output (Figure 20). There was no significant difference between the 2 meals but the postprandial increase tended to be higher after the high-salt meal (from 460.6 +/- 24.5 to 563.5 +/- 32.6, representing a peak increase of 22.34 %) than after the low-salt meal (from 462.8 +/- 22.4 to 546.9 +/-31.0, representing a peak increase of 18.2 %). In the high-salt meal, the response already peaked after 20 minutes and then slowly decreased but remained significantly elevated throughout the postprandial period (140 min). On the opposite, the response only reached its peak-value after 40 minutes in the low-salt meal, then slowly decreased and was no more significantly elevated during the last 60 minutes of the postprandial period.

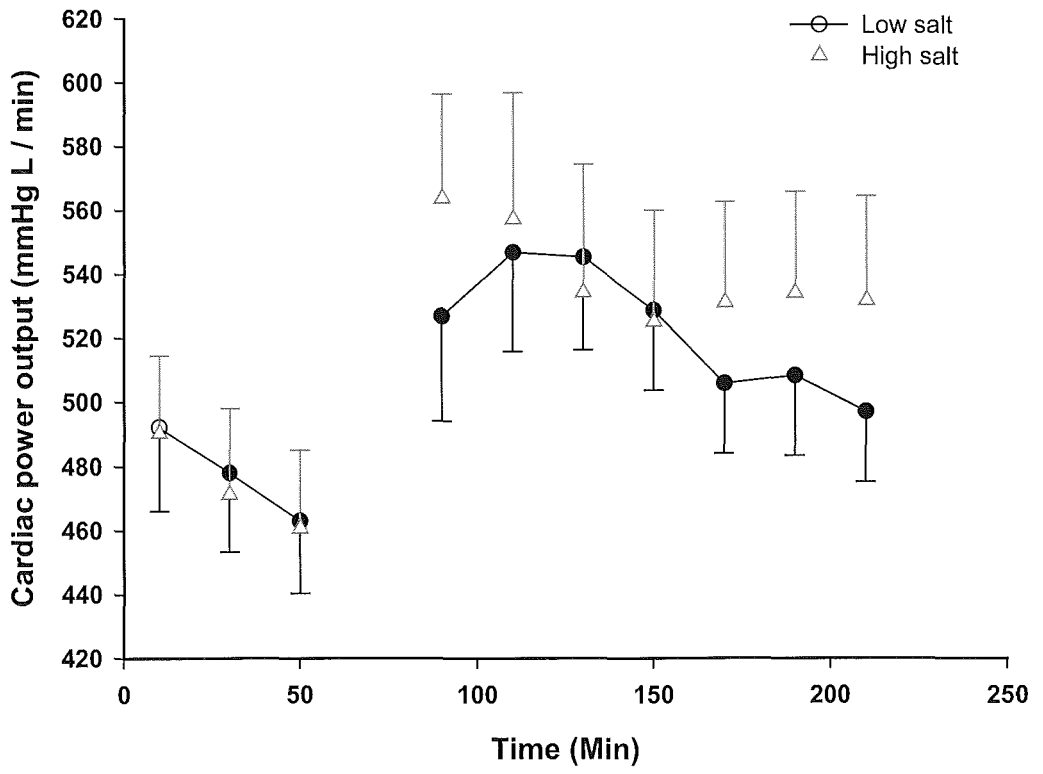


Figure 20. Cardiac power output for both low and high salt protocols.

Finally, changes in **baroreflex sensitivity** (BRS) after the meals are shown in Figure 21. BRS decreased significantly with the high-salt meal only during the first 20 minutes of the post-prandial period from a baseline value of 23.1 ± 2.5 ms/mmHg to 17.2 ± 2.3 ms/mmHg. Thereafter, BRS tended to return towards control. With the low salt meal, the decrease in BRS during the first 20 minutes postprandial from 21.5 ± 2.1 ms/mmHg to 16.4 ± 2.3 ms/mmHg was not quite significant. Although BRS tended globally to be lower during the high-salt meal compared to the low salt-meal, this was not significant.

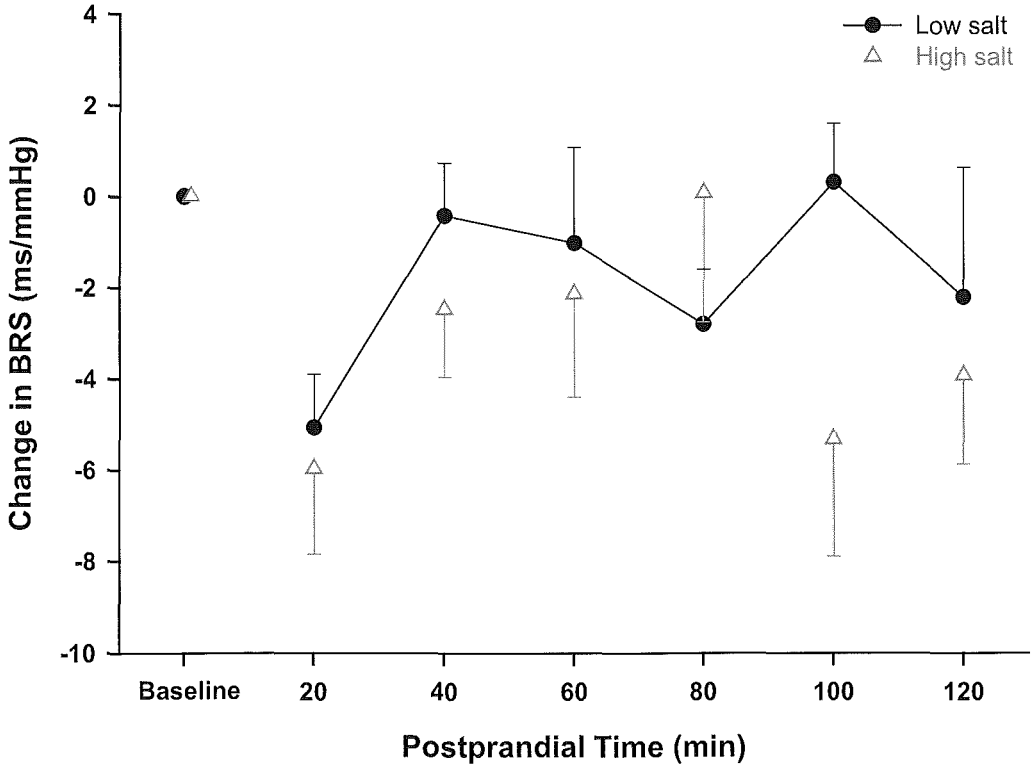


Figure 21. Baroreflex sensitivity for both low and high salt protocols.

3.3 Theoretical simulation of a postprandial hypernatremic peak

With our high-salt meal, each subject ingested an added amount of 120 mmol of sodium chloride within the 20 minutes of the meal. Assuming a complete intestinal absorption of the salt ingested and no additional urinary excretion of sodium during that time, those 120 mmol of NaCl would have been diluted in about 15 L of extracellular fluid volume (since sodium crosses very slowly the cell membrane). This would have lead to an increase in plasma sodium by **8 mmol/L** (= 120/15). However, the concomitant increase in extracellular osmolality would have drawn water from the intracellular compartment (30 L) into the extracellular compartment, which would attenuate the increase in plasma sodium, according to the computations below.

With an initial extracellular fluid volume of 15 L, an initial intracellular fluid volume of 30 L and an initial body osmolality of 290 mosmol/L water, one can compute an osmotic pool of 4'350 mosmol (= 15 L x 290 mosmol/L) for the extracellular compartment, of 8'700 mosmol (= 30 L x 290 mosmol/L) for the intracellular compartment, and 13'050 mosmol (= 4'350 + 8'700) for both compartments. Adding 120 mmol of NaCl (without any additional ingested water) into the system would have raise to total osmotic pool of the body by 240 mosmol (assuming a complete dissociation of NaCl into the body fluids). The resulting osmolality after osmotic equilibrium between the extracellular and the intracellular compartments would thus be of (13'050 + 240) mosmol / (30 + 15) L, or 295.3 mosmol/L water. Since all osmotic particles remain in the extracellular compartment, the extracellular volume would now be of (4'350 + 240) mosmol / 295.3 mosmol/L = 15.55 L, and the intracellular volume would be 8'700 mosmol / 295.3 mosmol/L = 29.45 L. The results are illustrated in Figure 22 using the classical Darrow-Yannet diagram of body fluid volumes versus osmolality. In conclusion, the expected increase in plasma sodium would be of 120 mmol of sodium divided by 15.55 L or **7.7 mmol/L**.

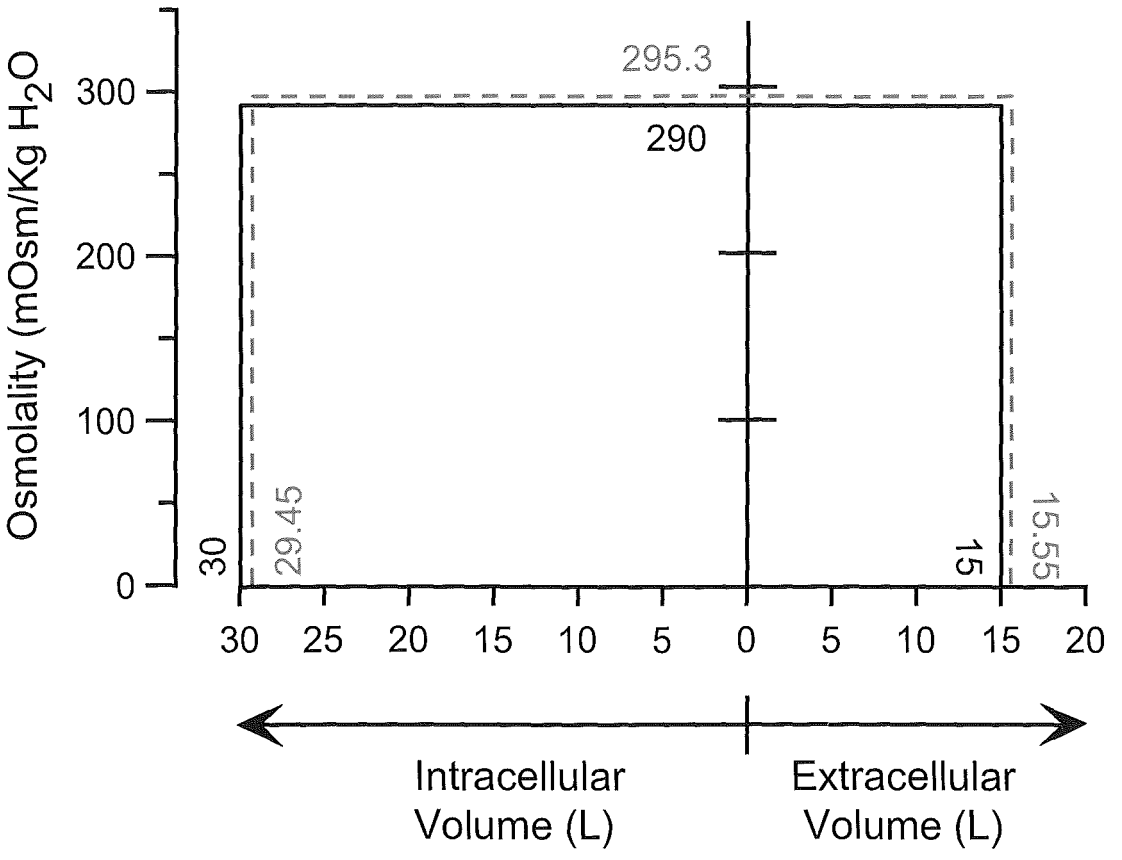


Figure 22. Classical Darrow-Yannet diagram illustrating the effects of adding 120 mmol of NaCl to the extracellular volume (see text for details).

4. DISCUSSION

Based on reports from the literature that a salt-rich diet may lead to left ventricular hypertrophy independently of changes in blood pressure (2), the present study was designed to test the hypothesis that a high-salt meal leads to a greater load on the cardiovascular system than a low-salt meal. Both meals elicited a series of cardiovascular changes including increases in heart rate, stroke volume, cardiac output, cardiac work and a fall in total peripheral resistance. The most important findings of our study is that, compared to the low-salt meal, the high salt meal induced: **(1)** a larger (when averaging the first 2 hours postprandial values) and significantly longer increase in stroke volume; **(2)** a trend ($p = 0.08$) for a larger increase in cardiac output when averaging the first 2 hours postprandial values; and **(3)** a larger increase in cardiac work. In addition, the high-salt meal elicited a significant postprandial increase in pulse pressure that was not found during the low-salt meal. We speculate that repetition of additional hemodynamic loads induced by regular salty meals over a period of months or years could favor cardiac hypertrophy independently of basal blood pressure. In addition, since each salty meal is expected to be associated with an increase in plasma sodium, repetition of post-prandial hypernatremic peaks may further potentiate the development of cardiac hypertrophy by a direct effect of salt on cardiomyocyte growth.

4.1 General cardiovascular responses to a standard meal

Both our meals (739 kcal = 3089 kJ) lead to an increase in heart rate (peak increase of ~12%) and cardiac output (peak increase of 24.1% with the high-salt meal and 18.6% with the low-salt meal) and a fall in total peripheral resistance. This is consistent with the classical response to a standard meal reported in the literature. For example, the ingestion of a standard 800-kcal meal in healthy volunteers was characterized by 18% increase in heart rate, a 27% increase in cardiac output and a fall in systemic vascular resistance (78). The increase in cardiac output was partially explained by a gastro-intestinal vasodilatation (increase in superior mesenteric blood flow by 84% above baseline levels) and a small increase in calf blood flow. The increase in cardiac output can be observed in various types of meals, whether it is composed primarily of carbohydrate, protein or fat (54). An increase in renal blood flow (and glomerular filtration rate) has also been described after a beef and chicken meal (99) and could contribute to the increase in cardiac output. Food ingestion of various meals (carbohydrate or fat) evokes also an increase (for at least 90 min) in

muscle nerve sympathetic activity as measured by microneurography (31). The post-prandial increase in cardiac output does not require an intact autonomous nervous system to the heart since it is preserved in patients with transplanted denervated hearts (110) and in young subjects pretreated with atenolol, a β_1-selective blocker (23).

Blood pressure post-prandial changes in healthy subjects are minor (78),(31) and often very variable among individuals. In our test meals, the postprandial response of blood pressure was very variable; some subjects showed an increase whereas others showed a decrease in arterial pressure, but this was not related to the level of salt-intake.

4.2 Does a high-salt meal lead to a greater load on the cardiovascular system?

In our study, the observed postprandial changes in several parameters suggest a more pronounced load on the cardiovascular system after ingestion of a salty meal.

4.2.1 Higher postprandial stroke-volume and cardiac output after a high-salt meal

Stroke volume (SV) increased significantly in both meals, but the increase was greater for the high-salt meal (+15.2 %, peak value) than for the low-salt meal (+10.9 %). Furthermore, SV remained significantly elevated throughout the postprandial period for the high-salt meal (up to 140 min of measurements) whereas the increase in SV was no longer significant after 80 minutes of the low-salt meal. Indeed, when averaging the first 2 hours postprandial values, the increase in SV was 4 mL/beat higher during the high-salt meal, accounting for a cumulative additional volume that is pumped by the heart of about 30 L over the 120-min post-prandial period. Similarly, cardiac output (CO) increased significantly in both meals. Although the 2hr-increase in CO was greater with the high-salt meal (+18.1 %) than with the low-salt meal (+13.9 %), the increase was at the limit of statistical significance ($p = 0.08$).

Note that both SV and CO were still significantly higher than baseline at the last post-prandial recording period (120-140 min) of the high-salt meal ($p < 0.01$), whereas during the low-salt meal the increase in SV was no longer significant and the increased in CO was milder ($p < 0.05$). Including the last recording period (120-140 min) in the average post-prandial CO analysis made the increase a little closer to statistically significance ($p = 0.06$) than when only the 2hr-average was considered ($p=0.08$). Therefore, had we recorded post-prandially longer, the cumulative increase in SV and CO (equivalent to an "area under the curve" analysis) would have been more pronounced.

The reasons of the increase in stroke volume and cardiac output are multiple. According to the Guytonian graphical analysis of CO (47), cardiac output stabilizes at the equilibrium point of two function curves, the **cardiac function curve**, which represents the ability of the heart to pump greater amounts with increasing filling pressures, and the **venous return curve**, which describes the amount of blood that returns to heart based on resistance to venous return and filling pressures of the cardiovascular system (Figure 23). According to this analysis, an increase in cardiac strength alone (Figure 23, left panel) will raise cardiac output by only a small amount. For cardiac output to increase significantly, it is necessary to act on the venous return curve either by *decreasing the resistance to venous return* (by dilating resistance vessels for example) and thus increasing the slope of the venous return curve, or by *increasing the mean circulatory filling pressure* (for example by increasing blood volume) and thus shifting the venous return curve to the right, or *by a combination of both* (Figure 23, right panel).

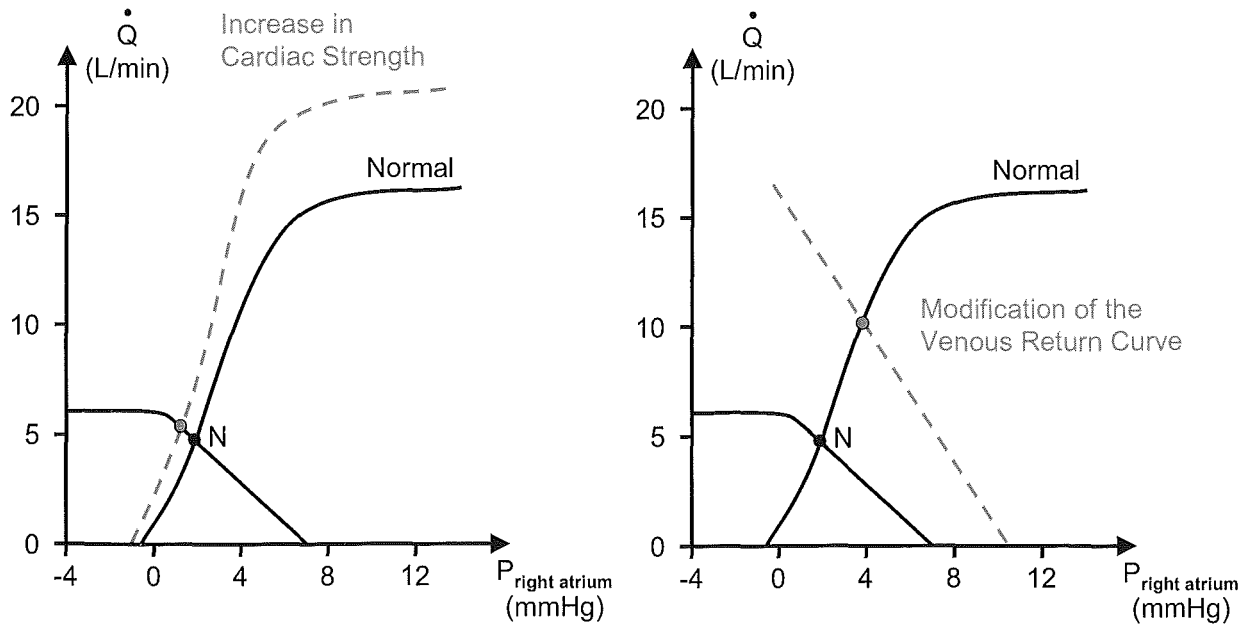


Figure 23. Guyton's graphical analysis of cardiac output. Adapted from Guyton ,1973

With the high-salt meal, we found only a tendency for a greater decrease in total peripheral resistance, which in itself could lead to a slightly greater slope of the venous return curve. In addition, a right shift of the venous return curve due to a greater increase in blood volume with the high-salt meal is expected, for the following reason. As computed in the Results section, the additional 120 mmol sodium supplied in the high salt meal should have lead to an increase in

plasma sodium, since an equal quantity of drinking tap water (300 ml) was given with both meals. We did not take any blood samples, but an increase in plasma sodium has been well documented after a high-salt meal. (see Figure 6). This should lead to secondary osmotic fluid shift from the intracellular to the extracellular compartment, increasing the extracellular fluid volume (ECFV) by 550 mL (or 3.6%) as computed in the Results section. In addition, the raised plasma osmolality stimulates the secretion of vasopressin, so that more water is retained by the kidney, further increasing ECFV. Theoretically, the increased osmolality should also increase thirst. However, none of our subjects expressed a thirst feeling with the high-salt meal, most probably because the expected rise in plasma osmolality was modest, below the threshold of thirst as depicted in Figure 24.

In contrast, ingesting the same amount of water during a low-salt meal (as in the study of Drummer et al. (27), and in our study) would tend to decrease plasma sodium concentration (see Figure 6), which tends to suppress endogenous ADH and increase urinary water excretion, thereby attenuating any meal-induced volume load. Indeed, in the study of Dummer, subjects taking the low-salt breakfast showed a much greater increase in urine flow and decrease in urine osmolality.

An increase in plasma volume is thus expected with the high-salt meal. Indeed, an oral ingestion of 164 mmol sodium in healthy volunteers led to a 3.1 % expansion in plasma volume (14). Plasma volume remains elevated with a more chronic high-salt intake as demonstrated by the following studies. In healthy male subjects submitted to one of four possible salt intakes at random (50, 200, 400 and 550 mmol/day for 7 days), plasma volume did not change under the low salt intake, but there was a dose-dependent increase in plasma volume with higher salt intakes, with increases of 8.7 ± 2.7% (under 400 mmol/day) and of 13.8 ± 2.9% (under 550 mmol/day) (52). When young normotensive subjects were shifted from a low (70 mmol/24h) to a high (250 mmol/24h) sodium intake over 10 days, there was a significant increase in plasma volume (determined by Evans blue dye dilution technique) with parallel decrease in plasma protein concentration and hematocrit (20). The intravascular volume expansion after salt intake is also supported by the fact that salt supplementation for 3 to 7 days (120 mmol/day) increased orthostatic tolerance (defined as time to presyncope after tilting) and plasma volume measured by Evans blue dilution technique (77).

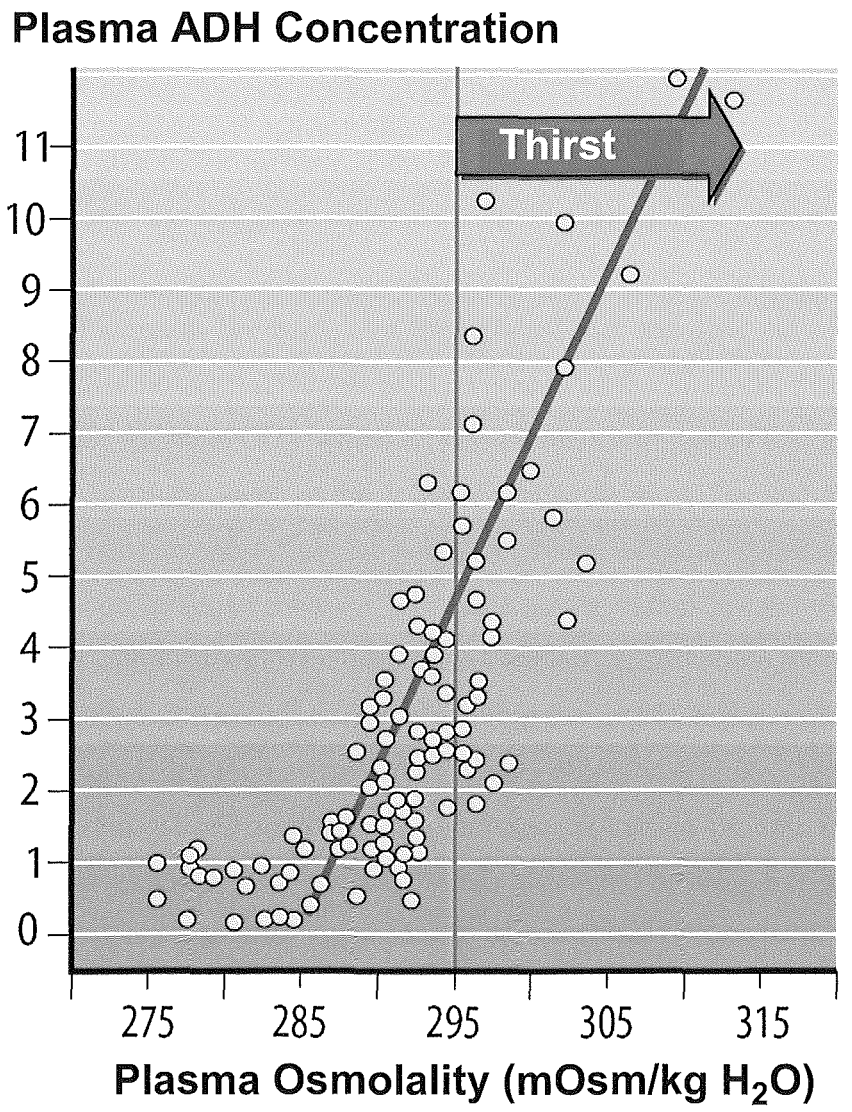


Figure 24. Relationship between plasma osmolality and plasma antidiuretic hormone (ADH) concentration with a threshold at ~285 mOsm/kg, below the threshold of thirst (~295 mOsm/kg). From Schmidt-Lang-Thews, Physiologie des Menschens, Springer, 2005

4.2.2 Higher stroke work and cardiac power output after high-salt meal

In our study stroke work was computed as the product of plethysmographic systolic blood-pressure and stroke volume. Both meals elicited a postprandial increase in stroke work: the response was greater after the high salt meal (+ 18.2% peak value) than after the low-salt meal (+11.3% peak value). Furthermore the postprandial stroke work remained significantly elevated throughout the postprandial period after the high-salt meal ($P<0.01$) whereas it was only significantly higher than baseline during 2 periods (20 min to 60 min) after the low-salt meal. Finally we found a statistically significant difference in stroke work for the first postprandial period ($p < 0.05$) and when averaging

the first 2 hours postprandial values, the increase in stroke work was significantly higher ($p < 0.01$) during the high-salt meal than during the low-salt meal.

Therefore, our results showed a higher stroke work after a salty meal. Stroke work was predominantly augmented by an increase in stroke volume because systolic blood pressure showed great variability among the subjects without conclusive trend or increase when averaging all subjects.

Stroke work represents the work of a single heart beat and does not incorporate heart rate. To that purpose, cardiac power output was also computed as the product of mean arterial pressure and cardiac output (W'=MAP x CO), which represents the mean rate of energy input the systemic vasculature receives from the heart at the level of the aortic root, i.e., the hydraulic power that would produce the same average flow in a steady stream without pulsation (11). Both meals induced a significant postprandial increase in cardiac power output but the response tended to be greater after the high-salt meal and remained significant during the whole postprandial period after the high-salt meal whereas it was significant only during the first 80 minutes after the low-salt meal. In both meals, the postprandial increase in cardiac power output was almost completely achieved by a raise in cardiac output. Since heart rate changes were similar in the two meals, cardiac power output showed similar trends as stroke work, but the difference between meals were more striking when using stroke work (as SBP x SV) than cardiac output power (as MAP x CO).

4.2.3. Does blood pressure contribute to the higher cardiovascular load after a high salt-meal?

4.2.3.1 Blood pressure response to both meals

By averaging all 10 subjects, there were no clear changes in **systolic blood pressure**. With the high salt meal, there was a small increase in SBP in the period 20-40 min (+2.8 ± 1.5 mmHg), but this increase was not significant. With the low salt in contrast, SBP tended initially to decrease during the first 20 min (-3.2 ± 2.3 mmHg), but this decrease was not significant and not sustained. With both meals, **mean arterial pressure** tended to decrease slightly and transiently, by 4.9 ± 1.6 mmHg in the low salt meal (first 20 min) and by 3.5 ± 1.5 mmHg in the high salt meal (period 40-60 min). Similar changes were seen with **diastolic blood pressure**.

The postprandial response of systolic, mean and diastolic pressure showed great variability among the 10 subjects, with some subjects exhibiting an increase whereas others showed a decrease. We could not observe a clear postprandial trend respective increase in systolic, mean and

diastolic pressure after the high-salt meal. But our results are similar to results described in the literature. In 12 normotensive healthy subjects ingesting a low-salt breakfast (4.6 mmol) and a high-salt breakfast (104.6 mmol) at a 1-week interval, there was no increase in blood pressure, measured by sphyngomanometry, after the high-salt meal (BP was 119.2 ± 3.2 mmHg over 77.7 ± 4.1 mmHg before the meal and 120.5 ± 1.85 mmHg over 75.5 ± 2.9 mmHg 90 minutes after the meal) nor any difference in pressure between the high-salt breakfast and the low-salt control experiment (27). In another study, a progressive increase in daily sodium intake by step increase of 50 mmol/day, from 10 mmol/day to 350 mmol/day over 7 days in 6 normotensive subjects, did not induce significant changes in mean blood-pressure. Mean BP was 88.4 ± 2.2 mmHg after 4 days of stabilization at 10 mmol sodium /day and 84.2 ± 4.0 mmHg under 350 mmol/day (93).

Since changes in BP can be caused by parallel changes in plasma sodium (35), one could expect that a high-salt meal, that leads to a hypernatremic peak, would be characterized by some increase in BP. However, large changes in plasma sodium (by 10 or 15 mmol/L) are required to observe changes in BP within a few hours (24). For smaller increases in plasma sodium, BP usually does not increase unless the changes in plasma sodium are sustained for several days. Similar observations are made when analyzing CSF sodium concentration. In 30 Sprague Dawley rats submitted during 7 days to intracerebroventricular infusion of respectively 0.15 M, 0.5 M and 1.5 M NaCl solutions, the blood-pressure rose significantly at day 1 (+ 22.7 mmHg) in the 1.5 M group whereas the increase in systolic pressure was slower and not significant (approximately + 4 mmHg) in the 0.5 M group (61).

In our study, we computed a maximal theoretical postprandial increase in plasma sodium of 7.7 mmol/L. However, this assumes an instantaneous intestinal absorption of sodium, no leak of sodium into the cells and other fluid compartments and no increase in sodium excretion. Thus, the increase in plasma sodium with a single high-salt meal was probably much less, so that the possible pressor effect of plasma sodium would have take days to appear and therefore could not be observed during our postprandial recording (140min). Furthermore, the effect of an acute increase in plasma sodium is dependent of age. Young subjects under 26 years may increase plasma sodium, extracellular volume and blood volume without elevation of arterial blood-pressure when submitted to an acute increase in sodium intake (93). In contrast, individuals over 60 years of age may be more sensitive to rise in plasma sodium induced by a high-salt diet (60). In our study, all subjects were less than 26 years old except for one (29-year). The lack of an increase in BP with a high-salt meal is thus not unexpected.

4.2.3.2 Influence of pulse pressure

Since systolic pressure tended initially to increase whereas diastolic pressures tended to decrease after the high-salt meal, pulse pressure showed a significant increase from 41.2 ± 3.5 mmHg to 46.3 ± 5.0 mmHg, representing a peak increase of 12.4 %, 40 minutes after the high-salt meal. Pulse pressure also tended to increase after the low salt meal, but none of the 20-min period was significant from baseline.

Many studies have established that pulse pressure is an independent predictor of cardiovascular events. Benetos et al. observed in 19'083 men aged between 40 and 69 years that pulse pressure was a significant independent predictor of global cardiovascular and especially coronary mortality in all age groups whereas no significant relationship was found for cerebrovascular mortality (3). Furthermore, an increase in pulse pressure was shown to be an independent predictive factor of congestive heart failure so that each 10 mmHg elevation in pulse pressure was accompanied by 14 % increase in risk of congestive heart failure in an elderly cohort (10).

In the literature, an association between an increase in pulse pressure and ventricular hypertrophy is also described. Pulse pressure was directly related to left ventricular mass index and showed a significant and independent influence on left ventricular mass index after multiple regression analysis in 333 men and women aged in mean 47 ± 0.5 years with untreated arterial hypertension (109). However it remains controversial whether this association is really independent of the systolic pressure because the stiffness of arterial wall will not only raise the pulse pressure but will also increase systolic pressure which is a well known potent stimulator of ventricular hypertrophy. Therefore, Verdecchia et al. suggest that the ventricular hypertrophy seen with elevated pulse pressure is mediated by a parallel increase in systolic pressure. In their study, conducted on 2'545 untreated hypertensive subjects whose age spanned from 15 to 90 years, they found no independent association between pulse pressure and left ventricular hypertrophy after univariate and multivariate analysis (107).

However, one should be cautious in equating an increase pulse pressure with an increased cardiovascular risk. An increase in pulse pressure may result from a combination of two causes: an increase in stroke volume and/or a decrease in arterial wall elasticity. In our study, the higher SV after a high salt-meal could partially explain the higher PP values. To that purpose, we grouped all 20-min values of SV with the corresponding 20-min values of PP. When all individuals were included, there was no correlation ($r = -0.02$) between SV and PP. However, when excluding from the analysis 3 individuals (subjects 1, 4 and 5) who show little or no changes in PP despite large

changes in SV, there was a relatively good correlation between the increase in SV and the increase in PP ($r = 0.46$) in the remaining seven individuals. Thus, in our study, the cause of the increased PP seems to be explained, at least partially, by the increase in SV. However, a contribution of an increased stiffness due to postprandial neurohormonal changes cannot be excluded.

4.2.4 Changes in total peripheral resistance in response to a meal

Although the ingestion of any meal will induce a regional sympathetic activation in the skeletal muscles and various other organs (16), total peripheral resistance (TPR) classically decreases after a meal due to the metabolically-induced vasodilatation observed in the gastro-intestinal tract and in the renal circulation (78). In our initial hypothesis, we postulated that the decrease in TPR may be less pronounced with the high-salt meal, leading possibly to an increase in BP. This hypothesis was based on the consecutive raise in CSF-sodium seen after a high-salt intake, which in turn induces central sympathetic stimulation so that we would expect a greater sympathetic stimulation after the high-salt meal leading to higher vascular resistances. However, our data does not support this hypothesis as the salty meal tended to lead to a similar fall in total vascular resistance than after the low-salt meal. Yet, this does not exclude a possible higher sympathetic stimulation with the high-salt meal. Indeed, an increase in plasma osmolality related to the high salt intake may have a vasodilator effect that may offset any increase in sympathetic vasoconstriction. The vasodilator effect of hypertonic saline could be mediated by rheological (A) and humoral factors (B).

A) The postprandial hypernatremic pick provokes a hemodilution by a fluid retention in the vascular system. Inoue et al. (59) were able to demonstrate in rats that a volume expansion with saline solution leads to an increased CO without detectable changes in MAP because the consecutive hemodilution elicits a fall in total peripheral and renal vascular resistances. In healthy adult subjects, the infusion of 0.15 ml/kg/min 3% NaCl during 60 minutes induced an increase in MAP whereas vascular resistances decreased 20 and 40 minutes after the infusion started (32). Further, when young normotensive subjects are shifted from a low (70 mmol/24h) to a high (250 mmol/24h) sodium intake over 10 days, there is a significant increase in plasma volume (determined by Evans blue dye dilution technique) with parallel decrease in plasma protein concentration and hematocrit, consistent with an hemodilution. The salt-induced increase in CO and SV was compensated by a decrease in TPR so that MAP was unaltered in both diets (20). Thus, hemodilution by decreasing blood viscosity may decrease vascular resistance. Hemodilution will also decrease oxygen transport capacity per liter of blood, leading to metabolic vasodilatation (17).

Both a decreased viscosity and metabolic vasodilation could contribute to a higher blood flow after a high-salt meal. In addition, hemodilution-induced higher blood flow will increase shear rate, favoring the release of NO from endothelial cells.

B) ANP is also an important mediator of vascular resistances. The intravascular volume expansion secondary to salt-intake leads to atrial wall distension, which is a potent stimulator for ANP secretion. In turn, ANP will induce natriuresis, suppress the renin-angiotensin-aldosterone system and provoke a vasodilation which could contribute to the lower resistances observed in our study after the high-salt meal. This is supported by the fact that the ingestion of a high salt meal (with 104.6 mmol NaCl) induced a short-term increase in plasma ANP within 30 minutes after the meal ingestion whereas no significant changes in plasma ANP could be observed after the ingestion of a low-salt (4.6 mmol NaCl) meal (27). However the postprandial response of ANP is influenced by the previous sodium diet. So in subjects submitted to initial normal (150 mmol/day) or high-salt intake (350 mmol/day), the ingestion of an additional 300 mmol NaCl load induced no significant rise in plasma ANP. Only subjects put previously under a low-salt diet (10 mmol NaCl /day) showed a significant increase in plasma ANP (100).

The effect of salt on TPR could also be time-dependent. Intravenous infusion of 3% NaCl during 60 minutes led to a biphasic response in TPR in young healthy subjects, while MAP increased continuously (32). Vascular resistances first fell during the first 40 minutes and only showed a rise during the last 20 minutes. The late increase of TPR was associated with a 30% increase in plasma norepinephrine suggesting that the increase in TPR was mediated by the sympathetic nervous system. Plasma sodium showed a significant raise from 137 mmol/l to 139 mmol/l and 141 mmol/l after 15, respectively, 30 minutes. So even during the intravenous administration of sodium, there is a time interval between the increase in plasma sodium and the consecutive sympathetic activation. We can postulate that this interval may be longer if sodium is given orally so that we could not observe the raise in vascular resistances during our postprandial recordings (140 minutes). In fact, Huang et al. (57)demonstrated a progressive, but delayed rise in CSF Na^+ concentration when Dahl S rats are switched to an high salt-intake with 8% NaCl rat chow. CSF Na^+ concentration increased slowly in the first night, reaching a significant increase at day 2 and then to stabilize at + 6 mmol/L in the third night after beginning the high salt diet. The rise in resting blood pressure only appeared in the daytime of day 3 and reached its plateau on day 5, i.e. 0.5-2 days after sodium concentration in CSF had increased.

4.2.5. Changes in baroreflex sensitivity

The baroreflex is an important mechanism for acute arterial pressure regulation. An increase in arterial pressure activates the stretch sensitive nerve fibers located in the adventicia of the carotid arteries and the aortic arch (baroreceptors), causing an increased neural input to brainstem nuclei. This is in turn leads to an inhibition of the sympathetic vasomotor center and to a stimulation of the vagi, resulting in bradycardia and an attenuation of the increase in BP.

In our study, we found during the first 20 minutes following the meal a significant reduction in baroreflex sensitivity after the high-salt meal and a not quite significant decrease after the low-salt meal. Thereafter, BRS was no longer different from the baseline. However, BRS tended to be generally lower (although not significantly) throughout the high-salt post-prandial period in comparison to the low-salt meal, as shown on Figure 21. This could be related to the expected higher plasmatic sodium concentration and osmolality after the high-salt meal as increases in plasma sodium / osmolality are known to inhibit the central control of cardiac baroreflex.

For example, Bealer et al. tested the effects of an acute infusion of NaCl on baroreflex function in male Sprague Dawley rats fed a normal chow (1). BRS was determined by continuously recording BP and heart rate (HR) in the conscious rat while raising BP with phenylephrine or lowering BP with sodium nitroprusside, and fitting a sigmoidal logistic curve through the BP-HR data (Figure 25). In each rat, BRS was monitored twice, first in the basal state and then at the end of a 30 minute infusion of NaCl 2.5 molar (which raised plasma osmolality from 297 to 311 mOsm/kg H_2O). Results show that an acute rise in plasma osmolality decreases HR maximum, increases HR minimum and decreases the slope of the BP-HR relationship (gain of the baroreflex). An increase in peripheral osmolality by about 14 mosmol/ leads to a significant reduction in cardiac baroreflex sensitivity.

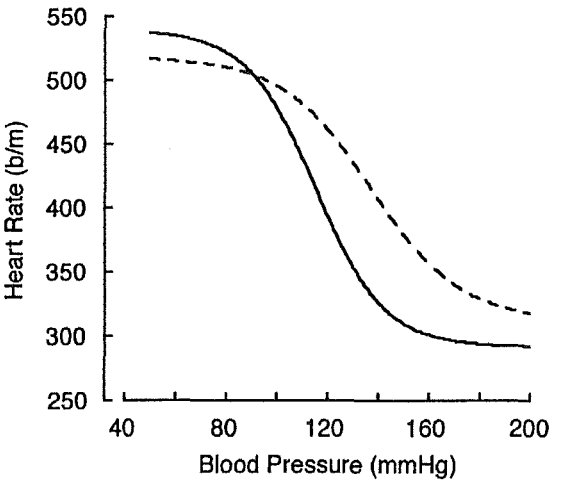


Figure 25. Sigmoid logistic function curves before (solid line) and following hypertonic saline (dashed line).

In our experiments (as described above in **section 3.3**), we computed an increase in osmolality by 5.3 mmol/l water consecutive to the ingestion of the high salt meal. This milder increase in plasma osmolality may have contributed to the greater decrease in BRS after a high-salt meal.

4.3 Hypothesis that post-prandial hypernatremic peaks could contribute to LVH

There is evidence that plasma sodium may be increased after a single high-salt meal. The ingestion of a high-salt breakfast (104.6 mmol NaCL) caused a mild but significant increase in plasma sodium that was still evident during the 4th hour after the high-salt meal (27). In another study performed by Gill et al. (39), 8 young healthy male volunteers ingested a large English breakfast containing 3.7g of sodium chloride (63 mmol). The postprandial plasmatic sodium rose gradually from 141.9 ± 0.8 mmol/l preprandially to 144.6 ± 0.8 mmol/l four hours after the meal. In our study, we did not perform any measurements of preprandial and postprandial plasma sodium, but based on the literature, significant changes in plasma sodium were expected. Thus, if a subject repeatedly ingests high-salt meals, repeated postprandial hypernatremic peaks are expected and could subject the heart to a hypernatremic environment during several hours per day.

In addition, there are evidences that during chronic high-salt intake plasma sodium may not return to normal between meals, leading thereby to a sustained postabsorptive hypernatremic environment. The change in plasma sodium may be observed already within 24 hours of high-salt intake. Indeed, plasma sodium was 145.7 ± 1 mmol/L 24 hours after instauration of a high-salt diet (82.0 mmol/day) in normal dogs, whereas it was significantly lower at 142.8 ± 0.4 mmol/L in dogs kept on a low salt diet (7.5 mmol/day) (96). Studies in humans have also demonstrated an increase in basal plasma sodium concentration during high salt intake. For example, when young normotensive individuals are switched from a daily sodium intake of 20 mmol for 5 days to a daily intake of 200 mmol for another 5 days, plasma sodium raises from 143 ± 1 mmol/l to 145 ± 1 mmol/l (91). Further, an increase in daily consumed sodium from 50 mmol/day to 300 mmol/day for each 14 days caused a statistically significant raise in plasma sodium in both normotensive (+1.6 mmol/L) and hypertensive (+3.0 mmol/L) subjects (60). These and other studies reviewed by de Wardener et al. (24) confirm in both normotensive or hypertensive subjects that increasing salt intake from a low (10-50 mmol/day) to a high (200-400 mmol/day) intake over relatively short periods (3 to 14 days) in general leads to a significant increase in plasma sodium (up to 3 mmol/L). Inversely, studies of acute salt reduction in normotensive and hypertensive subjects found a consistent fall in plasma sodium (50).

The increase in plasma sodium during high salt intake over time may be small (1 to 3 mmol/L), but this represents basal levels in the morning before the first meal. With each high-salt meal taken during the day, a supplementary hypernatremic peak is expected, which could lead to

larger increases in postprandial sodium concentration and a sustained hypernatremic environment throughout the day. These fluctuations in plasma sodium under high salt are important since plasma sodium may be directly implicated (i.e. independently of changes in BP) in the genesis of left ventricular hypertrophy as described below

Sodium is able to induce ventricular hypertrophy by a direct stimulation of cardiomyocyte growth (44). Similar results were also obtained in cultured endothelial cells of umbilical vein where an increase by 10 mmol/L of sodium concentration induced after 3 days a rise in RNA expression of many hypertrophy related factors, including endothelin, IGF and TGF (45). The trophic, pressure-independent, effect of sodium is also suggested by Du Cailar et al. (28). The authors found in hypertensive subjects that urinary sodium excretion is higher in the subgroup of subjects in whom the left ventricular mass was "inappropriately high" to the level of arterial pressure than in subjects with appropriate left ventricular mass. Subjects with inappropriate left ventricular mass were characterized by a higher concentric ventricular hypertrophy despite a lower mean arterial pressure and similar known duration of hypertension compared with subjects with adapted left ventricular mass. As stated by the authors, "these results suggest that a possible trophic effect of sodium on cardiac mass may be superimposed on the level of arterial pressure in essential hypertension".

The activity of the Na^+/H^+ exchanger (NHE) seems to have a key role in the development of ventricular hypertrophy, providing further evidence for the direct influence of sodium on ventricular structure. The NHE is responsible for 50 % of the Na influx into the cells. It exchanges one intracellular H^+ for one extracellular Na^+, resulting in an intracellular decrease in $[H^+]$ and increase in $[Na^+]$. Intracellular Na stimulates protein synthesis and inhibits protein degradation, but also stimulates Ca^{2+} influx via the Na^+-Ca^{2+} exchanger, resulting in a rise in intracellular calcium (which is known to be a potent signal for cell growth and hypertrophy). The NHE isoform 1 (NHE-1) has been implicated in different models of hypertrophy, such as aortic constriction-induced hypertrophy and postinfarction myocardial hypertrophy. Male CD-1 mice submitted to a banding of the thoracic aorta developed an enhanced LV-weight after 2 weeks whereas mice treated concomitantly with cariporide (a selective inhibitor of NHE-1) had a marked attenuation of left ventricular hypertrophy. At the histological level, cariporide attenuated the development of cardiomyocyte hypertrophy (70). Treatment with cariporide during 1 month in spontaneous hypertensive rats induced a slight decrease in systolic blood pressure (6 mmHg) and a regression of the heart weight to body weight ratio, whereas a larger decrease in BP with nifedipine did not lead to ventricular regression (8). Finally, various studies also report a rise in Na-influx or upregulation of Na-influx transporters with consecutive increase in intracellular sodium in cardiac hypertrophy (108).

4.3.1 Possible reasons for the difference between computed and observed postprandial raise in plasma sodium

In our theoretical simulation, we computed that the supplementary sodium load of 120 mmol should generate a postprandial increase in plasma sodium by 7.7 mmol/L. However, the ingestion of 104.6 mmol of sodium with a breakfast induced a maximal postprandial increase in plasma sodium between 1-2 mmol/L (27). Various mechanisms may attenuate the theoretical postprandial increase in plasma sodium and, therefore, may explain the difference between our computed and the effectively observed raise in plasma sodium:

a) The intestinal absorption of sodium is not instantaneous (as assumed in our model), but occurred probably over more than one hour in our subjects. Gastric emptying of ingested saline was measured in rhesus monkeys by indwelling intra gastric cannula. After the infusion of 150 ml of isotonic saline into the gastric cannula, gastric emptying was variable, but took usually between 10 to 20 minutes (90). Since our subjects did not drink saline, but ingested it with the meal, gastric emptying was delayed, resulting in delayed intestinal sodium reabsorption.

b) During that time, some of the ingested sodium may enter the intracellular compartment.

c) An acute sodium load will induce a progressive increase in urinary sodium excretion over hours. When subjects previously submitted to a normal salt diet (150 mmol salt/day) during 5 days, drink an 800 ml soup containing 300 mmol sodium and 1200 ml tap water over an hour period, their urinary sodium excretion increases from 100 μmol/min before the salt load to a peak value of approximately 325 μmol/min 2 hours after the ingestion of the soup (90; 100). When the same dose of salt was given intravenously (2 liters of physiological saline over 60 min), a situation that would simulate an instantaneous intestinal ingestion, urinary sodium excretion had already doubled in the second 30-min period after starting the salt load. This rapid increase in sodium excretion would thus attenuate any increase in plasma sodium. In the same study, comparison of the time course of sodium excretion after intravenous or oral salt loading also provides strong evidence for the delayed intestinal absorption of the salt ingested.

Altogether, these observations may explain why plasma sodium after a salty meal may not increase by more than 2 mmol/day. However, this increase may be sufficient to stimulate cell growth. Indeed, in the study of Gu et al.(44), increasing the sodium concentration by only 2 mmol/L above normal (146 mmol/L) caused the cellular protein content of cultured dog coronary artery smooth muscle cells to increase by 85% ($P<.01$). If the same is true for cardiac myocytes, small increments in plasma sodium repeated with every salty meals could be sufficient to trigger cardiac hypertrophy.

5. Conclusions

In our study, we compared in the same healthy young individuals the cardiovascular response of a moderately salted meal (45 mmol) and of a high-salt meal (165 mmol sodium). Our results revealed statistically significant differences and additional trends demonstrating a differential cardiovascular response after the ingestion of a low-salt and a high-salt meal. The high-salt meal induced a longer and significant higher increase in SV and stroke work. Further, CO also tended to be higher after the high-salt meal. There was no clear trend for the arterial blood-pressure (in particular no clear increase after the high-salt meal) except a significant raise in pulse pressure during 40 minutes after the high salt-meal.

We can speculate that if every salty meal is characterized by a greater increase in SV, CO, cardiac work and pulse pressure, this should lead to repetitive loads on the heart and may thus, in the long run favor cardiac hypertrophy. We did not measure plasma sodium, but according to the literature a postprandial increase in plasma sodium is also expected after a high salt meal. In turn, the expected, repetitive hypernatremic peak after each high-salt meal may additionally promote cardiac hypertrophy since sodium has various direct growth stimulating effects on myocardial cells and is able to directly stimulate the sympathetic nervous system.

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