Autonomic nervous system mediated effects of food intake

Interaction between gastrointestinal and cardiovascular systems

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Autonomic nervous system mediated effects of food intake

Interaction between gastrointestinal and cardiovascular systems

Door het autonome zenuwstelsel tot stand gebrachte effecten van voedselinname

Wisselwerking tussen het gastrointestinale
en het cardiovasculaire systeem

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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door

Narender Pieter Van Orshoven
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Co-promotor:
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aan mijn ouders

CONTENTS

CHAPTER 1

General introduction

The autonomic nervous system and the nervous control of the gastrointestinal and cardiovascular systems have been studied extensively, but knowledge about their interactions during and after food intake is limited. The studies presented in this thesis focus on the mechanisms behind these interactions and the potential consequences of their failure. This chapter describes the background and aims of these studies and provides a short description of the methods used.

1 BACKGROUND

1.1 The somatic and autonomic nervous system

The elementary functions of the human organism can be classified into vegetative and animal functions (Bernards and Bouman, 1988). The animal functions are directed at the exchange of information with the external environment and are carried out by the somatic nervous system, which is under the influence of the will. Information is gained via stimulation of the sensory organs and is processed in the sensory nervous system; reactions are mediated by the motor system. The somatic nervous system can be divided into a central section, consisting of the brain, brainstem and spinal cord, and a peripheral section, consisting of the peripheral sensory and motor nerves. The vegetative functions are directed at maintaining a constant internal environment and are mediated by the autonomic (vegetative or visceral) nervous system (ANS) and the hormonal system. These two systems interact and for this reason they are also referred to as the neuroendocrine system. Their function is mainly involuntary. Hormones are produced by a number of glands in the body, and their production is regulated mainly by feedback mechanisms between the hormone-producing gland and the hypothalamus. The hypothalamus is the highest neuroendocrine integrative center: it coordinates autonomic and endocrine responses in order to enable organ systems to function as an integrated system (Guyton and Hall, 2000).

The autonomic nervous system consists of central and peripheral sections. A number of nuclei in the hypothalamus form the primary vegetative center. Functionally, most reactions of the autonomic nervous system are based on feedback loops made up of reflex arches. Afferent fibers transport information from painreceptors, mechanoreceptors (stretch or pressure), or chemoreceptors from

the peripheral to the central parts of the autonomic nervous system, and responses are transported via efferent pathways from the central to the peripheral part of the autonomic nervous system. There are two major efferent pathways, called the sympathetic and parasympathetic nervous systems. They usually have opposite (but sometimes synergistic) effects on the end organs they innervate. In the hypothalamus, there is no clear differentiation between the sympathetic and the parasympathetic systems. Figure 1 shows a schematic overview of the innervations and function of the sympathetic and parasympathetic systems. The efferent nerve fibers of the parasympathetic system are distributed to specific organs via the vagal nerve and the sacral nerves. The sympathetic fibers leave the spinal cord in the thoracic and lumbar segments and form the prevertebral sympathetic trunk. The extensive neural network of the gastrointestinal system, the enteric nervous system (see below), is usually considered a third component of the autonomic nervous system.

In addition to feedback systems between organs and the neuroendocrine system, organ function is also regulated via local feedback systems within the organ (Bernards and Bouman, 1988). This is called autoregulation. The term is usually reserved for the capacity of organs to maintain a constant blood flow, despite changes in perfusion pressure. This autoregulatory response occurs in the absence of neural and hormonal influences and therefore is intrinsic to the organ. Organs with a strong autoregulatory response are the brain, the heart, and the kidney.

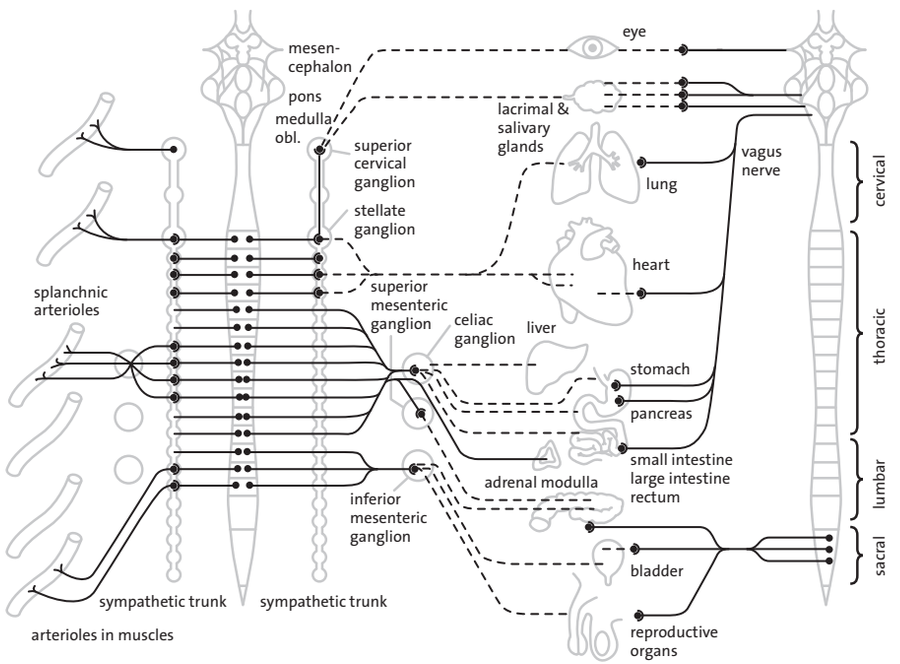
1.2 Cardiovascular and gastrointestinal systems: separated or integrated functions?

The cardiovascular and gastrointestinal systems are under the control of the autonomic nervous system. Although the enteric nervous system is usually described as a separate and independent part of the autonomic nervous system (Woods, 1987), it has many interactions with the cardiovascular system, as the latter must adjust blood flow to the constantly changing metabolic needs of the gastrointestinal system. Previous studies have suggested that there is a direct relation between the gastrointestinal and cardiovascular systems. Animal studies (Hodgson et al., 1992) and a study on healthy young subjects (Rossi et al., 1998) showed that gastric distension causes a reflex increase in arterial pressure and a sympathetically mediated increase in heart rate and peripheral vascular resistance, which suggests there is a gastrovascular reflex activated by mechanoreceptors in the stomach wall. Several other studies have also described a similar direct functional relation between gastrointestinal distension and cardiovascular function. For example, swallow syncope (Levin and Posner, 1972) and paroxysmal hypertension after irritation of the large bowel in patients with high spinal cord lesions (Mathias and Frankel, 1988) have shown that gastrointestinal

function affects blood pressure. Furthermore, it has been demonstrated that bladder distension increases muscle sympathetic nerve activity (MSNA) in humans, which suggests that there is a vesicovascular reflex that is probably induced by stretch receptors in the bladder wall.

The exact nature of these interactions is, however, unclear – does the cardiovascular system only react indirectly to the gastrointestinal system, for example in reaction to changes in splanchnic autoregulation? Or are there direct, neural mechanisms between the gastrointestinal and cardiovascular systems such that their functions are not separate, but integrated? These questions have not yet been answered.

FIG. 1 Schematic view of the autonomic nervous system. Special attention is drawn to the sympathetic outflow to arterioles in different vascular beds (e.g. splanchnic vascular bed; muscular vascular bed) as these might be different, contradicting the hypothesis that there is one general sympathetic tone. Adapted from The integrative action of the autonomic nervous system: neurobiology of homeostasis (Janig, 2006).



1.2.1 Autonomic regulation of the cardiovascular system

The cardiovascular system transports a constant flow of blood containing oxygen and metabolites to the organs. This flow is diverted over a number of vascular beds, arranged in a parallel circuit (Bernards and Bouman, 1988). The most extensive vascular beds are those of the brain, the splanchnic system (i.e., the vascular system of the abdominal organs), the muscles, and the kidney.

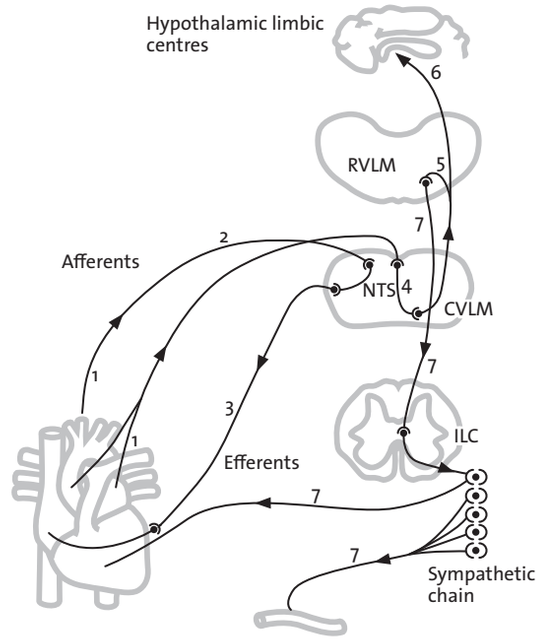
Arterial blood flows toward the organs as a result of the perfusion pressure. Pressure in the arterial system can be increased by increasing cardiac output or peripheral arterial resistance. Cardiac output is controlled by autoregulatory mechanisms and by sympathetic and parasympathetic fibers innervating the heart. Arterioles account for about 50% of the total peripheral arterial resistance. The arterial wall has a high content of smooth muscle, which is innervated by sympathetic nerves (Figure 1). It is generally thought that sympathetic outflow is similar to all arterioles, but theoretically, regional differences in sympathetic tone could exist in the arterioles of different vascular beds (see Figure 1). While sympathetic outflow to arterioles in muscles and skin can be measured directly in humans with microneurography (see below), this is not possible for the sympathetic outflow to other vascular beds.

In addition to sympathetic nervous innervations, the heart and the arterioles are also influenced by free (nor-) adrenalin in the circulation. Noradrenalin is produced in the adrenal glands, which are innervated by sympathetic nerves.

A number of regulatory mechanisms ensure that the perfusion pressure is maintained at a constant level. One such mechanism is a reflex arch called the arterial baroreflex (Figure 2; Joyner and Shepherd, 1997; Wieling and van Lieshout, 1997). The baroreflex consists of afferents from mechanoreceptors in the heart (vagus nerve) and the carotid sinus (glossopharyngeal nerve) that are activated by an increase in arterial blood pressure. They have an inhibitory effect on vasomotor centers in the brain stem, lowering sympathetic outflow and consequently heart rate and blood pressure. For this reason, the arterial baroreflex is termed a negative feedback system. In addition to the baroreflex, mechanoreceptors in the lung, axon reflexes, and noradrenalin in the circulation are also involved in the regulation of blood pressure.

At rest about 30% of the cardiac output goes to the brain. An upright posture, muscular exercise, and digestion of food are activities that challenge circulatory homeostasis in humans: during vigorous exercise about 60% of the cardiac output goes to the muscles and during digestion about 60% of the cardiac output goes to the splanchnic system (Silbernagl and Despopoulos, 1994).

FIG. 2 This schematic drawing shows the pathways of the baroreflex, which is a reflex within the autonomic nervous system that continuously measures blood pressure and adjusts peripheral arterial resistance by activation of the sympathetic system. It is an example of a feedback system.

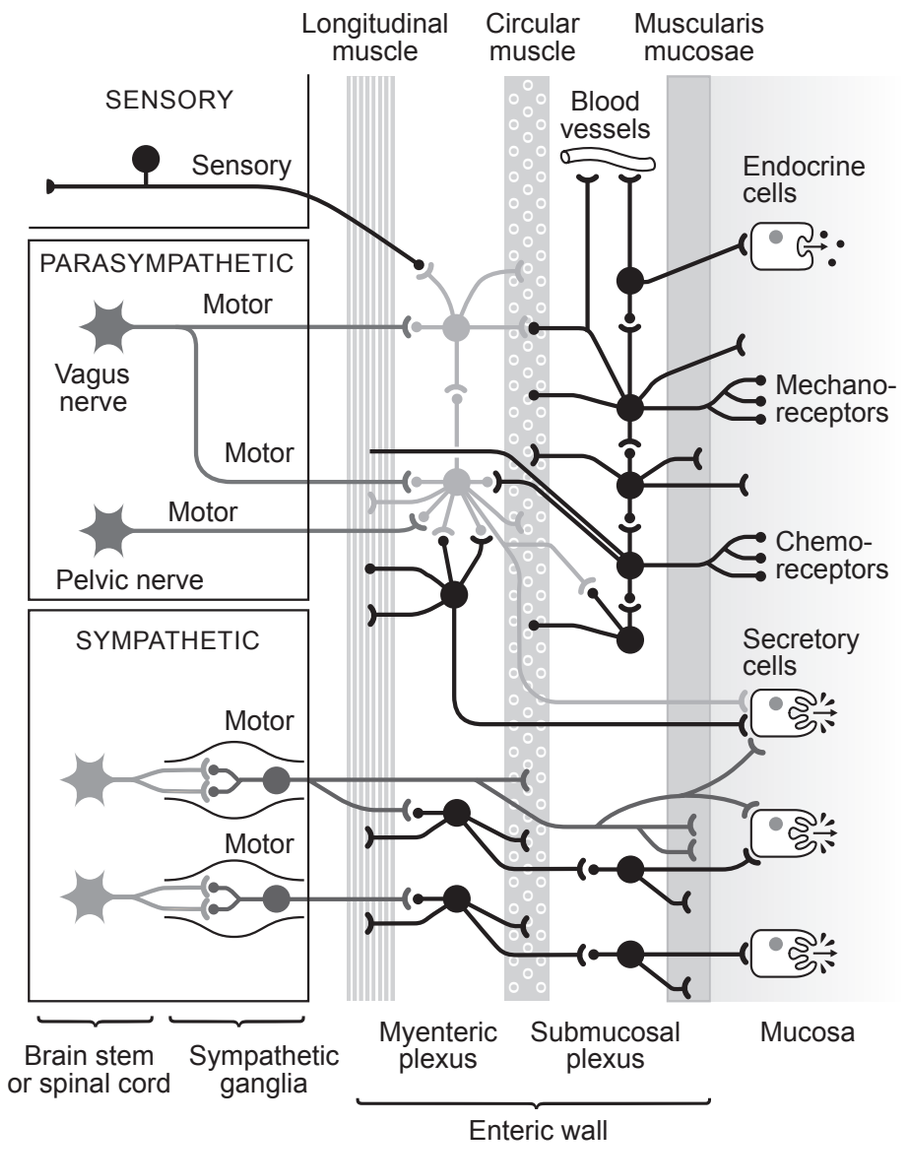


1 Carotid sinus and aortic arch stretch receptors.
2 Unmyelinated fibers running in glossopharyngeal and vagus nerve synapsing at nucleus tractus solitarii (NTS).
3 Parasympathetic preganglionic fibers running in vagus nerve emerging from nucleus ambiguous.
4 Intermediate neurons
5 Inhibitory neuron from caudal ventrolateral medulla (CVLM) to rostral ventrolateral medulla (RVLM).
6 Afferent pathway for release of vasopressin.
7 Sympathetic cardiac and vasomotor fibers passing intermediolateral collum (ILC) of spinal cord and sympathetic chain ganglia. In healthy subjects, a decrease in blood pressure signaled by the stretch receptors elicits an increase in heart rate and an increase in sympathetic vasomotor tone. An increase in blood pressure will cause the opposite. Adapted from Smit et al. (1996).

1.2.2 Autonomic regulation of the gastrointestinal system

The gastrointestinal system extracts water, electrolytes, and nutrients from food and delivers them to the circulation. In this process, the food bolus moves through the alimentary tract and is exposed to digestive juices secreted by different parts of the gut. Oxygenated blood has to circulate through the gastrointestinal organs to provide oxygen for these processes and to transport the absorbed substances. To regulate these complex functions, the gastrointestinal system is strongly controlled by the autonomic nervous system and the endocrine system. In addition, it has its own nervous system, the enteric nervous system. The enteric nervous system lies in the wall of the gut and is made up of about 100 million neurons, about the same number of neurons as in the entire spinal cord (Guyton and Hall, 2000). The enteric nervous system controls gastrointestinal motility and secretion and is composed of two interconnected plexuses, an outer plexus, lying between the longitudinal and circular muscle layers, and an inner submucosal plexus (Figure 3). The enteric nervous system can function on its own, but stimulation by the parasympathetic or sympathetic nervous system can further activate or inhibit gastrointestinal function. In general,

FIG. 3 The enteric nervous system, showing the myenteric and submucosal plexuses. Extrinsic control of these plexuses is by the sympathetic and parasympathetic nervous systems and sensory fibers passing from the luminal epithelium and gut wall to the enteric plexuses and from there to the prevertebral ganglia of the spinal cord an directly to the spinal cord and brain stem. Adapted from Boron and Boulpaep (2002).



stimulation of the sympathetic nervous system inhibits gastrointestinal activity, whereas stimulation of the parasympathetic system stimulates gastrointestinal activity. Afferent information comes from sensory neurons that have their nerve endings in the gastrointestinal epithelium or gut wall.

These neurons are stimulated by distension of the gut or by specific chemical substances in the gut and transmit information not only to the two plexuses of the enteric system, but also to the prevertebral ganglia of the sympathetic nervous system, the spinal cord, and via the vagus nerves to the autonomic centers in the brain stem. These sensory nerves can elicit local reflexes within the gut and other reflexes that are relayed back to the gut from either the prevertebral ganglia or the basal regions of the brain.

There are 3 types of gastrointestinal reflexes (Guyton and Hall, 2000). The first type consists of reflexes that occur within the enteric nervous system and which are important in the control of gastrointestinal secretion and motility. The second type consists of reflexes from the gut to the prevertebral sympathetic ganglia and then back to the gastrointestinal tract. These reflexes transmit signals over long distances; for example, signals from the stomach to cause contraction or relaxation of the colon (the gastrocolic reflex). The third type consists of a number of reflexes that pass from the gut to the spinal cord or brain stem and then back to the gastrointestinal tract. These include pain reflexes that cause general inhibition of the entire gastrointestinal tract. In addition to the nervous regulation of the gastrointestinal system, hormones are important in regulating secretion and motility.

1.3 Physiological response of the cardiovascular system to food intake

While eating seems a simple and often pleasurable act, many integrated processes are needed to guarantee a continuous stream of metabolites to the end organs, especially the brain. In response to food intake, splanchnic blood vessels dilate (Mathias and Bannister, 1999b) to divert blood to the gastrointestinal tract (Kooner et al., 1989), in order to meet the oxygen demands of digestion. Blood flow in the mesenteric artery increases after food intake (Fujimura et al., 1997), although the mechanism responsible for this increase is unknown. We do know, however, that in healthy older subjects and in patients with type II diabetes, the hypotensive response to oral glucose is related to the rate of gastric emptying and the subsequent interaction of glucose with the small intestine (Jones et al., 2001a; O'Donovan et al., 2002; O'Donovan et al., 2005; Russo et al., 2003). Furthermore, when gastric emptying and exposure of the small intestine to glucose are delayed or slowed by oral (Jones et al., 2001a; Russo et al., 2003) or intraduodenal (O'Donovan et al., 2005) administration of the viscous polysaccharide, guar gum, both the fall in blood pressure and the

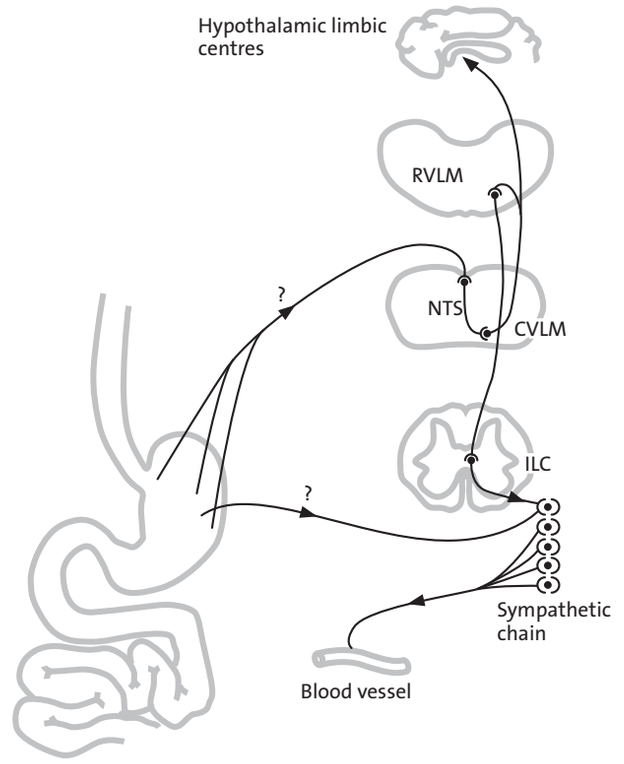
increase in heart rate are attenuated. These findings suggest that when the gastric contents empty into the small intestine, receptors are activated by carbohydrates and decrease vasoconstrictor tone, causing local splanchnic vasodilatation.

In order to maintain blood pressure despite the reduction in vascular resistance in the splanchnic system, other vascular beds need to show vasoconstriction after food intake. MSNA and blood pressure have been shown to increase in response to an oral glucose drink (Berne et al., 1989) or a fat- or protein-containing drink or a mixed meal (Fagius and Berne, 1994). This pressor effect might be related to gastric distension by the food bolus. A previous study by our group showed that MSNA and blood pressure increase after gastric distension (Rossi et al., 1998). In this respect, gastric distension is similar to other conditions involving somatic and visceral stimuli, such as bladder distension (Fagius and Karhuvaara, 1989), cold pressor test (Victor et al., 1987) and the diving response (Fagius and Sundlof, 1986), in which nonbaroreceptor input induces pressor effects of MSNA. Our hypothesis is that gastric distension activates the tension or stretch receptors lining the walls of the stomach, which stimulate the central nuclei through vagal afferents to increase MSNA, leading to an increase in vascular resistance, followed by an increase in blood pressure. This suggested autonomic reflex arch is called the 'gastrovascular reflex' (Figure 4). The results of animal experiments support the suggestion that the observed increase in sympathetic outflow is related to stomach distension via mechanoreceptors in the stomach wall (Pozo et al., 1985). In fact, in anesthetized animals, gastric distension increases arterial pressure and causes peripheral vasoconstriction, and both effects are abolished by stomach afferent sensory denervation (Pozo et al., 1985; Vacca et al., 1996). At present, it is not known whether the increase in MSNA in response to oral glucose (Berne et al., 1989) is mediated by the gastrovascular reflex, the baroreflex, or the response to carbohydrate load in the small intestine.

1.4 Effects of aging on responses to food intake

Aging is associated with physiological changes in the cardiovascular and gastrointestinal systems. Healthy aging is associated with attenuation of the baroreflex control of heart rate (Matsukawa et al., 1996) and reduced MSNA responses to the Valsalva maneuver (Matsukawa et al., 1998), gravitational stress (Iwase et al., 1991), and vestibular stimuli (Ray and Monahan, 2002). Although orocecal and total gut transit times are similar in healthy young subjects and healthy old subjects, aging is associated with a slowing of solid and liquid gastric emptying (Clarkston et al., 1997). The transit time of the food bolus from the fundus to the antrum of the stomach is prolonged in the elderly, and this might lead to prolonged activation of receptors for satiety, located in the fundus, causing a diminished sensation of hunger

FIG. 4 Schematic drawing of the gastrovascular reflex. For details, see text; for abbreviations, see figure 2.



and desire to eat. In elderly people, blood pressure falls after eating (Jansen and Hoefnagels, 1989; Peitzman and Berger, 1989). The pathophysiology of this phenomenon has thus far not been clarified.

1.5 Failure of normal responses in reaction to food intake: postprandial hypotension

Postprandial hypotension (PPH), defined as a fall in systolic blood pressure of 20 mmHg or more within 2 hours of eating (Jansen and Lipsitz, 1995), occurs in people with autonomic impairment. In severe cases of autonomic failure, patients may even lose consciousness when standing up after eating. The mechanism of PPH is unknown but is probably multifactorial and might involve an attenuated baroreflex, an attenuated reflex increase in sympathetic activity by activation of stretch receptors in the stomach (gastrovascular reflex, Rossi et al., 1998), sympathetic dysfunction (e.g., autonomic neuropathy in diabetes mellitus, Parkinson disease), and a decreased cardiac output. For this reason, PPH is likely to occur in frail elderly with extensive

comorbidity. There is evidence that PPH occurs especially often in frail, older ambulant (Aronow and Ahn, 1994; Jansen et al., 1995) and hospitalized patients (Vloet et al., 2005). This poses an additional problem with regard to the choice of method to diagnose PPH in these individuals (intermittent or continuous blood pressure measurement).

1.6 Failure of normal responses in reaction to food intake: irritable bowel syndrome (IBS)

Several studies indicate that abnormal brain-gut interactions play a role in the pathophysiology of IBS (Chelimsky and Chelimsky, 2001; Elsenbruch and Orr, 2001; Heitkemper et al., 2001); however, results are often contradictory. IBS is one of the most common syndromes seen by gastroenterologists, accounting for 10-15% of their patient population (Drossman et al., 2002). The lack of methods to assess vagal and sympathetic effects on the gastrointestinal tract directly has forced investigators to monitor the autonomic regulatory mechanisms of the cardiovascular system as being representative of general autonomic function. According to the Rome criteria, IBS patients suffer from abdominal pain associated with defecation or changes in bowel habit, with features of disordered defecation, abdominal distension, and/or abdominal bloating (Camilleri, 2001). The disorder is 'functional' in that the symptoms cannot be explained by structural or biochemical abnormalities. The majority of patients with IBS experience their symptoms during and after food intake (Dapoigny et al., 2003). It is not known whether the increase in MSNA in response to food intake differs between patients with IBS and healthy subjects.

1.7 Can postprandial hypotension cause focal brain ischemia?

A postprandial fall in blood pressure (O'Donovan et al., 2002) might influence the blood flow to the brain. In healthy subjects, cerebral blood flow is kept constant within a large range of blood pressure, by cerebral autoregulation. If the blood pressure falls below the lower limit of cerebral autoregulation, blood flow to the brain decreases and the patient loses consciousness. In patients with a disturbed cerebral autoregulation, even small changes in blood pressure might change blood flow to the brain. Although blood flow to the brain is closely regulated in healthy subjects, this might not be the case in patients with obstructive disease of the main arteries (carotid and vertebral) supplying the brain, in which certain areas in the brain (the watershed areas) might have a marginal perfusion. In these patients, small changes in blood pressure might lead to focal brain ischemia. It is not known whether food intake triggers the occurrence of focal brain ischemia in subjects with a compromised cerebral blood flow.

2 METHODS

2.1 Gastrointestinal methods

At present, it is not possible to measure the activity of the enteric system directly. Brain and gut interactions have been investigated experimentally in animals and humans, by using vestibular and central autonomic stimuli, by measuring intraluminal pressures with manometry, by measuring oro-rectal transit time with radioscintigraphy, and by evaluating sensory function with barostat (see below; Camilleri, 1990). This inability to measure the autonomic activity of the enteric system directly means that there have been few studies of the interactions between the gastrointestinal and cardiovascular systems.

The effect of food on the cardiovascular and autonomic nervous systems might be determined by its volume (by causing distension of the stomach) and by its composition (carbohydrates, fat, proteins, osmolality). Dilatation of a flaccid bag placed in the stomach, by means of a barostat, can be used to mimic the effect of a food bolus, while intraduodenal infusion of nutrients via a nasogastroduodenal tube can be used to mimic the effect of the composition of the food bolus.

2.1.1 Barostat

The barostat technique was developed in the 1980s (Azpiroz and Malagelada, 1985). The barostat is a device for providing a constant pressure in a flaccid bag, using a pneumatic pump. Barostat-bag systems have been used in the esophagus, stomach, small bowel, colon, and rectum. Since the gut wall can relax and contract very rapidly, the bag has to be inflated or deflated very rapidly by a feedback air pump system to keep the pressure inside the bag, and consequently within the gut, constant.

2.1.2 Intraduodenal glucose infusion

The effect of nutrients in the duodenum can be evaluated by using a special silicone rubber catheter with built-in duodenum infusion port, and channels for measuring pressure and the transmucosal potential difference (TMPD). The catheter is introduced through a nostril, and its position can be checked by X-ray examination. The exact position of the catheter tip can be controlled continuously by measuring the potential difference between the catheter tip and a point a few centimeters proximal to the tip: the TMPD is usually negative in the stomach and neutral or positive just distal to the pylorus.

2.2 Evaluation of autonomic function

Under resting conditions, sympathetic and parasympathetic activity in end organs is usually in equilibrium. A way to test autonomic function is by disturbing this balance and interpreting the reaction of the end organ, which reflects the reaction of the autonomic nervous system. To test an autonomic reflex, efferent neural activity can be recorded directly from nerves, or measured indirectly by recording responses from the relevant end organ (Oey, 2004). It should be emphasized that assessment of autonomic function depends not only on reflex arches and on efferent nerve activity but also on end-organ responsiveness, with factors including the metabolic clearance and disposition of transmitters, postsynaptic receptors, postreceptor translation, and second messenger systems influencing the final response (Mathias and Bannister, 1999a). In addition, the results of tests of autonomic function are influenced by multiple factors, such as the subject's emotional state, posture during the test, and medication (Oey, 2004). For this reason, multiple testing is advised and results should be interpreted in the context of the whole clinical picture.

2.2.1 Assessment of sympathetic activity

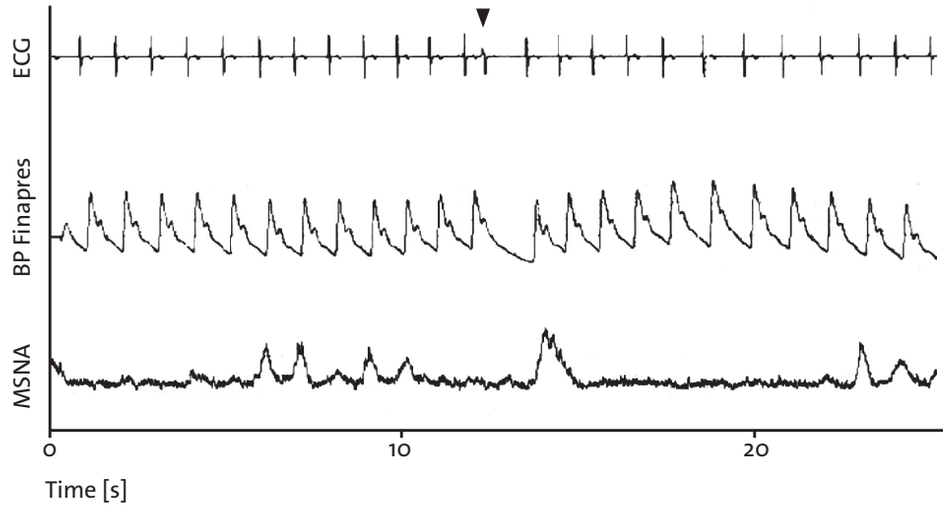
The activity of the sympathetic nervous system can be evaluated indirectly, by measuring plasma catecholamine levels, or directly, by microneurography.

CATECHOLAMINE AND ITS METABOLITES While both adrenaline and noradrenalin are released from the adrenal medulla, noradrenalin is the major neurotransmitter at sympathetic nerve endings. Stimuli that result in sympathoneural activation increase plasma levels of noradrenalin. The difference between the plasma levels of catecholamine at baseline and after stimulation is a measure of sympathetic activation. However, because the concentration of noradrenalin in plasma is the net result of a number of processes (such as noradrenalin secretion, neuronal uptake, intraneuronal and extraneuronal metabolism, and clearance), changes in plasma level are only an indirect measure of sympathetic activation and should be interpreted with care (Mathias and Bannister, 1999a).

MICRONEUROGRAPHY It has become possible to measure sympathetic activity in humans directly, by recording postganglionic nerve impulses in peripheral nerves with percutaneous microelectrodes inserted into a peripheral nerve. The technique, microneurography, was developed by Vallbo and Hagbarth in the 1960s (Vallbo and Hagbarth, 1967) and was later refined and validated, mainly by Wallin (1999). This method enables the direct measurement of postganglionic nerve traffic to arterioles in muscle (MSNA) and skin (skin sympathetic nerve activity, SSNA; Mano, 1999). Increases in MSNA or SSNA are associated with vasoconstriction of arterioles in muscle and skin, respectively. A tungsten-recording electrode is inserted into a peripheral nerve to

measure spontaneous sympathetic activity, and a reference needle electrode is placed subcutaneously 2 to 3 centimeters away from the recording electrode. Spontaneous bursts of sympathetic activity can be recorded in the nerves innervating the skin and muscles and have a characteristic morphology and relationship to R-waves (ECG; see Figure 5). The correct position of the electrode can be confirmed by the characteristic response to the Valsalva maneuver. In the mid-1990s, Dr. P.L. Oey introduced this technique to the Department of Clinical Neurophysiology, UMC Utrecht, to study the autonomic nervous system. The peroneal nerve, which lies directly under the skin, was used.

FIG. 5 Example of raw data, showing heart rate [ECG], finger arterial blood pressure [BP Finapres] and integrated muscle sympathetic nerve activity [MSNA]. The inverse relation between MSNA and blood pressure is visible: when blood pressure tends to fall, MSNA increases (especially after the marked blood pressure fall due to the compensatory pause following the premature beat at the arrow), followed by an increase in blood pressure.



A common hypothesis is that the sympathetic nervous system acts as a global system for the maintenance of sympathetic tone. There is, however, evidence that sympathetic outflow to different tissues is controlled separately. This hypothesis is supported by the finding that while simultaneously measured bursts in activity in nerves running to different muscles show clear similarities, the bursts in activity of nerves running to muscle and skin are completely different (Mano, 1999). It is now thought that the sympathetic nervous system should be regarded as consisting of a number of subdivisions that can be activated in varying combinations and to different degrees depending on the functional demand (Figure 1).

2.2.2 Autonomic function tests

As described above, autonomic function can be tested by disturbing the balance between sympathetic and parasympathetic activity and by measuring the effect on end organs. In the 1980s, Ewing and Clark developed a test battery for the assessment of autonomic function (Ewing and Clarke, 1982). This battery was later revised by the American Academy of Neurology (no authors listed, 1996). Most of those tests are used in routine clinical practice and are described briefly below.

SPECTRAL ANALYSIS OF HEART RATE AND BLOOD PRESSURE It is well known that blood pressure and heart rate are not constant, but vary beat by beat. For instance, respiration causes heart rate to vary with a frequency equal to the respiration rate. In the 1970s, a method was developed to analyze this complex heart rate pattern, using spectral analysis. This mathematical technique separates the total variability into components at different frequencies (Hyndman et al., 1971), thereby providing insight into the relative strength of the various sources of variation. Analysis of blood pressure together with heart rate provides even more information. The availability of devices to noninvasively measure beat-to-beat blood pressure, such as the Portapres (TNO-TPD Biomedical Instrumentation, Netherlands Organization for Applied Scientific Research) and Finapres (Ohmeda) devices, has contributed to the popularity of spectral analysis.

Spectral analysis of the variability in heart rate and blood pressure using a Fourier transform results in a power spectrum, from which the power in the low frequency (LF: around 0.1 Hz) and high frequency (HF: around 0.3 Hz) ranges, and the LF/HF power ratio, can be calculated. In a simplified model, the spectral power of the LF and HF ranges of heart rate is considered a marker of sympathetic and vagal cardiac tone, respectively, and the ratio between them represents the sympathovagal balance. This model is debated. In a previous study, we found that heart rate variability, measured as the LF/HF ratio using short-term spectral analysis, shows considerable intra-individual variability, expressed as inter-observer variability, which suggests that the results of these calculations should be interpreted with caution (van Schelven et al., 2000), and that a combination of different methods for assessing the autonomic nervous system is to be preferred.

VALSALVA MANEUVER The Valsalva maneuver is considered as a test of baseline vagal cholinergic function. The test induces changes in the interbeat interval of heart rate during elevated intrathoracic pressure, which is achieved by forced expiration with nearly closed glottis. In the laboratory, this is done by forced expiration with open glottis in a tube connected to a manometer, so that intrathoracic pressure can be kept constant.

DEEP BREATHING TEST The deep breathing test is another measure of vagal responsiveness. The interbeat interval of heart rate shows a natural rhythmic change during breathing, the so-called sinus arrhythmia. This can be exaggerated by deep breathing. We used a standard protocol of 6 deep breaths per minute, using a metronome.

COLD PRESSOR TEST Cold stress is a strong sympathetic stimulus, leading to an increase in heart rate and blood pressure. We measured heart rate and blood pressure during a baseline period, followed by a 2-minute period during which the right hand was immersed in ice water (cold pressor period), and a 2-minute recovery period. The effect of a cold pressor test was assessed by measuring changes in MSNA, mean arterial pressure, and heart rate.

3 AIMS AND OUTLINE OF THIS THESIS

The studies described in this thesis focus on autonomic nervous system mediated interactions between the gastrointestinal and cardiovascular systems in response to food intake and on the potential consequences of failure of these interactions. The aims were:

(1) To determine the effect of aging on the 'gastrovascular reflex' (chapter 2).
(2) To establish the effect of nutrients, in particular glucose, administered by means of an intraduodenal catheter on heart rate, MSNA, and blood pressure in healthy young and healthy old individuals (chapter 3).
(3) To study the effect of a meal on heart rate, MSNA, blood pressure, and pre- and postprandial autonomic function tests in patients with IBS (chapter 4).
(4) To investigate whether PPH occurs more frequently in patients admitted to a geriatric ward than in healthy elderly individuals, how often it is associated with symptoms, and what the optimal interval between blood pressure measurements is in order to diagnose PPH (chapter 5).
(5) To find out whether food or alcohol intake triggers the occurrence of an ischemic cerebral event in patients with compromised cerebropetal blood flow (chapter 6).
(6) To discuss the results and to formulate some hypotheses concerning the integration of the different mechanisms (chapter 7).

CHAPTER 2

Effect of gastric distension on cardiovascular parameters: Gastrovascular reflex is attenuated in the elderly

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ABSTRACT

Stretching the stomach wall in young healthy subjects causes an increase in muscle sympathetic nerve activity and in blood pressure, the gastrovascular reflex. We compared healthy elderly subjects with healthy young subjects to find out whether the gastrovascular reflex attenuates in normal ageing and we studied whether there was a difference in autonomic function or gastric compliance that could explain this possible attenuation. Muscle sympathetic nerve activity, finger blood pressure and heart rate were continuously recorded during stepwise isobaric gastric distension using a barostat in 8 healthy young (6 men and 2 women, 27 ± 3.2 years, mean ± s.e.m.) and 8 healthy elderly subjects (7 men and 1 woman, 76 ± 1.5 years). Changes in cardiac output and total peripheral arterial resistance were calculated from the blood pressure signal. The baseline mean arterial pressure and muscle sympathetic nerve activity were higher in the elderly group (both $P < 0.05$) and muscle sympathetic nerve activity increase during the cold pressor test was lower in the elderly group ($P = 0.005$). During stepwise gastric distension, the elderly subjects showed an attenuated increase in muscle sympathetic nerve activity compared to the young subjects ($P < 0.01$). The older group tended to show a higher increase in mean arterial pressure ($P = 0.08$), heart rate ($P = 0.06$) and total peripheral arterial resistance ($P = 0.09$). The cardiac output rose slightly in both groups without significant difference between groups. The fundic compliance did not differ between groups. We conclude that stepwise gastric distension caused an increase in muscle sympathetic nerve activity in both groups, but the increase in the elderly was attenuated.

INTRODUCTION

Digestion of food places a burden on the cardiovascular system. The blood flow in the superior mesenteric artery increases during a meal (Fujimura et al., 1997), indicating an increase in total splanchnic blood flow. To prevent a fall in systemic blood pressure during and after a meal, an increase in cardiac output and peripheral arterial resistance is needed.

In a normal condition, the systolic blood pressure does not fall after a meal in young healthy subjects (Mathias et al., 1989). This indicates that the cardiovascular system is activated in an early stage to prevent this fall in blood pressure. Directly after swallowing food, the stomach wall relaxes and is distended by the food bolus. This might be the trigger for the activation of the cardiovascular system.

Stepwise proximal gastric distension causes an increase in Muscle Sympathetic Nerve Activity (MSNA) and blood pressure in young healthy subjects. This phenomenon was named gastrovascular reflex (Rossi et al., 1998). The physiological relevance of this reflex may be to increase peripheral arterial resistance to compensate for the decrease of splanchnic arterial resistance that occurs during eating. The increase in blood pressure is possibly a consequence of baroreflex resetting caused by mechanoreceptor stimulation in the stomach wall (Rossi et al., 1998).

Healthy ageing is associated with changes in physiology with respect to food processing as well as to blood pressure regulation. Ageing is probably associated with impaired receptive relaxation and accommodation of the gastric fundus after a meal (MacIntosh et al., 2000; Rayner et al., 2000). In contrast to the effects of age on gastric mechanics, it seems that age has little, if any, effect on small intestinal or colonic motor function, and orocecal and whole-gut transit time are not affected in the healthy elderly (MacIntosh et al., 2000). The question remains whether ageing is associated with changes in the response to purely mechanical gastric distension without the effects of a meal mentioned above.

The aim of this study was to compare the influence of proximal gastric distension on sympathetic outflow, total peripheral arterial resistance, cardiac output, heart rate and blood pressure in young and in older healthy humans. Furthermore, we studied whether possible differences between the groups might be explained by a difference in fundic compliance or in autonomic status. Autonomic status was screened with the cold pressor test, Valsalva maneuver and spectral analysis of heart rate variability.

MATERIALS AND METHODS

POPULATION

Volunteers were recruited by advertisements in the local newspaper. All were free of overt cardiopulmonary disease as assessed from medical history taken by telephone. Older subjects were invited to the outpatient clinic to undergo a screening program consisting of medical history and physical examination. Inclusion criteria for the elderly group were age > 70 and good health. The latter was based on a detailed health questionnaire and physical examination, in accordance with the modified exclusion criteria defining medically stable elderly subjects for exercise studies (Greig et al., 1994). To exclude postprandial hypotension in the elderly, older subjects underwent a standard "meal-test" (75 g glucose in 300 ml water; Jansen et al., 1987) after an overnight fast on the day prior to the experiment. Postprandial hypotension was defined as a fall in systolic blood pressure of more than 20 mmHg within two hours after a standard meal. The Ethics Committee of the Utrecht University Hospital approved the protocol. The subjects all gave their written informed consent for participation in this study.

MEASUREMENT INSTRUMENTATION

Blood pressure was recorded continuously from the right hand with finger photoplethysmography (Finapres, Ohmeda, Englewood, CO, USA). Cardiac output (L/min) and total peripheral resistance (medical units [MU]; mmHg.s/ml) were obtained by pulse contour analysis (software: Beatscope 1.0; TNO TPD biomedical instrumentation, Academic Medical Centre, Amsterdam, the Netherlands). This method is accurate for measuring changes over time, but not for absolute values (Stok et al., 1993; Stok et al., 1999). For this reason we only report changes from baseline for cardiac output and total peripheral resistance. The electrocardiogram (ECG) was recorded using bipolar chest leads. Multifibre recordings of muscle sympathetic activity were made with a Tungsten microelectrode inserted in the peroneal nerve of the right leg (Vallbo et al., 1979; Wallin, 1999). A reference needle electrode was placed subcutaneously 2 - 3 cm away from the recording electrode. Small adjustments of the recording electrode were made until a site was found at which spontaneous sympathetic activity was recorded. Sympathetic bursts were identified by their characteristic morphology and relationship to R-waves (ECG). The correct position of the electrode was confirmed by

the characteristic response to Valsalva maneuver (Ligtenberg et al., 1997).

The stomach was distended by a non-compliant intragastric bag (maximal capacity: 700 ml) connected to a G&J Electronics Distender Series II (TM) barostat, a computer controlled injection-aspiration air pump (Protocol Plus Data Scanner software; G&J Electronics, Toronto, Ontario, Canada). A similar approach was described in our previous report (Salet et al., 1998). In the current study the stomach was distended at fixed intragastric pressures (isobaric), using a staircase protocol (Whitehead and Delvaux, 1997). This method allows assessment of the function of tension receptors in the stomach wall. The barostat was connected to the intragastric bag by a single-lumen polyvinyl stomach tube (12 CH Levin stomach tube, Argyle, Sherwood Medical, Tullamore, Ireland). Pressure and volume transducers in the barostat allow real-time monitoring of gastric distension.

Gastric sensory function was assessed at the end of each distension step using visual analogue scales (VAS; Sriwatanakul et al., 1983) for sensation of fullness, nausea and pain. Subjects were asked to quantify these three sensations from 'no sensation' to 'unbearable fullness' or 'unbearable nausea, to the point of vomiting' and 'unbearable pain'.

ECG, respiration, finger arterial blood pressure and MSNA (mean rectified voltage neurograms) were recorded and monitored with an IBM compatible PC, using POLY 5 (samples at 200 Hz; POLY 5, Physiocal Analysis Package, Inspector Research Systems B.V., Amsterdam, the Netherlands). Intragastric pressure and volume curves were monitored on-line with G&J Electronics Protocol Plus TM control software.

EXPERIMENTAL PROTOCOL

The study was performed after an overnight fast. All subjects were instructed to refrain from eating, drinking, and smoking after 21:00 h the previous day. Just before the experiment, subjects were asked to empty their bladders. The experiment took place at 08:00 h in an air-conditioned room. The intragastric bag was introduced through the mouth into the proximal stomach. The throat was sprayed with a Lidocaine solution (100 mg/ml, 1 spray equals 10 mg) which resulted in a maximum anaesthetized period of 20 min (the actual measurement started about 45 min after the intragastric bag was introduced). The intragastric bag was insufflated with 200 ml of air to unfold the bag. After complete deflation of the bag, the catheter was connected to the barostat. Subjects lay on a bed at an angle of 30° in an anti-Trendelenburg supine position. The hand that was used for the finger photoplethysmographic blood pressure recording (right hand) was kept warm with a cherrystone pillow to avoid vasoconstriction (Jagomagi et al., 2001) and was kept at a fixed vertical heart – hand distance to eliminate the influence of gravity on

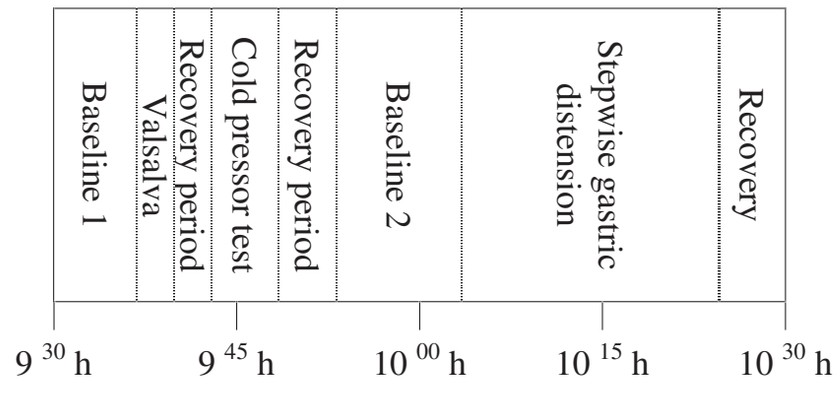
the observed blood pressure. The appropriate heart – hand height was determined by comparing the mean arterial blood pressure value of the Finapres with the mean arterial blood pressure value of at least two measurements with a sphygmomanometer.

After signals of good quality were obtained, baseline MSNA, blood pressure, heart rate and respiration rate were recorded for 5 min (first baseline period). During this period the subject was asked to sustain a comfortable breathing frequency of 15 breaths per minute assisted by a metronome. To assess baseline vagal and sympathetic responsiveness, the following provocation tests were subsequently performed: Valsalva maneuver (keeping a manometer inflated to 40 mmHg for 15 s) and a Cold Pressor Test (CPT; the subject's left hand was immersed in ice water up to the wrist for 2 minutes). Each test was started only after signals had returned to baseline level.

The provocation tests were followed by a second baseline (pre-distension) period for a duration of 10 min. During the second baseline period the minimal distension pressure (MDP; minimal pressure inside intragastric bag to overcome the intra-abdominal pressure; Mearin et al., 1991) was determined and sustained. One or two baseline blood pressure measurements with a sphygmomanometer were done to adjust the Finapres and to use for later analysis. Subsequently, intragastric pressure was increased in 3-minute steps of 2 mmHg each, using a staircase protocol. In the last minute of each distension step, the subject was asked to quantify sensations of fullness, nausea, and pain on a VAS, ranging from 1 to 7. Gastric distension was ended after the 7th distension step (intragastric pressure = 14 mmHg) or after reaching maximal bag volume (700 ml) or maximum tolerable volume. Recording was continued after the gastric distension period for 15 minutes (recovery period) to ascertain normalization of the parameter values to baseline. Figure 1 shows the time line of the experiment protocol.

FIG. 1 **Time line of the protocol**

Not shown is the preparation period, which started at 08.00 h and was used for placing the intragastric bag and the finding of signals (MSNA, Finapres, ECG and pneumogram). During the first baseline period stable signals were obtained and used for comparison of baseline values. The recovery periods served to let all signals return to baseline levels. The values during the second baseline period served as baseline for the distension period. The heart rate variability during the second baseline period was used for spectral analysis.



DATA ANALYSIS

Mean arterial blood pressure (MAP; mmHg) was calculated per heartbeat (time average MAP per beat) from the finger blood pressure curve. Heart rate (beats/min) and beat length (interbeat interval (IBI); ms) were calculated beat to beat from the ECG using automatic R-wave detection. The stored integrated MSNA signal was analyzed by software specially developed by our group. This software detected MSNA bursts based on their characteristic, more or less triangular shape using criteria on the slopes, duration and amplitude of the bursts. For instance, 'EMG pulses' were recognized from their steep front slope, and automatically rejected. Detected bursts were assigned to independently detected R-waves in the ECG, requiring the delay between the R-wave and the burst top to be within an a priori defined time window. Only one burst was assigned per R-wave. The results were shown graphically as stylized MSNA bursts together with the raw MSNA and ECG for visual inspection and, if necessary, correction.

Burst counts were calculated as burst frequency (number of sympathetic bursts per minute, NBM; bursts/min) and as burst incidence (number of sympathetic bursts per 100 heartbeats, NBR). The burst area was calculated as the area under the curve (arbitrary units) per minute (ABM; a.u.c./min) and as the area per 100 heartbeats (ABR; a.u.c./100 heartbeats).

Autonomic status:

Information on autonomic status of all subjects was obtained by the following tests:

1 *Spectral analysis of the heart rate variability:*
Five consecutive minutes of the baseline period were used for spectral analysis of the heart rate variability. Detection of R-waves in QRS complexes was used to obtain a list of interbeat intervals. The list of interbeat intervals was manually corrected for extra-systolic as well as missing heartbeats by interpolation. The linear trend was removed from the list and a cosine tip window was applied. The power spectrum was estimated as the squared amplitude of the result of a fast Fourier transform. From that, the power in the low frequency (LF: 0.04-0.15 Hz) and high frequency (HF: 0.15-0.40 Hz) ranges, and the LF/HF power ratio were calculated.

2 *Valsalva:*
Baseline vagal cholinergic function was determined by calculating the IBImin/IBImax ratio (Valsalva ratio) from the minimum IBI in the ECG during elevated intrathoracic pressure (phase II) and the maximum IBI during the first 30 s after the release of intrathoracic pressure (phase IV; Mathias and Bannister, 1999a).

3 *Cold pressor test:*
The effect of a cold pressor test was assessed by observing changes in MSNA, mean arterial pressure and heart rate. Two minutes of baseline were directly followed by 2 min of immersion of the right hand into ice water (cold pressor period) and 2 min of recovery. The cold pressor and recovery periods were divided into 30-s epochs. Mean absolute (mean arterial pressure, heart rate and NBM) or proportional (ABM) changes from baseline were calculated for each epoch. These delta values per epoch were used in a repeated measures ANOVA with age-group as the between-subject factor. The significance of changes over time and the difference between the groups were calculated.

Effect of gastric distension:
Comparison of the baseline values of the young with that of the elderly was obtained for the following parameters: mean arterial pressure (sphygmomanometer), average heart rate and MSNA (NBM).

The effect of gastric distension was calculated by dividing this part of the measurement into separate periods. From each recording, an artifact-free 2-min period was selected from the second baseline period (barostat bag at MDP) and from the recovery period. Parameters (mean arterial pressure, heart rate, cardiac output, NBM, NBR and total peripheral resistance) were averaged over these 2-min periods to obtain the baseline and recovery values. For the 7 barostat distension steps (+2 to +14 mmHg), the parameters were averaged over 3-min periods. All parameter step values were expressed relative to their baseline values (Δ; change from baseline). For the burst area parameters ABM and ABR, which were expressed in arbitrary units, proportional changes (%Δ) from baseline were calculated.

The Δ-values of both groups showed a normal distribution. Repeated measures ANOVA was performed for each parameter to compare changes from baseline and differences between groups during distension of the barostat bag. Data from the recovery period were not included. The (proportional) Δ-values of the 6 distension steps were analyzed with distension step as the within-subject factor and age-group as the between-subject factor. Since the data did not fulfill the assumption of compound symmetry in most of the cases only multivariate results are shown (all subtests gave similar results).

Linear regression slopes:
Most variables showed an approximately linear change during gastric distension. An estimation of this change was made using linear regression of the Δ-values against the distension step number (independent variable). The slope of the regression line was calculated for each group (constant not in equation; baseline and recovery periods were not included).

Visual analogue perception scores:
The association between the degree of gastric distension and the perception of pain, nausea and fullness, registered as VAS scores, was calculated using the Spearman correlation test. Changes over time and differences between groups in VAS scores were calculated with repeated measures ANOVA.

Gastric wall compliance:
An estimation of the compliance of the gastric wall (fundic compliance) was made by calculating the regression coefficient of the 'intragastric pressure – volume' ($\Delta V/\Delta P$) curves that were measured with the barostat. To minimize the influence of tension of the abdominal muscles, subjects were asked to relax those muscles as far as possible. Because distributions were normal, the group results were compared using a Student's t-test.

Missing data:
All original signal curves were reviewed carefully off line. Parts with missing data (for instance caused by turning off the Finapres for 30 s to avoid painful congestion of the finger) or with artifacts (for instance in the MSNA signal, caused by involuntary muscle tension) were marked and omitted in further analysis.

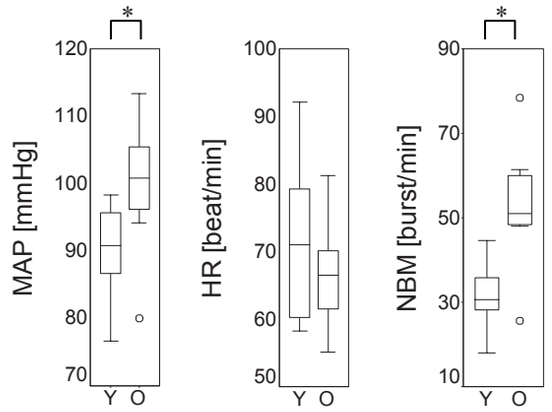
Missing data were considered in 1 – min epochs, prior to calculating the 2- or 3-min averages mentioned above. A 1-min epoch was considered 'missing' if it contained significant artifacts. Inter- or extrapolation replaced these missing points using the group average trend. Of the numbers used, 5.6% consisted of replaced missing data, two thirds of which occurred in the final (7th) distension step, due to measurements stopped early. For this reason, the 7th distension step was left out of all calculations.

General:
Differences were considered significant using a cut-off of $P < 0.05$. All statistics were performed with SPSS version 11. All results that showed normal distribution are expressed as the mean ± standard error of the mean (s.e.m.), other results as median and range.

Because the baseline heart rate did not differ between groups (Figure 2) and the difference between results of burst counts (NBM and NBR) and burst size (ABM and ABR) were not significant, only results of NBM and ABM during CPT and gastric distension are shown.

FIG. 2 **Baseline values**

Baseline MAP (mean arterial pressure measured with sphygmomanometer), HR (heart rate), MSNA in burst frequency (NBM) per group (Y, Young; O, Old).
Boxplots show median, range and interquartile range
○ = indication of excluding outliers
✱ = indication of a significant difference between groups with Mann Whitney U tests



RESULTS

Eleven healthy young subjects were recruited and they could all be included. The measurement failed in 3 subjects, due to practical problems (unable to swallow the barostat bag, MSNA signal could not be found or urge for micturition). Seven out of 16 recruited elderly could not be included due to hypertension, diabetes or postprandial hypotension and in one subject the MSNA signal could not be traced.

Experiments were completed on 8 healthy young subjects (6 men and 2 women, mean age ± SEM: 27 ± 3.2 years, range 21 – 40 years) and 8 healthy elderly subjects (7 men and 1 woman, 76 ± 1.5 years, range 71 – 82). Body mass index was not different between young and older subjects (young, 23.23 ± 0.46 kg/m² versus older, 25.61 ± 1.37 kg/m²; P = 0.12). All subjects were normotensive (supine blood pressure ≤ 160 / 90 mmHg) and none took medication that could affect autonomic circulatory function. Fourteen subjects (6 of the young and all of the elderly) tolerated 7 steps of distension (MDP + 14 mmHg) and the remaining 2 subjects, 6 steps. For technical reasons, recordings were ended in one young subject before the end of the recovery period.

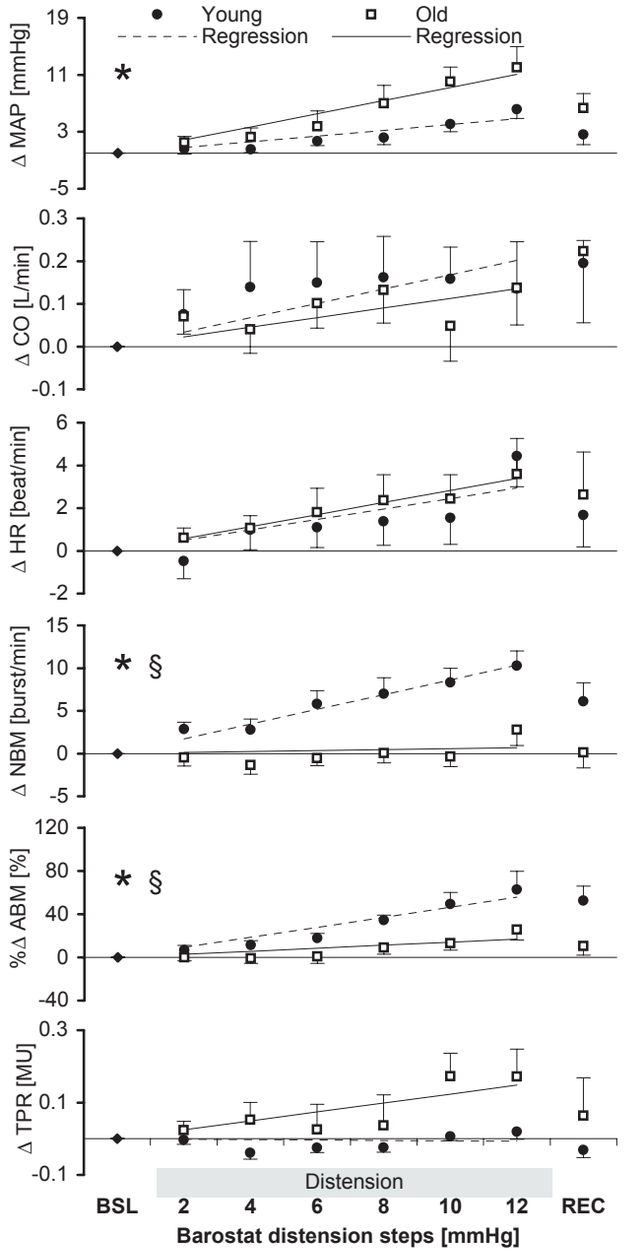
TABLE 1 **Course of parameters (absolute values) during barostat gastric distension**

	Barostat distension steps							
	Baseline	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Recovery
MAP young	92 ± 3.8	93 ± 3.7	93 ± 3.9	94 ± 3.9	95 ± 3.7	97 ± 3.8	**99 ± 3.7**	95 ± 3.3
MAP old	107 ± 7.6	108 ± 7.3	109 ± 7.7	110 ± 8.0	114 ± 8.5	117 ± 8.4	**119 ± 9.2**	113 ± 8.2
HR young	72 ± 4.3	71 ± 4.4	72 ± 4.3	73 ± 4.4	73 ± 4.4	73 ± 4.8	**76 ± 4.4**	73 ± 4.6
HR old	67 ± 2.8	67 ± 2.9	67 ± 2.9	68 ± 2.7	69 ± 2.7	69 ± 2.5	**70 ± 2.5**	69 ± 3.4
NBM young	31.4 ± 2.8	34.3 ± 2.7	34.2 ± 2.1	37.3 ± 1.7	38.4 ± 2.1	39.8 ± 2.1	**41.7 ± 2.3**	37.5 ± 1.9
NBM old	52.9 ± 5.3	52.4 ± 5.1	51.5 ± 5.3	52.3 ± 4.8	52.9 ± 4.8	52.5 ± 5.0	**55.7 ± 4.0**	53.0 ± 4.4

Changes of mean arterial pressure (Finapres; MAP), heart rate (HR) and sympathetic burst frequency (NBM) in reaction to barostat distension steps. Values are mean ± standard error. Maximal mean difference from baseline is shown in bold characters. Significance of changes was calculated on the delta values, not on the absolute values. For statistical results, see text and Figure 3.

FIG. 3 **Course of parameters (Δ) during barostat gastric distension**

Changes in MAP (mean arterial pressure), HR (heart rate) and MSNA in NBM (burst frequency) and ABM (total area under the curve per minute) in reaction to stepwise stomach, ★= indication of significant changes in time as calculated with repeated measures ANOVA. § = indication of a significant difference between the groups or a significant interaction. For details, see text.



EFFECTS OF GASTRIC DISTENSION (BAROSTAT)

Baseline parameters

Baseline mean arterial pressure (sphygmomanometer) and MSNA were higher in the elderly (Figure 2; mean arterial pressure: $P < 0.01$; MSNA: $P < 0.01$). The heart rate did not differ significantly between the groups ($P = 0.4$). All parameters showed a trend to return to baseline level after the barostat distension was stopped (Figure 3).

Changes of parameters during stepwise barostat distension

The absolute values of variables are shown in Table 1 and the observed changes in mean arterial pressure, cardiac output, heart rate and MSNA (NBM and ABM) and total peripheral resistance during distension are illustrated in Figure 3.

During distension, an approximately linear increase in all the variables was found. However, the standard errors indicate that the responses vary appreciably among the subjects. Mean arterial pressure increased during the barostat distension (Figure 3). The slopes of the regression lines were 0.8 and 1.8 mmHg per distension steps for the young and elderly respectively. Repeated measures analysis showed a significant change during distension ($P = 0.003$), but no significant interaction between distension step and group ($P = 0.19$) and no significant difference between subject effect for group ($P = 0.08$). This means that the increase in MAP during subsequent distension steps (as shown in Figure 3) is significant, but both groups reacted in the same way statistically to the barostat distension steps. Cardiac output rose slightly in both groups (maximal increase 0.14 L/min in both groups), but this increase was not significant ($P = 0.6$) and there was no significant difference between groups ($P = 0.6$).

The heart rate showed a trend ($P = 0.06$) towards increase in both groups (Figure 3; slope in the young subjects 0.5 beats/min per distension step; in the elderly subjects 0.6 beats/min per distension step). There was no interaction between distension steps and group ($P = 0.4$) nor a between-subject effect for group ($P = 0.7$), indicating that there was no difference between the groups.

The change in number of sympathetic bursts (NBM) in reaction to the gastric distension was different in the two groups. The young subjects showed a steady increase, whereas the elderly did not seem to change at all (Figure 3; slope in young 1.7 bursts/min, in elderly 0.1 bursts/min). Maximal increase in the young subjects was reached in the 6th step (mean 10 ± 1.7; range +2 to +16 bursts/min). The increase of both groups together was significant (repeated measures analysis: $P = 0.005$). The interaction between distension steps and group was not significant ($P = 0.05$), but the between-subject effect (group) was ($P = 0.002$), indicating that the increase in the young was significantly higher than in the old group.

The difference between the groups in the area under the curve of the sympathetic bursts was less obvious compared to the number of burst measures of MSNA. Both

groups showed an increase (Figure 3; regression slope in young 9.3% per distension step, in the elderly 2.8%/step, both P < 0.01). In the elderly the increase started only after the 4th distension step. Maximal increase was reached in the 6th step in both groups. The interaction between distension steps and the group was not significant (P = 0.3), but the between-subject effect of the group was (P = 0.007). The observed increase in both groups was significant (P = 0.002) as well.

Total peripheral resistance did not increase in the young (0.02 MU increase in the 6th step), but the elderly did show an increase (0.17 MU in the 6th step). The difference between the groups was not significant (P = 0.09)

Comparable results were found when barostat volume and stomach tension (measure that combines pressure and volume inside the gastric barostat bag) were used as dependent instead of barostat pressure (because isobaric distension steps were used, there is no difference in using 'steps' or 'pressure').

Gastric sensory function:

All subjects in the younger group and most subjects in the elderly group reported an increased feeling of fullness during distension (ANOVA: P < 0.001; Spearman correlation coefficient young: 0.85; elderly: 0.66). Most subjects did not feel nauseous, especially in the elderly group (ANOVA: P = 0.2; Spearman correlation young: 0.41; elderly: 0.35) or experience pain except at the higher distension steps (ANOVA: P = 0.05; Spearman correlation younger group 0.68; elderly: 0.45). Only the VAS scores on nausea were significantly higher in the young (ANOVA: fullness: P = 0.19; nausea: P = 0.03; pain: P = 0.9).

Fundic compliance

Fundic compliance was determined by calculating the linear regression coefficient of the pressure – volume curves ($\Delta V/\Delta P$) for each individual. Mean compliance of the young was 43.5 ± 2.4 ml/mmHg, of the elderly 43.9 ± 5.1 ml/mmHg. This difference was not statistically significant (P = 0.9; independent samples t-test).

AUTONOMIC FUNCTION TESTS

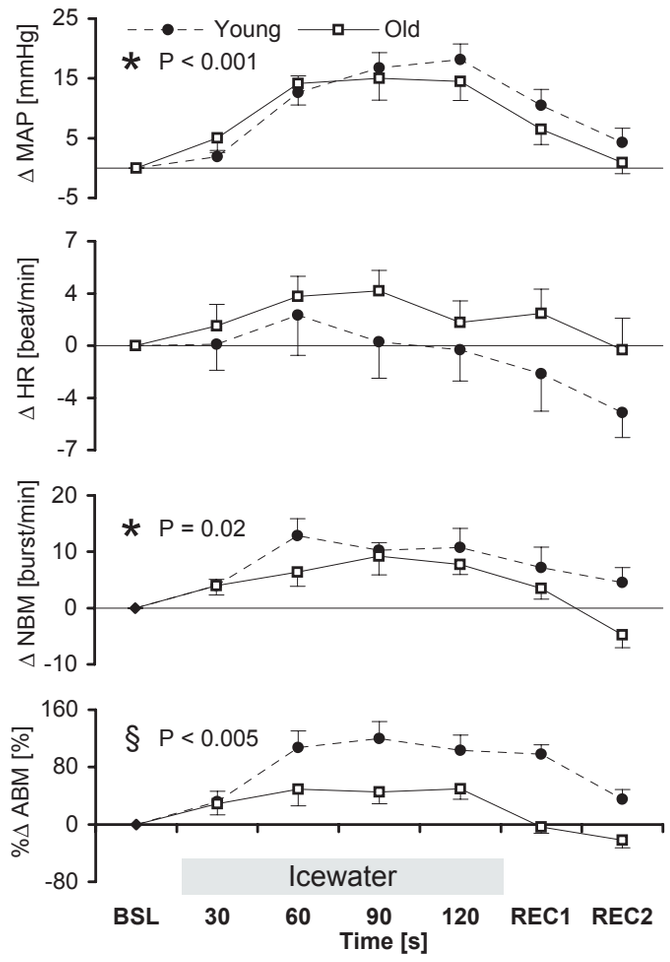
Heart rate variability was calculated from IBIS in the baseline period. LF power, HF power did not differ significantly between the groups (all P ≥ 0.4, data are not shown).

Valsalva ratios of all subjects were above 1.0, which is considered normal in our laboratory. The mean value of the younger group was slightly higher than that of the elderly, but this difference was not statistically significant (P = 0.4, data are not shown).

During the cold pressor test, NBM, ABM and MAP rose steadily, whereas the HR peaked at 1 - 1.5 min and declined slightly thereafter (Figure 4). Repeated measures ANOVA showed a significant change in time in mean arterial pressure and sympathetic burst frequency (NBM), a trend in sympathetic burst area (ABM: P = 0.05), but no significant change in time in heart rate (P = 0.13; Figure 4). The change in number of sympathetic

FIG. 4 **Course of parameters (Δ) during cold pressor test**

Changes in MAP (mean arterial pressure), HR (heart rate) and MSNA in burst frequency (NBM) and burst area (ABM) in reaction to a cold pressor test. BSL = baseline. REC = recovery. ★= indication of significant changes in time as calculated with repeated measures ANOVA. § = indication of a significant difference between the groups or a significant interaction.



bursts (NBM) in reaction to the CPT tended to be different in each group, the interaction between time and group was nearly significant (P = 0.05), but the between subject effect (group) was not significant (P = 0.10). The sympathetic burst area (ABM) showed a statistically different reaction between the groups. The young group showed a significantly higher increase compared to the elderly (P = 0.005).

DISCUSSION

The main finding of the present study is that the elderly subjects showed reduced reactivity of MSNA during stepwise gastric distension. This reduced reactivity to gastric distension could be part of a more generalized difference in autonomic function between young and old, as the reactivity of sympathetic burst area (ABM) in reaction to a cold pressor test was reduced in the elderly as well. Fundic compliance did not differ between the groups. Therefore, it is unlikely that reduced reactivity of MSNA in reaction to gastric distension is related to fundic compliance.

Although the effect of proximal gastric distension induced by food intake is different from that of stretching the stomach wall by means of barostat in this study, both result in an elongation of the gastric wall and activation of stretch receptors (Scheffer et al., 2002).

The higher baseline MSNA, which was found in the older group (Figure 2), has been described by others as well, and is considered an age-related physiological change (Sundlof and Wallin, 1978; Iwase et al., 1991). Ageing also reduces the MSNA responses to Valsalva (Laitinen et al., 1999), to gravitational stress (Iwase et al., 1991) and to vestibular stimuli (Ray and Monahan, 2002). In contrast with our result, Ray and Monahan (2002) found that the increase in total activity of MSNA in response to a cold pressor test in the elderly was not attenuated, although the change in burst frequency in older subjects was less than that in young subjects. It should be noted that they did not examine subjects above the age of 70. Regarding higher baseline MSNA in the elderly, it is our experience that the difference in baseline MSNA between young and older subjects is the most manifest in the 70+ age group. This is also the reason why the elderly group in this study consisted of subjects who were over 70.

Superior mesenteric artery-flow and -diameter increased in response to a meal (Fujimura et al., 1997). These phenomena can be interpreted as an effect of a decrease in splanchnic arterial resistance, possibly by the release of vasoactive intestinal hormones. The gastrovascular reflex (increase in MSNA and blood pressure in reaction to distending of the stomach) prevents a fall in blood pressure by increasing local arterial resistance in muscles. With regard to the physiological mechanism of the gastrovascular reflex, it was hypothesized in our previous study that the sympathetic response is a direct (neural) effect of stomach distension mediated by tension receptors in the stomach wall through vagal afferent fibers, comparable with a spinal

reflex (Rossi et al., 1998). This hypothesis is supported by the findings from another study (O'Donovan et al., 2002) and an ongoing experiment (Van Orshoven et al., personal communication) in which intraduodenal glucose infusion is applied as a trigger. In these experiments the MSNA and blood pressure started to change only after 10 min, whereas in the present study the increase of MSNA already occurred within 1 min. We hypothesize that stretching the stomach wall increases MSNA through a direct, fast acting neural pathway and that intraduodenal glucose infusion increases MSNA through a slow, indirect pathway by the release of vasoactive intestinal peptides. This might cause splanchnic blood pooling and subsequently a fall in venous return of blood to the heart and a lower cardiac output, which activates the baroreflex and thus increases MSNA.

The slope of the regression line of MSNA during the stepwise gastric distension was less steep in the elderly. On the other hand, total peripheral resistance and blood pressure tended to increase more in the elderly than in the young. These findings seem to be in contradiction with each other: a stronger increase in MSNA in the young group would be expected to lead to a stronger increase in total peripheral arterial resistance and subsequently more increase in blood pressure. A likely explanation is that in older subjects the decrease in splanchnic arterial resistance induced by gastric distension is less pronounced than in young subjects. This may be caused by a reduced compliance of the splanchnic arteries in the elderly. This assumption is in line with the well-known fact that ageing causes reduction in central arterial compliance (Monahan et al., 2001). In non-compliant arteries, the ability to show vasodilatation is reduced. More pronounced vasodilatation in the splanchnic system of the young group might explain why total arterial resistance and blood pressure did not increase that much. Although this explanation seems reasonable, the current study does not give direct evidence for it. Further experiments with a direct measurement of splanchnic flow, for instance by the non-invasive duplex ultrasound method, are desirable.

Furthermore, a small increase of sympathetic activity has a larger effect on blood pressure in non-compliant arteries than in compliant arteries. This may explain why the blood pressure in the elderly increased strongly despite their attenuated MSNA response. Reduced vascular compliance may also explain the finding that mean arterial pressure has a significant positive correlation to an increase in MSNA in elderly, but not in young subjects (Watanabe et al., 1993).

It could be argued that the attenuated increase in MSNA in the elderly during distension relates to their higher baseline. MSNA consists of rhythmic spontaneous discharges that are synchronous to the heartbeat, and NBM cannot exceed the heart

rate (Sundlof and Wallin, 1978). Our results of the cold pressor test, also found by others (Ray and Monahan, 2002), strongly suggest that the 'ceiling effect' is unlikely, since the elderly showed a similar NBM increase to the young.

It could be argued that the higher increase of MSNA in young subjects is due to an indirect effect (discomfort) of gastric distension since the VAS scores in nausea during gastric distension is a significant difference between the young and the elderly group. However, pain and other stress reactions are characterized by a prompt and immediate increase in heart rate (Freyschuss et al., 1990) and in the present study the minor increase in heart rate was not significantly different between the groups. We consider the difference in the results of the gastric sensory test as an expression of decreased perception of gastric distension in the elderly, which was also found by others (Rayner et al., 2000).

It has been suggested that decreased fundic compliance could be a cause of postprandial hypotension (Morley, 2001) by attenuating the incentive of the gastrovascular reflex. The results of the present study, as well as of another study (Rayner et al., 2000) showed no difference in fundic compliance between the young and the elderly. These results cannot reject the hypothesis, since we only studied healthy elderly subjects and not patients suffering from postprandial hypotension. However, we think this explanation is less likely, because we would then expect to find at least a minor difference between young and old.

In the present study, normal results for both young and older groups on Valsalva maneuver and similar results of the spectral analysis of the heart rate variability suggest normal autonomic function. The findings that the area under the curve of the MSNA of the elderly group showed an increase during proximal gastric distension and during CPT and that the variables decreased toward initial values after the end of provocations indicate an intact baroreflex control of MSNA.

In normal subjects superior mesenteric artery flow increases postprandially which causes an increase in splanchnic-mesenteric blood volume. The systemic blood pressure is maintained due to compensation by baroreflex-mediated decrease in the volume of the muscle and probably the skin resistance bed. In patients with autonomic failure, the superior mesenteric artery flow increase response to feeding is mostly preserved, but the auto-regulatory response is reduced or absent, and therefore systemic blood pressure passively falls as a larger blood volume is transferred into the splanchnic bed (Fujimura et al., 1997). We would emphasize that MSNA is dominated by vasoconstrictor impulses destined for muscle blood vessels and the main control of MSNA is exerted by arterial baroreceptors (Wallin and Elam, 1997). An increase in

MSNA is associated with intramuscular vasoconstriction. The increase in MSNA and blood pressure during stretching of the stomach wall in both the young and the elderly found in the present study might be considered as a reflex preventing a fall in systemic blood pressure during the ingestion of a meal.

Limitation of the present study

The performance of the experiments was technically demanding, which limited the number of subjects studied and thereby the power of the statistical tests. As an illustration, observed powers were calculated for some repeated measures analyses of the gastric distension effect (for $P = 0.05$, using SPSS 11.0). For mean arterial pressure, the observed power was 0.4 for both the between-groups-effect and the interaction between the distension step and the group. For HR, the observed powers were 0.6 for the effect of the step, and smaller than 0.3 for the interaction and group effect. Low powers indicate a low chance of finding a significant difference in the available sample, assuming a stated difference exists in the population.

Although we do not know how reproducible the results of this study are, it is a well-known fact that intraindividual variations in MSNA are small (Wallin and Elam, 1997). In our previous study we also found a high reproducibility of MSNA in healthy subjects and chronic renal disease patients, both at rest as well as in reaction to provocations (Ligtenberg et al., 1999).

The measurement of cardiac output and total peripheral resistance by means of pulse contour analysis has its limitation for assessing the absolute values (Remmen et al., 2002). Therefore we used this method only to express the relative changes of cardiac output and total peripheral resistance during gastric distension. This has proven to be a valuable non-invasive method, particularly when the body-position has not changed during the measurement (Stok et al., 1999).

To summarize, the present study shows that stepwise gastric distension causes an increase in MSNA in young and older subjects. However, the increase in MSNA is attenuated in the elderly group. Further studies are needed to determine whether the gastrovascular reflex is altered in patients with postprandial hypotension, a disorder which frequently occurs in the elderly.

CHAPTER 3

The effect of intraduodenal glucose on muscle sympathetic nerve activity in healthy young and older subjects

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ABSTRACT

OBJECTIVE The cardiovascular response to a meal is modulated by gastric distension and the interaction of nutrients, particularly carbohydrate, with the small intestine. We tested the hypothesis that the depressor effect of small intestinal glucose is greater in older than in young subjects and that this reflects a blunted increase in muscle sympathetic nerve activity (MSNA).

METHODS The effects of intraduodenal glucose infusion (IDGI) on blood pressure, heart rate and MSNA were evaluated in 8 healthy young subjects (4 women; mean age ± s.e.m.: 28.8 ± 3.4 years), 8 healthy elderly (4 women; 75.3 ± 1.6 years) and in two patients with symptomatic postprandial hypotension (PPH), one young (21 years), and one old (90 years).

RESULTS In both young and elderly healthy subjects, IDGI decreased blood pressure ($P < 0.05$), but the fall in systolic blood pressure was greater in the older subjects (17.0 ± 4.1 versus 6.5 ± 1.6 mmHg, $P = 0.03$). The increases in MSNA in both groups during IDGI were comparable (old versus young: 9.0±3.4 versus 7.8±1.0 bursts/minute). Baroreflex sensitivity for numbers of sympathetic bursts was attenuated in the elderly ($P < 0.03$). The increase in burst area in the young patient with PPH was attenuated (18 versus 63% in the healthy young group).

INTERPRETATION The fall in blood pressure induced by IDGI is greater in healthy elderly when compared to young. The reason for this is unclear, as they have similar increases in MSNA.

INTRODUCTION

The apparently simple act of eating and digesting a meal generates a host of autonomic changes. After eating (or drinking glucose in the laboratory setting), splanchnic blood vessels dilate (Mathias and Bannister, 1999b) to divert blood to the gastrointestinal tract (Kooner et al., 1989) in order to meet the oxygen demands of digestion. Maintaining blood pressure after consuming a meal is therefore a balance between the amount of blood diverted to the gastrointestinal tract and the degree of reflex mediated sympathetic vasoconstrictor discharge. A series of mechanisms (Van Orshoven et al., 2004; Van Orshoven et al., 2006), which sense the volume and carbohydrate content of the meal (Vloet et al., 2001; O'Donovan et al., 2002; Lipp et al., 2005), regulate the amount of blood that is diverted to the gut. This effectively reduces circulating blood volume. Normally, in healthy young people, the arterial baroreceptors sense this temporary "hypovolaemia" and increase sympathetic nerve outflow to other vascular beds in order to maintain blood pressure (Lipsitz et al., 1993; Fagius et al., 1996). In elderly people, for reasons that are not completely known, blood pressure falls after eating (Jansen and Hoefnagels, 1989; Peitzman and Berger, 1989). Postprandial hypotension (PPH), defined as a fall in systolic blood pressure of 20 mmHg or more within 2 hours of eating (Jansen and Lipsitz, 1995), occurs in people with autonomic impairment. In severe cases of autonomic failure, patients can, and frequently do, loose consciousness when standing up after eating.

The amount of splanchnic vasodilatation depends upon the glucose content of the meal. It is thought that when the gastric contents empty into the small intestine, receptors are activated by carbohydrates and regulate the withdrawal of vasoconstrictor tone and the degree of local splanchnic vasodilatation. In healthy older subjects and in patients with type II diabetes, the hypotensive response to oral glucose is related to the rate of gastric emptying and the consequent interaction of glucose with the small intestine (Jones et al., 2001a; O'Donovan et al., 2002; Russo et al., 2003; O'Donovan et al., 2005). Furthermore, when gastric emptying and small intestinal glucose exposure are slowed, by oral (Jones et al., 2001a; Russo et al., 2003) or intraduodenal (O'Donovan et al., 2005) administration of the viscous polysaccharide–guar, the fall in blood pressure and rise in heart rate are both attenuated. It has also been suggested that the osmolarity of the carbohydrate liquid may contribute to the hypotensive response (Lipp et al., 2005; Raj et al., 2006). However, a recent study

indicated that the hypotensive response to intraduodenal glucose in healthy older subjects is dependent on load and not concentration (Gentilcore et al., 2006).

In addition to the arterial baroreflex, the degree of reflex mediated sympathetic outflow is probably regulated by a mechanical mechanism arising from the stomach, the so-called "gastrovascular reflex". Gastric distension activates the stretch receptors lining the walls of the stomach, which fire afferent signals to the central nervous system to increase muscle sympathetic nerve activity (MSNA), heart rate, cardiac output and blood pressure (Rossi et al., 1998; Scott et al., 2001; Shannon et al., 2002; Van Orshoven et al., 2004). Therefore, distension of the stomach has a pressor effect. The function of the gastrovascular reflex is blunted by age (Van Orshoven et al., 2004), and probably contributes to the fall in blood pressure after eating in the elderly. At present it is not known if the increase in MSNA induced by oral glucose (Berne et al., 1989) is mediated by the gastrovascular reflex, the baroreflex, or due to the effect of a carbohydrate load with the small intestine. The aim of this present study was to "bypass" the effects of gastric distension and determine whether there is a cardiovascular response to the presence of nutrients in the small intestine. To do this we measured blood pressure, heart rate and MSNA in subjects after intraduodenal glucose infusion (IDGI). Second, we wanted to determine if aging had any effect on this mechanism. To achieve this we compared the cardiovascular responses following IDGI in healthy young and elderly subjects. Third, we wanted to evaluate the effect of intraduodenal glucose in two patients with symptomatic PPH.

MATERIALS AND METHODS

POPULATION

HEALTHY SUBJECTS Twelve young (age < 35 years, free of overt cardiopulmonary disease) and 13 older (age > 70 years) subjects were recruited by advertisement. Health status of the elderly was confirmed by a detailed questionnaire and physical examination, using exclusion criteria applicable to medically stable elderly subjects undergoing exercise studies (Greig et al., 1994).

The protocol was not completed in 4 young subjects (vasovagal collapse during introduction of the catheter in 1, nausea / vomiting during the test in 1 and failure to find MSNA in 2) and in 5 older subjects (2 unwilling to complete the protocol, urge for micturition in 1 and in failure to find MSNA in 2). Measurements were, accordingly, completed in 8 healthy young (4 women, 4 men; mean age ± s.e.m.: 28.8 ± 3.4 years, body mass index (BMI): 24.2 ± 1.3 kg/m²) and 8 healthy elderly subjects (4 women, 4 men; 75.3±1.6 years, BMI: 25.1 ± 1.0 kg/m²).

SYMPTOMATIC PPH PATIENTS Ten subjects with symptoms suggestive of PPH were referred by a neurologist and a geriatrician at University Medical Center Utrecht. They underwent a standard 'meal-test' within a week prior to the experiment: ingestion of 75g glucose in 300 ml water (Jansen et al., 1987) after an overnight fast. Four of the subjects could be included as they showed PPH (20 mmHg or greater fall in systolic BP within 2 hours after this drink (Jansen and Lipsitz, 1995). Of those 4 subjects, 1 subsequently withdrew from the study and another failed to complete the protocol. Two patients with PPH completed the protocol: patient A, a 22 year old female, had a history of 'light-headedness' and tiredness after meals, particularly those high in carbohydrate. Patient B, a 90 year old female, had suffered from fainting / near collapse after meals for a number of years and was otherwise healthy. Both patients had no relevant previous medical history, were not taking any medication and physical examination showed no abnormalities.

The Ethics Committee of the Utrecht University Hospital approved the protocol. All subjects provided their written, informed consent for participation in the study.

MEASUREMENT INSTRUMENTATION

Glucose was administered intraduodenally using a silicone rubber catheter (Dentsleeve, Wayville, South Australia, Adult Assembly, diameter 3.5 mm), introduced

through a nostril. Two extra catheter ports were used for measuring the transmucosal potential difference (TMPD), to monitor the catheter position (Fone et al., 1990). The infusion port for glucose was 5 cm distal to the duodenal TMPD channel. Basal blood pressure was measured with a sphygmomanometer. Continuous BP was recorded using finger photoplethysmography (Finapres, Ohmeda, Englewood, CO, USA) at a fixed vertical heart – hand distance. The electrocardiogram (ECG) was recorded using bipolar chest leads. Multifibre recordings of MSNA were made with a microelectrode inserted in the peroneal nerve of the right leg, as described previously (Van Orshoven et al., 2004).

Electrocardiogram, finger arterial BP with derived systolic, mean and diastolic arterial blood pressures (Sys BP, Mean BP, Dias BP; mmHg) from the Finapres and MSNA were recorded continuously and monitored on-line (sampling at 200 Hz with POLY 5, Physiological Analysis Package, Inspector Research Systems B.V., Amsterdam, the Netherlands). Baroreceptor-heart rate reflex sensitivity (inter beat interval to systolic BP) was evaluated with HemoLab software (http://www.intergate.com/~harald/HemoLab/HemoLab.html).

EXPERIMENTAL PROTOCOL

The study was performed in an air-conditioned room. All subjects were instructed to refrain from eating, drinking, and smoking after 9:00 p.m. the previous day. All medications known to affect BP were discontinued for at least 24 hours.

The nasoduodenal catheter was initially positioned. Subjects were then asked to empty their bladder and placed in half sitting position on a bed at an angle of 70° with the horizontal plane. A maximum of 1 hour was required to find the optimal MSNA signal.

Figure 1 summarizes the time line of the experimental protocol. During the 'baseline' period, all signals were recorded in the resting state, with NaCl 0.9% infused intraduodenally. At t = 30 minutes an IDGI at a rate of 3 kcal / minute was commenced and continued for 60 minutes. During the 'recovery' period, NaCl 0.9% was administered again.

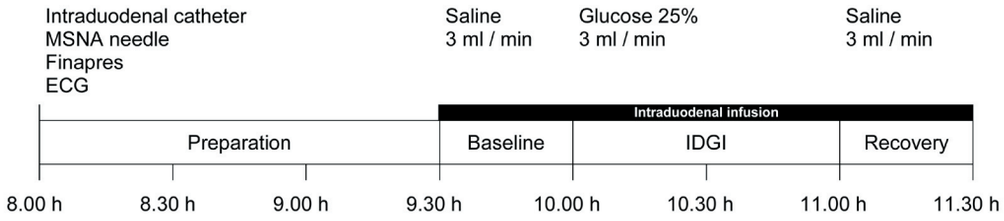
DATA ANALYSIS

All signals were carefully checked off-line and epochs with missing data or with artifacts were marked and omitted in further analysis.

Heart rate (beats/minute) and beat duration (RR interval; ms) were calculated beat to beat from the ECG. Sympathetic bursts in the MSNA were identified by their characteristic morphology and relationship to R-waves in the ECG. The sympathetic burst frequency was calculated as the number of bursts per minute [bursts/minute]

FIG. 1 **Experimental protocol**

The start of the baseline period was variable, while the duration of all infusion periods was standardized..



and per 100 heart beats [bursts/100 beats]. The area of the sympathetic bursts was expressed in arbitrary units per minute [area/minute] and per 100 heart beats [area/100 beats]. Cardiac output (CO; l/minute), stroke volume (SV; ml/minute) and total peripheral resistance (TPR; mmHg/(l/minute) were obtained by pulse contour analysis of the BP signal (software: Beatscope 1.0; TNO TPD biomedical instrumentation, Academic Medical Centre, Amsterdam, the Netherlands) and quantified as relative changes.

The short term BP-MSNA relationship, defined as the sensitivity of MSNA for changes in diastolic BP, was calculated from spontaneous blood pressure variations, using a method adapted from Kienbaum et al. (2001). Sensitivities were calculated over 5 minute epochs from the baseline, IDGI and recovery periods. Heartbeats with extrasystole or other large BP variations were removed manually. For each beat, the diastolic BP was plotted against the corresponding MSNA (approximately 1.4 seconds later) value. In some cases, beats with extreme diastolic BP values were present, at which MSNA saturated: a curve fitted through all points would be a sigmoid. Only the linear part of the sigmoid curve was used in the calculation of a linear regression and the slope of this regression line was used as an index of baroreflex sensitivity. At least 100 heartbeats were used for the regression, and sensitivity values were accepted only if the correlation was not too low. Four variants of the sensitivity were calculated, corresponding to the four ways of expressing MSNA (bursts/minute, bursts / 100 beats, area/minute and area/100 beats, all per mmHg of Dias BP).

Baroreceptor-heart rate reflex sensitivity was evaluated by the sequence technique, using selected 5-minute periods with verified signal quality. This was done at 5 instances in the protocol: 1 period during baseline, 1 in the first 20 minutes of intraduodenal glucose infusion, 1 between 20 and 40 minutes, 1 between 40 and 60 minutes and in 1 during the recovery period.

For the analysis of changes of the parameters during the IDGI period, the measure-

ment was divided into 2-minute epochs. Mean values of all parameters were calculated per epoch. Epochs containing artifacts (6% of the data, evenly distributed over subjects and measurement duration) were replaced by interpolated values (SPSS version 11.0.1). For the IDGI period, all parameters were expressed relative to their baseline values (Δ = change from baseline). For the MSNA burst area (expressed in arbitrary units), and for CO, SV and TPR, proportional changes (%Δ) compared to baseline were calculated.

In each individual, the extreme values (e.g., the greatest blood pressure fall) were considered most relevant to assess the effect of IDGI. As maximum changes occurred at different times in each individual, averaging over subjects for a given time point would obscure any effect. Accordingly, the effect of IDGI was evaluated by calculating individual maximal increases (MSNA, CO, HR) or decreases (Sys BP, Dias BP).

The mean changes from baseline were also calculated at the nadir of the systolic blood pressure: for each individual, mean values of all parameters were taken at the time of the maximum systolic blood pressure fall, using the same 2-minute epochs mentioned above.

Prior to calculation of the extrema, data were smoothed using a 10-minute rectangular time window (5 blocks of 2 minutes). Maximum decreases were calculated by subtracting the minimum value during baseline from the minimum value during IDGI (the result is negative, indicating a decrease). Maximum increases were calculated by subtracting the maximum value during baseline from the maximum value during IDGI. For the parameters that were expressed as relative changes from baseline, the mean value of the baseline was set at 100%, followed by subtraction of the extreme relative value during baseline from the extreme relative value during IDGI.

STATISTICS

All analyses were performed with SPSS version 11.0.1. We considered differences to be significant using a cut – off point of $P < 0.05$. Bonferroni correction was used in cases of multiple t-tests. Results that showed a normal distribution are expressed as mean ± standard error of the mean (s.e.m.), and other results as median and range or specified percentiles.

The group results for baroreceptor-heart rate reflex sensitivity and baseline BP, HR, MSNA values were compared with independent samples t-tests. The BP-MSNA relationship measures were compared with a Mann-Whitney U test. T-tests were used to compare maximum changes between groups and to determine whether maximum changes were different from baseline.

RESULTS

BASELINE RESULTS

Cardiovascular parameters and blood pressure – MSNA relationship

Baseline values in young and older healthy subjects and in patients A and B are summarized in Table 1. There were no differences between the young and older groups in any parameter, except for the sphygmomanometric systolic BP and for MSNA (bursts/100 beats). Baseline HR was higher in both patients. Baseline MSNA (bursts/minute) did not differ from the healthy controls in patient A, and was not available in patient B.

The BP-MSNA relationship, used as a measure of baroreflex sensitivity, was calculated for all data during baseline, IDGI and recovery. Thirty-five percent of the sensitivity values had to be rejected, mostly because the correlation was too low. For 2 elderly subjects, no useful data remained. There was no significant effect of protocol stage (baseline, IDGI or recovery) on any of the BP-MSNA relationship measures. Therefore, average values per subject were used, giving 4 numbers per subject, as plotted in Figure 2. Significant differences between young and old were evident for the BP-MSNA relationship for MSNA expressed as the number of bursts per minute or per 100 heartbeats.

TABLE 1 Baseline parameters

Results at baseline in healthy young and older subjects (mean ± s.e.m. and in two patients with PPH. Comparison between the two healthy groups is by independent samples t-test). Blood pressure results are from sphygmomanometer. Sys BP = systolic blood pressure; Dias BP = diastolic blood pressure; HR = heart rate, MSNA = muscle sympathetic nerve activity.

Parameter	Healthy young N=8	Healthy old N=8	Young vs Old P	Patient A (young)	Patient B (old)
Systolic BP [mmHg]	132±3.6	149±3.9	0.005	138	157
Diastolic BP [mmHg]	87±4.4	87±3.1	0.98	82	89
Heart Rate [beat/minute]	68±5.3	61±2.2	0.3	84	79
MSNA [bursts/minute]	41±4.5	47±2.8	0.3	48	-
MSNA [bursts/100 beats]	59±4.3	78±5.5	0.02	57	-

For cardiac baroreflex sensitivity, the HemoLab software detected true baroreflex sequences in all 80 selected 5-minute periods except for 1 (1% of the data used). The mean value of the baroreflex gains found for delays of 0 or 1 heartbeat was used in further analyses. Repeated measures ANOVA showed a significant difference between the groups (P = 0.005), but no relevant effect of time (P = 0.2), and no interaction of time and group (P = 0.6). Therefore, pooled data (mean value of all 5-minute periods for each subject) were used as a measure of the baroreceptor-heart rate reflex sensitivity. This showed a higher baroreceptor-heart rate reflex sensitivity in the young group (16.4 ± 2.7 ms/mmHg of Sys BP versus 6.7 ± 1.1 ms/mmHg of Sys BP, P = 0.004; analysis with only the delay = 0 or delay = 1 heartbeat showed similar results).

TABLE 2 **Maximum changes in cardiovascular parameters in response to intraduodenal glucose infusion in healthy young and older subjects**

The mean maximal fall (decreasing parameters) or mean maximal rise (increasing parameters) of the healthy young (Y) and older (O) groups. All parameters changed significantly during IDGI, relative to baseline (P<0.05), except for MSNA (bursts/100 beats) (young P = 0.04, old P = 0.1) and MSNA (area/100 beats) (young P = 0.008, old P = 0.06). BP = blood pressure; MSNA = muscle sympathetic nerve activity.

Parameter	Change from baseline (units as specified per parameter)			Time from the start of IDGI to maximum change [minute]		
	Young N=8	Old N=8	P	Young N=8	Old N=8	P
Max↓Systolic BP [mmHg]	-6.5 ± 1.6	-17.0 ± 4.1	0.03	23.6 ± 8.1	33.8 ± 6.4	NS
Max↓Diastolic BP [mmHg]	-7.3 ± 2.3	-7.0 ± 1.5	NS	32.3 ± 8.7	32.0 ± 5.0	NS
Max↑Heart Rate [beat/minute]	7.2 ± 1.6	9.4 ± 2.2	NS	48.8 ± 4.7	44.5 ± 4.7	NS
Max↑Stroke Volume [%]	12.1 ± 5.0	6.4 ± 1.6	NS	42.1 ± 7.7	31.0 ± 5.1	NS
Max↑Cardiac Output [%]	22.3 ± 7.4	17.2 ± 3.5	NS	44.0 ± 5.5	38.3 ± 4.5	NS
Max↓Tot Peripheral Resistance [%]	-21.5 ± 4.4	-18.9 ± 3.1	NS	43.8 ± 5.8	43.5 ± 4.2	NS
Max↑MSNA [burst/minute]	7.8 ± 1.0	9.0 ± 3.4	NS	43.3 ± 5.3	29.6 ± 6.2	NS
Max↑MSNA [bursts/100 beats]	8.7 ± 1.9	6.8 ± 3.6	NS	42.7 ± 4.8	27.0 ± 6.4	NS
Max↑MSNA (area/minute) [%]	62.5 ± 14.4	59.8 ± 23.9	NS	50.1 ± 3.8	31.3 ± 4.9	0.01
Max↑MSNA (area/100 beats) [%]	56.0 ± 14.2	44.6 ± 18.8	NS	50.7 ± 3.9	28.4 ± 6.7	0.01

EFFECTS OF INTRADUODENAL GLUCOSE INFUSION

The maximal effects of IDGI are shown in Table 2. The only parameter for which there was a different maximal response to IDGI in the two healthy groups was systolic BP; in the older group the mean maximal fall in Sys BP was substantially greater than in the younger group (17.0 ± 4.1 mmHg versus 6.5 ± 1.6, P = 0.03). The timing of the maximal increase / fall, shown in Table 2, did not differ between the two groups for any parameter, except for the peak in MSNA area, which occurred later in the young

healthy group (whether MSNA was expressed as area/minute or area/100 beats). In the young group, the maximal increase in burst occurrence (times for MSNA expressed as bursts/minute and bursts/100 beats taken together) was earlier than the peak in the burst area measures, but the difference was not statistically significant (43 ± 5.0 versus 50 ± 3.8 minutes, P = 0.1).

Table 3 shows the results of changes from baseline at the nadir of the blood pressure fall. Stroke volume increases in the young subjects, and decreases in the elderly. Cardiac output shows a greater increase in the young group, although this difference is not statistically significant.

Both PPH patients exhibited a fall in Sys BP that was greater than in the healthy controls. In patient A there was a gradual fall in Sys BP with a maximum of 28 mmHg. At this time, she complained of dizziness. In patient B there was a maximum blood pressure fall of 92 mmHg at 45 minutes. She complained of dizziness and fatigue and, at the maximum fall, briefly lost consciousness and was put in the Trendelenburg position. In contrast to the healthy individuals, who showed an increase in HR during IDGI, there was a decrease of 4 beats/minute at 31 minutes after the start of IDGI in patient A and a decrease of 38 beats/minute at 39 minutes in patient B. The maximal increase in MSNA (area/minute) in patient A was 18% compared to a mean maximum increase of 63% in the healthy young group. The timing of the maximal changes did not differ between patients A and B and their respective age control groups, except for MSNA (area/minute), which occurred at 29 minutes in patient A, similar to that in the healthy older subjects. MSNA (bursts/minute) increased in patient A to a maximum of 5.3 bursts/minute, compared to a mean maximum increase of 7.8 in the healthy young

TABLE 3 **Changes from baseline at the nadir of the systolic blood pressure**

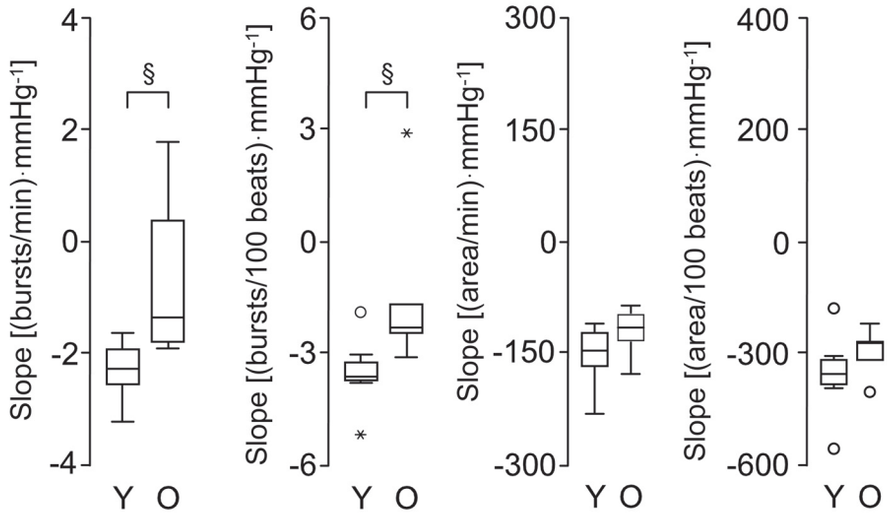
Same parameters as in table 2, values averaged at individual time points of maximal decrease of systolic blood pressure.

Parameter	Healthy young N=8	Healthy old N=8	Young vs Old P	Patient A (young)	Patient B (old)
Systolic BP [mmHg]	-6.5 ± 1.6	-17.0 ± 4.1	0.03	-28.4	-92.2
Diastolic BP [mmHg]	-6.1 ± 1.9	-6.4 ± 1.3	NS	-7.3	-50.4
Heart Rate [beat/minute]	2.7 ± 0.7	5.7 ± 2.1	NS	-1.6	-36.2
Stroke Volume [%]	8.9 ± 4.5	-2.5 ± 2.4	0.04	-12.8	-40.0
Cardiac Output [%]	14.1 ± 5.9	5.6 ± 3.0	NS	-14.4	-11.3
Total Peripheral Resistance [%]	-15.9 ± 4.4	-11.9 ± 2.5	NS	2.2	-28.4
MSNA [bursts/minute]	0.9 ± 2.4	1.7 ± 1.9	NS	4.4	-
MSNA [bursts/100 beats]	1.1 ± 2.9	-1.4 ± 2.6	NS	5.3	-
MSNA (area/minute) [%]	20.6 ± 13.2	22.6 ± 17.2	NS	14.3	-
MSNA (area/100 beats) [%]	17.1 ± 12.4	15.2 ± 15.2	NS	15.5	-

group. CO increased with 4% in patient A and 7% in patient B compared to a mean maximum increase of 22% in the young and 17% in the older subjects. TPR showed a decrease of 3% in the young patient and 11% in the older patient, compared to about 20% in both healthy groups.

FIGURE 2 **BP-MSNA relationship in healthy young and older subjects**

Box plots showing measures of the BP-MSNA relationship (sensitivity of changes in MSNA for spontaneous changes in diastolic BP). The measures were calculated over multiple 5-minute periods during the entire protocol and averaged in each subject. There are significant differences (§, $P < 0.05$) between young (Y) and old (O) for MSNA expressed as the number of bursts per minute and as burst per 100 heartbeats (Mann-Whitney U test, $P < 0.03$). For the MSNA (area/minute) and MSNA (area/100 beats) measures, the differences between the age groups were not significant ($P > 0.2$). Box plots show median, interquartile range, outliers (o=values between 1.5 and 3 box lengths from the upper or lower edge of the box) and extremes (*=value > 3 box lengths from the upper or lower edge of the box).



DISCUSSION

The main findings of this study of healthy young and older subjects and 2 patients with idiopathic PPH were: (1) in both healthy young and older subjects IDGI induces a fall in BP, a rise in HR, and an increase in MSNA; (2) the fall in systolic BP in response to IDGI was smaller in the healthy young, compared to older subjects, but there is no difference in the magnitude of the HR or MSNA responses, although the maximum increase in MSNA burst area occurs earlier in the older subjects; (3) the short-term sensitivity of MSNA (number of bursts) for BP and the baroreceptor-heart rate reflex sensitivity was less in older subjects; and (4) in PPH patients IDGI is associated with a marked fall in BP, a decrease in HR and (in the one patient in which data were available) attenuation of the increase in MSNA burst area (area/minute). The above observations provide novel insights into the mechanisms responsible for the greater postprandial blood pressure fall in healthy elderly compared to young adults, and an even greater fall in PPH patients.

An important response to oral glucose is activation of MSNA. Fagius et al. (1996) reported that the increase in MSNA in response to oral glucose was blunted in the elderly. We have now shown that the 'gastrovascular reflex' is not the only meal-related mechanism that increases MSNA, in that an increase in MSNA also occurs in response to the interaction of glucose with the small intestine. Furthermore, there was no difference in the MSNA response to IDGI between healthy young and older subjects, suggesting that the blunted MSNA response to oral glucose (300 ml) in healthy elderly, as reported by Fagius et al. (1996), reflects an impaired 'gastrovascular reflex', rather than a difference in the effects of glucose per se.

We cannot determine whether the observed increase in MSNA in response to IDGI represents a secondary, baroreflex-mediated, response to the BP fall, or that the interaction of glucose with the small intestine directly causes an increase in MSNA. The well-characterized baroreflex mechanism may be sufficient to account for the MSNA increase.

The healthy elderly exhibited a greater fall in blood pressure, while their MSNA activation was comparable to the young. Five, not mutually exclusive, mechanisms / hypotheses may account for this: (1) reduced baroreflex sensitivity in the healthy elderly, (2) reduced peripheral vascular responsiveness to MSNA, so that there is a lesser increase in vascular resistance in response to a given increase in MSNA, (3) a

more pronounced fall in splanchnic vascular resistance in response to IDGI, (4) a lesser increase in cardiac output in response to IDGI and (5) a difference in the release of vasoactive hormones / peptides.

The measurements performed in our study support the hypothesis 1, i.e. that the greater fall in blood pressure in elderly reflects a reduced baroreflex sensitivity. That a greater fall in BP in the healthy elderly did not evoke a greater MSNA increase, could be interpreted as indicative of decreased sympathetic baroreflex sensitivity. More directly, our study provides evidence that sympathetic baroreflex sensitivity is diminished in the elderly (Figure 2, effect of diastolic BP on burst incidence), as has also been observed by others (Jones et al., 2001b). This reduced sensitivity might be related to the reduced sensitivity of the baroreceptor-heart rate reflex in the elderly, as was confirmed in our study.

Hypothesis 2 (i.e., reduced vascular responsiveness to MSNA) can neither be supported nor refuted by our observations. However, this hypothesis is supported by the observation that the reflex-mediated increase in limb vascular resistance during hypovolemia is less in older adults, because of attenuated vasoconstrictor responsiveness to sympathetic stimulation (Davy et al., 1998). While our observation that there was no difference between the groups in TPR does not support this hypothesis, the reliability of this indirect measure is uncertain.

Hypothesis 3 (about splanchnic vascular resistance) is also supported by previous observations. In healthy subjects superior mesenteric artery-flow increases postprandially leading to an increase in splanchnic-mesenteric blood volume (Lipsitz et al., 1993). Fujimura et al. (1997) reported that in patients with autonomic failure, the increase in superior mesenteric artery-flow in response to feeding is essentially preserved, but the autoregulatory response of the splanchnic system is reduced or absent and, therefore, systemic BP passively falls as a larger blood volume is transferred into the splanchnic vascular bed. In these patients the decrease in superior mesenteric artery blood flow in response to the cold pressor test is attenuated, indicative of diminished vasoconstrictor nerve activity in the mesenteric vessels (Fujimura et al., 1997). The more pronounced fall in BP in the older group that we tested may have the same explanation, i.e., the postprandial sympathetic activity of the splanchnic system is attenuated.

Hypothesis 4 (about cardiac output) is supported by the findings that in healthy young subjects, meal ingestion increases cardiac output (Hoost et al., 1996; Takamori et al., 2007) and that, with aging, the cardiovascular reserve capacity decreases (Lakatta, 1994). Our study failed to reveal significant differences in CO between the healthy young and older subjects (Table 2), although at the nadir of the systolic blood pressure, stroke volume was significantly higher in the young group. The mean value

of CO was also higher in the young group, but the difference was not statistically significant. However, it cannot be excluded that this indirect measure did not show a significant difference in our relatively small sample due to a type 2 error. The 2 PPH patients showed a decrease in HR, instead of an increase, and decrease in CO at the nadir of the systolic blood pressure, suggesting the concept that hypothesis 4 plays a role, at least in PPH patients.

Hypothesis 5 (about vasoactive hormones) was not evaluated in our study, but IDGI stimulates the release of a number of vasoactive hormones / peptides (Lavin et al., 1998) and blocking their release by octreotide is associated with prevention of PPH (Hoeldtke et al., 1991). Insulin has vasodilatory effects (Brown et al., 1989), and blunting the postprandial peak of insulin by acarbose, reduces PPH in patients with autonomic failure (Shibao et al., 2007), although this effect may also be due to slowing gastric emptying, or the release of glucagon-like peptide 1 (Jones et al., 1998; O'Donovan et al., 2002; Gentilcore et al., 2005; Gentilcore et al., 2007). It should also be noted that while plasma levels of a number of peptides with vasodilatory properties, including the neurotransmitter vasoactive intestinal polypeptide, do not change following ingestion of carbohydrate (Mathias et al., 1989), their potential role should not be discounted, as in patients with autonomic failure, the hypotensive response to vasodepressor agents is known to be enhanced (Mathias et al., 1977).

There was a striking difference between the patients with PPH and the healthy controls at the nadir of the blood pressure fall (Table 3): stroke volume and cardiac output show a large decrease, in patient A partly compensated by a relatively high MSNA and TPR. This suggests that a factor that decreases cardiac output, such as a decrease in heart rate, plays a role in PPH. In patient A with PPH, MSNA burst area did not increase further after 29 minutes of IDGI, whereas the increase in the younger group continued and reached its maximum after 50 minutes. Although the significance of this finding is uncertain, it suggests an inability to increase MSNA sufficiently to maintain the systemic BP.

The experimental protocol in our study was demanding for the subjects. This resulted in a number of potential candidates refusing to undergo the study and some failed studies. As there was deliberately a considerable amount of time between the potentially unpleasant procedures and the start of the data collection, and we attempted to ensure that at the subject was comfortable during the measurement, it appears unlikely that psychological / emotional factors influenced our results. However, we cannot discount the possibility that the cohorts studied may not be representative and that type 2 statistical errors occurred, although the outcome of the majority of the analyses appeared clear-cut.

In conclusion, our study has established for the first time that IDGI results in a

fall in BP in healthy young, as well as older, subjects and PPH patients and that in the healthy elderly the fall in BP is much greater than in healthy young, despite there being no difference in the MSNA response. This discrepancy is likely to be related to an attenuated sympathetic baroreflex sensitivity in the elderly. Furthermore, we postulate that in the elderly there may be a more pronounced fall in splanchnic resistance and / or an attenuated increase in cardiac output. These mechanisms add to the reduced 'gastrovascular reflex' in the elderly to increase the propensity for a meal-related fall in blood pressure. The latter appears to be of fundamental importance for the regulation of hemodynamics during a meal, in that even young subjects, when deprived from this reflex, exhibit a significant fall in blood pressure in response to enteral glucose. Our observations are likely to be of relevance to the management of patients with PPH: measures that increase gastric distension, cardiac output, splanchnic vascular resistance or baroreflex sensitivity may prove to be effective treatments.

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CHAPTER 4

Subtle involvement of the parasympathetic nervous system in patients with irritable bowel syndrome

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ABSTRACT

This study comprises assessment of autonomic function in irritable bowel syndrome (IBS) patients, focusing on meal-related changes. In 18 IBS patients (4 males, mean age 45 ± 3.0 [s.e.m.] years) and 19 healthy volunteers (6 males, mean age 41 ± 3.5 years) blood pressure, heart rate, heart rate variability and muscle sympathetic nerve activity (MSNA) were assessed before, during and after consumption of a standardized meal. In pre – and postprandial phase Valsalva maneuver, cold pressor test (CPT) and deep breathing test were carried out and Visual Analog Scale (VAS) scores for nausea, bloating and pain were obtained.

In the IBS group, the meal induced significantly higher VAS scores for pain (P = 0.002) and bloating (P=0.02). During food intake, the increase in blood pressure, heart rate and MSNA was equal in patients and controls, but the increase of LF/HF ratio of heart rate variability was significantly higher in the IBS group (median [quartiles] 2.29 [1.14 – 3.00] versus 0.77 [0.25 – 1.81]; P = 0.03). IBS patients scored lower on pre – and postprandial RRmax/RRmin ratio during deep breathing (DB ratio, P = 0.03). The increase in MSNA (burst frequency) in response to CPT tended to be higher in the IBS patients (P=0.07). We conclude that reactivity to food intake, measured as muscle sympathetic nerve activity, is normal in IBS patients. The lower DB ratio and higher LF/HF ratio during food intake in IBS patients is an indication of a reduced parasympathetic reactivity. These results suggest that reduced baseline activity as well as responsiveness of the parasympathetic system could play a role in the pathogenesis of IBS.

INTRODUCTION

Patients with Irritable bowel syndrome (IBS) suffer from abdominal pain associated with defecation or changes in bowel habit, with features of disordered defecation and with abdominal distension (Camilleri, 2001). The disorder is 'functional' in that the symptoms cannot be explained by structural or biochemical abnormalities. Previous studies have shown that, in comparison with healthy subjects, a subset of patients with IBS display altered visceral perception in various regions of the gut (Swarbrick et al., 1980; Accarino et al., 1995; Evans et al., 1996), can display different motility patterns (McKee and Quigley, 1993; Evans et al., 1996) or have a history of psychological trauma or an abnormal psychological profile (Ford et al., 1987; Whitehead and Palsson, 1998; Kanazawa et al., 2004). To date, no single shared etiologic factor has been found.

The autonomic nervous system may mediate abnormal brain - gut interactions in irritable bowel syndrome. Several studies seem to indicate that this relationship exists indeed (Smart and Atkinson, 1987; Heitkemper et al., 1998; Karling et al., 1998; Chelimsky and Chelimsky, 2001; Elsenbruch and Orr, 2001; Waring et al., 2004), however, the results are often contradictory. The lack of methods to assess the specific vagal and sympathetic influence on the gastrointestinal system forces investigators to monitor the autonomic regulatory mechanisms of the cardiovascular system as being representative of general autonomic function. Consequently, many conclusions about sympathetic and parasympathetic function had to be derived indirectly from spectral analysis of heart rate and blood pressure. In a previous study we showed that heart rate variability (HRV) measured as LF/HF-ratio using short term spectral analysis has a considerable intra-individual variability, displayed as inter-observer variability (van Schelven et al., 2000). To get a more reliable measure of changes in the sympathovagal balance we measured not only heart rate variability, but added a direct measure of the efferent sympathetic nerve activity to muscles (MSNA) by means of microneurography. Such measurements have shown sympathetic impulses in muscle nerves to be vasoconstrictor signals governed by baroreceptor reflexes and to be involved in cardiovascular homeostasis (Burke et al., 1999). In previous studies of healthy subjects (Rossi et al., 1998; Van Orshoven et al., 2004), we found a direct relation between non-perceived gastric distension and MSNA indicating a relationship between MSNA and visceral autonomic innervation. Furthermore, several studies have shown that MSNA

changes markedly during eating in healthy subjects (Fagius and Berne, 1994; Cox et al., 1995). To our knowledge, a study of muscle sympathetic nerve activity changes in IBS patients has not been reported in the literature so far.

The aim of this study was to test the hypothesis that the autonomic nervous system and, in particular, cardiovascular responses to eating, differ between IBS patients and healthy volunteers. The majority of IBS patients experience their symptoms during and after food intake (Dapoigny et al., 2003). Therefore we focused our study on the autonomic reaction in pre –, intra – and early postprandial (first 15 minutes after ingestion of the standardized meal) phases of food intake. For this purpose, we recorded MSNA, blood pressure, electrocardiogram (ECG) and respiration before, during and after a standardized meal. In addition, the response to Valsalva maneuver, deep breathing test and cold pressor test (CPT) were assessed in a fasting state and after consumption of the meal, to test the influence of food intake on autonomic reactivity.

MATERIALS AND METHODS

PARTICIPANTS

Forty three patients were recruited from the outpatient Gastroenterology Clinic, of whom eighteen patients (14 women and 4 men, mean age ± s.e.m.: 45 ± 3.0 years) diagnosed with IBS according to the Rome II criteria (Drossman, 1999) were included in the study. Organic disorders were excluded by means of appropriate tests, including colonoscopy in all patients. All subjects were screened for lactose intolerance. Thirteen patients were not willing to undergo the protocol, 12 were excluded for different reasons (e.g., drug-related, not in active disease state, other diseases finally explained the symptoms, such as M. Crohn). Duration of IBS symptoms was 16 ± 3.9 years (mean ± s.e.m.). Six patients suffered from constipation-predominant IBS (stool frequency < 3/ week) and 6 from diarrhea-predominant IBS (stool frequency > 3/day). The other 6 patients experienced alternate periods of diarrhea and constipation. All patients were studied in a period in which they experienced typical symptoms of IBS. None of the patients had a history of abdominal surgery or systemic, neurological or cardiovascular disease.

TABLE 1 **Baseline characteristics of patients and controls**

Data are presented as mean ± s.e.m. or as absolute number of patients [n]. Differences between IBS and control groups were calculated with independent samples t-test.

	IBS patients			Controls			Statistic
Characteristic	♀	♂	**Total**	♀	♂	**Total**	**P**
Number of subjects [n]	14	4	18	13	6	19	
Age [years]	43 ± 3.6	54 ± 2.8	45 ± 3.0	37 ± 3.6	49 ± 7.0	40 ±3.5	0.4
Length [cm]	169 ± 2.1	179 ± 1.5	170 ± 2.0	167 ± 2.4	183 ± 0.7	172 ± 2.4	0.7
Weight [kg]	65 ± 3.3	82 ± 3.6	68 ± 3.2	63 ± 2.8	78 ±3.5	68 ± 2.7	0.9
Baseline Systolic BP [mmHg]	126 ± 3.8	137 ± 7.2	128 ± 3.5	126 ± 1.6	122 ± 6.0	125 ± 2.1	0.4
Baseline Diastolic BP [mmHg]	78 ± 2.5	87 ± 2.9	80 ± 2.2	71 ± 1.1	85 ± 4.7	75 ± 2.2	0.14
Baseline Heart rate [beat/min]	77 ± 2.7	62 ± 7.1	74 ± 3.5	69 ± 2.6	75 ± 6.8	71 ± 2.8	0.4
Baseline MSNA [burst/min]	50 ± 2.3	47 ± 5.4	49 ± 2.1	43 ± 3.9	54 ± 3.2	47 ± 3.0	0.5

Twenty one healthy volunteers were recruited by advertisement in a local newspaper and by acquisition among the hospital personnel. Nineteen of them

(13 women and 6 men, age 40 ± 3.5 years) were included in the study and served as controls. Two were excluded for drug-related reasons. All volunteers were healthy and had no history of major disease or gastrointestinal disorder. None of the participants (patients and healthy volunteers) had diabetes, a history of alcohol abuse, cardiovascular or renal disease, and none was using medication known to affect autonomic function. Patients and controls were matched for age, sex, height and weight (Table 1). The study was approved by the Ethics Committee of the University Medical Center Utrecht and all subjects gave written informed consent.

MEASUREMENT INSTRUMENTATION

Blood pressure was continuously recorded from the right hand, held at the level of the heart, using finger photoplethysmography (Finapres, Ohmeda, Englewood, CO, USA). The ECG was continuously recorded from bipolar chest leads (Counterpoint, Dantec Elektronik Medicinsk, Skovlunde, Denmark). Multifiber recordings of sympathetic activity were made with tungsten microelectrodes inserted in a muscle nerve fascicle of the peroneal nerve of the right leg (Vallbo et al., 1979; Wallin, 1999). A reference needle electrode was placed subcutaneously 2 to 3 centimeters away from the recording electrode. The microneurographic signal was first amplified and bandpass filtered (500 – 2000 Hz; Dantec Counterpoint). Custom made electronics were used to rectify and integrate the signal (resistor capacitor network, 0.1 s time constant) to obtain the MSNA. Small adjustments of the recording electrode were made until a site was found at which spontaneous sympathetic activity was recorded. Sympathetic bursts were identified by their characteristic morphology and relationship to R-waves in the ECG. Correct position of the electrode was evaluated during a Valsalva maneuver (Vallbo et al., 1979). A mercury strain gauge around the chest was used to record respiration (pneumogram).

ECG, respiration, arterial blood pressure, and MSNA (mean rectified voltage neurograms) were sampled at 500 Hz, monitored on-line (software: POLY 5, Physiological Analysis Package, Inspector Research Systems, Amsterdam, the Netherlands) and stored on disk for off-line analysis with an IBM-compatible PC. All measurements were viewed off-line and portions of data containing obvious major artifacts, for example, due to coughing of the subject, were discarded.

EXPERIMENTAL PROTOCOL

The study was performed after an overnight fast. All subjects were instructed to refrain from eating, drinking, and smoking after 21:00 h the previous day. Just before the start of the experiment, subjects were asked to empty their bladder. The experiment started at 8:30 a.m. in a room with a temperature of 23 to 25° C.

Participants lay supine on a bed with the head tilted at an angle of 30°.

The following autonomic function tests were subsequently performed: Valsalva maneuver (forced expiration, keeping a manometer at 40 mmHg for 15 seconds), a resting period during which the subject was asked to sustain a comfortable breathing frequency of 15 breaths per minute assisted by a metronome (this period was used for spectral analysis of heart rate), a deep breathing test (breathing deeply with maximum effort at a frequency of 6 per minute for 1 minute, assisted by a metronome) and a CPT (the subject's left hand immersed in ice water up to the wrist for 2 minutes). Each test was started only after signals had returned to baseline. After the autonomic function tests, subjects ate a standardized meal consisting of two pancakes (total: 300 ml, 2500 kJ, 84 g carbohydrates, 17 g proteins, 22 g fat, not containing lactose) and a glucose drink (150 ml, 20% (w/w) glucose, 500 kJ). They were assisted with eating because they had to remain still and in a supine position to avoid displacement of the tungsten electrodes. Directly after finishing the meal, subjects were again asked to breathe at a rate of 15/min for 5 minutes (postprandial rest period). Then deep breathing test, CPT and Valsalva maneuver were repeated.

Visual Analog Scales (VAS; Sriwatanakul et al., 1983) were used to score the sensations nausea, bloating and pain. Participants were asked to quantify these three sensations before and directly after the meal, on a VAS ranging from 'no sensation' to 'unbearable nausea', 'unbearable fullness' and 'unbearable pain'.

DATA ANALYSIS

Mean arterial pressure (MAP; mmHg) was calculated per heart beat. The heart rate (beats/min) and beat length (interbeat interval (IBI); ms) were calculated beat to beat from the ECG using automatic R-wave detection. The stored integrated MSNA signal was analyzed by software specially developed by our group (Rossi et al., 1998; Van Orshoven et al., 2004). In short, the software detected MSNA bursts based on their characteristic, relatively triangular shape using criteria on the slopes, duration and amplitude of the bursts. For instance 'EMG pulses' were recognized from their steep front slope and automatically rejected. Detected bursts were assigned to independently detected R-waves in the ECG, requiring the delay between the R-wave and the burst top to be within an apriori defined time window. Only one burst was assigned per R-wave. The results were shown graphically as stylized MSNA bursts together with the raw MSNA and ECG for visual inspection and, if necessary, correction.

Burst counts was calculated as burst frequency (number of sympathetic bursts per minute = NBM; burst/min). The burst area was calculated as the area under the curve (arbitrary units) per minute (ABM; a.u.c./min). All raw data were checked manually as well.

Effect of food intake:
To determine changes during the meal, all parameters were recorded during a 2-minute baseline period, during the first 10 minutes of the meal and 3 minutes directly following the meal. Recorded values were averaged over 30-second periods. Missing data (1.6% of all data in IBS group and 6.5% in control group) were replaced using the linear trend method in SPSS. Because all variables showed a fast increase after the start of the meal (within 2 to 3 minutes) and then reached a new steady state, the groups were compared using the mean values of this steady state (mean value of 5th to 10th minute after start of the meal). Delta (Δ) values were calculated as absolute instead of relative changes from baseline, as groups did not differ significantly in baseline, except for ABM. This parameter was expressed in arbitrary units and for that reason relative changes from baseline (%ABM) were calculated. Because all subjects scored '1' on all pre-meal VAS scores (= no complaints), only post-meal VAS scores were compared (Mann Whitney U test).

Autonomic tests before, during and after the meal:

1 **VALSALVA** The Valsalva ratio was determined by calculating the ratio of the maximum RR interval in the ECG during the first 30 seconds after release of intrathoracic pressure (phase IV of the Valsalva maneuver) over the minimum RR interval during elevated intrathoracic pressure (phase II; Mathias and Bannister, 1999a).
2 **HEART RATE RESPONSE TO DEEP BREATHING** Vagal responsiveness was determined by calculating the RRmax/RRmin ratio using the maximum and minimum interbeat intervals per breath during 6 consecutive deep breaths.
3 **SPECTRAL ANALYSIS OF THE HEART RATE VARIABILITY** Five consecutive minutes of the pre- and postprandial rest periods and a selected 5 minute period of the intra-prandial period were used for spectral analysis of the heart rate variability. Detection of R-waves in QRS complexes was used to obtain a list of interbeat intervals. The list of interbeat intervals was manually corrected for extrasystolic as well as missing heartbeats by interpolation while inspecting the ECG. The linear trend was removed from the list and a cosine tip window was applied. The power spectrum was estimated as the squared amplitude of the result of a Fourier transform of the list of IBI values (direct method). The frequency axis was rescaled to Hz using the average heart rate in the 5 minute period. Powers were calculated for the low frequency (LF: 0.04-0.15 Hz) and high frequency (HF: 0.15-0.40 Hz) ranges, giving also the LF/HF power ratio.
4 **COLD PRESSOR TEST** The effect of a CPT was assessed by observing changes in MSNA, mean arterial pressure and heart rate. Two minutes of baseline were directly followed by 2 minutes of immersion of the right hand into ice water (cold pressor period) and two minutes of recovery. The cold pressor and recovery periods were divided into 30-second epochs. Mean values were calculated for each epoch. Because all values showed an increase, we calculated maximal increases by subtracting the epoch with the highest value during baseline from the epoch with the highest value during the test.

STATISTICAL ANALYSIS

Differences were considered significant when $P < 0.05$. All statistics were performed with SPSS version 11. Data that showed normal distribution were expressed as mean ± s.e.m., other results as median and quartiles. Independent samples t-tests, Mann-Whitney U (MWU) tests or Chi square tests were used to calculate significance of differences between groups. Repeated measures ANOVA was used to compare multiple group means of repeated tests.

RESULTS

VISUAL ANALOGUE PERCEPTION SCORES

Before the meal, all subjects (IBS and controls) scored '1' on the VAS for nausea, bloating and pain, indicating no complaints at all. Directly after finishing the meal, the IBS group had a significantly higher median score than the control group for bloating (5.5 versus 1; P = 0.02, MWU test) and for pain (3.5 versus 1; P = 0.002). Nausea was not reported frequently: median scores were 1.5 and 1, respectively, without significant difference (P = 0.7).

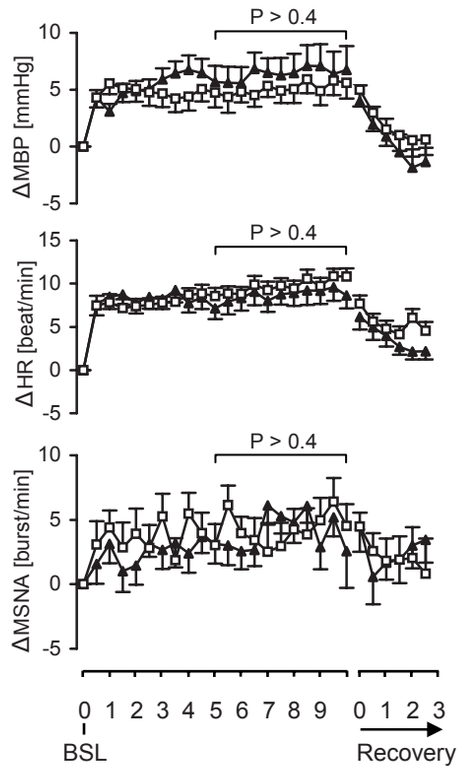
CHANGES IN BLOOD PRESSURE, HEART RATE, HEART RATE VARIABILITY AND MSNA DURING FOOD INTAKE

Baseline systolic and diastolic blood pressure, heart rate and MSNA did not differ between the groups (Table 1). Blood pressure, heart rate and MSNA increased significantly during eating in both groups (one sample t-test against zero per group, using mean delta values over period 5 - 10 minutes after start of meal: all P < 0.05). The increase took place within 1 minute for all parameters and remained high during eating (Figure 1). During the meal, there were no significant differences between the groups for all parameters (all P values > 0.4). After the meal, all parameters decreased towards baseline level. Results of ΔSBP, ΔDBP and ΔMBP were similar and therefore only ΔMBP results are shown. For the same reason ΔABM results are not shown. Patients took significantly longer to consume the meal compared to controls (14 ± 1.3 minutes versus 10 ± 1.1 minutes, P = 0.04), probably due to the fact that they experienced

TABLE 2 Spectral analysis of heart rate (median [25 – 75% quartiles]) in controls and patients with irritable bowel syndrome (IBS). Increases in intra- and postprandial phase compared to pre – prandial phase are shown. **bold** = significant.
The LF/HF ratio (sympathovagal balance) in patients with irritable bowel syndrome (IBS) and healthy controls increased markedly during the meal. In the intra-prandial phase, the LF/HF ratio increase was significantly higher in IBS patients than in controls (P = 0.03).

	Changes in LF/HF ratios	
	Intraprandial	**Postprandial**
Controls (n = 19)	0.77 [0.25 – 1.81]	0.40 [-0.24 – 1.03]
IBS patients (n = 18)	**2.29 [1.14 – 3.00]**	0.32 [-0.07 – 1.13]

FIG. 1 **Blood pressure, heart rate and MSNA changes during test meal**



Changes in mean arterial blood pressure (MAP), heart rate (HR) and muscle sympathetic nerve activity (MSNA; mean ± s.e.m.) from pre-prandial baseline values during the intra-prandial and early postprandial (recovery) period. Data of IBS group (▲) and controls (□) are shown. All parameters increased significantly during the meal (one sample t-test, mean value of 5th to 10th minute compared to baseline: all P < 0.04), but there were no differences between groups (independent samples t-test, all P > 0.4).

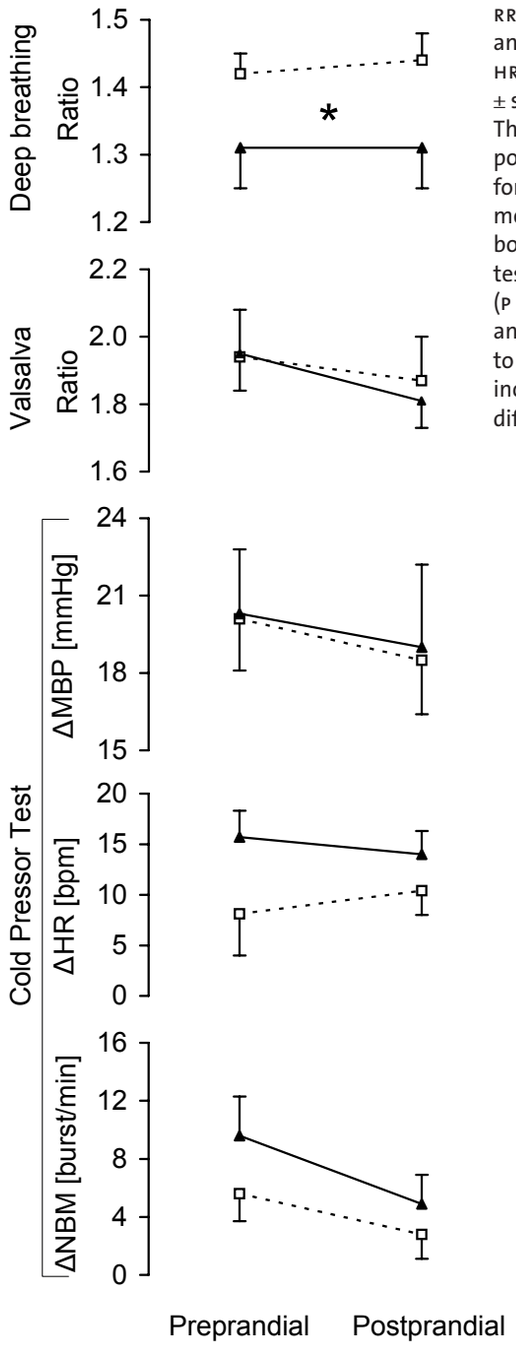
symptoms of bloating and pain that made it more difficult for them to continue eating at the same pace.

The mean LF/HF ratio increased during the meal and decreased thereafter in both the patients and the healthy controls (Table 2). There were no significant differences between the groups before or after the meal (MWU test). The increase of the LF/HF ratio (change compared to pre-prandial baseline) was significantly higher in the IBS patients than in controls (median [25 – 75% quartiles]: 2.29 [1.14 – 3.00] versus 0.77 [0.25 – 1.81]; MWU: P = 0.03). There was no significant correlation between VAS scores for pain/bloating and changes in LF/HF ratio.

AUTONOMIC FUNCTION TESTS BEFORE AND AFTER THE MEAL

All subjects were checked for autonomic neuropathy / vagal impairment by calculating heart rate range in response to deep breathing (mean value of 5 best breaths). Results (median + range) of IBS patients and controls were 17.3 [7.4-29.6] and 21.6 [7.6-36.0], respectively. Compared with normative data (Low, 1997) all subjects fell within normal range after correction for age.

FIG. 2 **Autonomic function tests**



RRmax/RRmin ratio during deep breathing and Valsalva maneuver, and changes of MAP, HR and MSNA during cold pressor test (mean ± s.e.m.) of IBS group (▲) and controls (□). There were no differences between pre- and postprandial tests. * = significant difference for both pre- and postprandial tests (repeated measures ANOVA). The RRmax/RRmin ratio in both pre- and postprandial deep breathing tests were significantly lower in the IBS group (P = 0.036). The increase in NBM in both pre- and postprandial cold pressor tests tended to be higher in the IBS group (P = 0.07). The increase in heart rate was not significantly different between groups (P = 0.2).

Figure 2 presents the ratio between maximum and minimum RR interval in the ECG during pre- and postprandial Valsalva maneuver and deep breathing test and the maximal changes from baseline of all parameters during the CPT. There were no differences between pre - and postprandial tests (repeated measures ANOVA, time effect all $P > 0.1$) and no interactions between time and group (repeated measures ANOVA, time*group all $P > 0.5$). The RRmax/RRmin ratio of heart rate during deep breathing was significantly lower in IBS patients compared to the controls (repeated measures ANOVA, group effect $P = 0.036$) in both pre-prandial (1.31 ± 0.03 versus 1.42 ± 0.06) and postprandial (1.31 ± 0.04 versus 1.41 ± 0.06) tests (Figure 2). This lower ratio during deep breathing indicates a shift in sympathovagal balance toward a lower parasympathetic tone in the IBS group. There was no significant correlation between DB ratio and VAS scores for pain/bloating. The maximal increase in NBM during pre-prandial (15.7 ± 2.6 versus 8.1 ± 4.1 bursts/min) and postprandial (14.0 ± 2.3 versus 10.4 ± 2.4 bursts/min) CPTs tended to be higher in the IBS group (repeated measures ANOVA, difference between groups $P = 0.07$). Maximal increase in heart rate and mean blood pressure during CPT was not significantly different between the groups ($P = 0.2$ versus $P = 0.9$). Results of the Valsalva maneuver were similar in pre – and postprandial state. There were no differences between IBS patients and controls for the results of Valsalva ratio (1.88 ± 0.08 versus 1.91 ± 0.13, mean of both occasions). Peak heart rate during phase II of the Valsalva maneuver was higher in the IBS patients (103 ± 4.9 versus 97 ± 3.6 beats/min) but the difference was not significant ($P = 0.3$). Findings were similar in trough heart rate during phase IV: 55 ± 1.7 (IBS) versus 53 ± 2.3, $P = 0.8$.

DISCUSSION

The main findings of the present study were as follows. First, IBS patients showed lower RRmax/RRmin ratios during deep breathing in both pre- and postprandial conditions suggesting reduced parasympathetic tone. Second, MSNA during as well as in response to active eating did not differ between groups. Third, a significantly higher LF/HF ratio of heart rate variability during eating was found in IBS patients, favoring sympathetic influence intra-prandially. Fourth, the IBS group showed a trend for a greater increase in the MSNA response to the cold pressor test, again favoring sympathetic tone. Taken together, these findings suggest lower overall parasympathetic tone in patients with IBS.

Assessment of MSNA by microneurography is the most direct method of recording efferent sympathetic activity. To our knowledge, there is no report on comparison of MSNA measurements in IBS patients before, during and after consumption of a standardized meal.

Several reports suggest that IBS might relate to an autonomic nervous system imbalance. However, there is no agreement on the nature of this shift, whether toward greater sympathetic or parasympathetic activity. Several studies showed a higher LF/HF ratio at rest, due to either increased sympathetic or decreased parasympathetic activity (Karling et al., 1998; Orr et al., 2000; Heitkemper et al., 2001; Thompson et al., 2002). Parasympathetic provocation tests such as cardiac response to deep breathing showed vagal withdrawal in most studies (Lee et al., 1998; Waring et al., 2004) and mild sympathetic excess was reported in reaction to strong sympathetic activation tests such as the isometric handgrip exercise or orthostatic stress tests (Lee et al., 1998). The fact that the IBS group showed a trend towards a higher increase in MSNA and heart rate in reaction to CPT in the present study is consistent with these reports.

Based on several lines of evidence, we hypothesize that an overall reduction in parasympathetic tone is the primary event that accounts for the observed greater increase in sympathetic activity in the patients with IBS during sympathoexcitatory stimulation in the CPT, as well as during the meal. First, the reduction in RRmax/RR/min, a measure that assesses parasympathetic function alone, is suggestive of a primary reduction in parasympathetic activity, rather than simply a skew in the ratio of sympathetic to parasympathetic tone. Second, IBS patients showed a higher LF/HF ratio during eating (Table 2). Yet, the MSNA increase during eating was similar in IBS

patients and controls (Figure 1) suggesting normal sympathetic activation to food intake. Thus, this higher LF/HF ratio is more likely due to attenuated parasympathetic activity rather than an enhanced sympathetic activity, in agreement with the findings of Elsenbruch et al. (2001).

The presence of a similar and normal Valsalva ratio in both groups could have several interpretations. The most important heart rate changes during Valsalva maneuver the phase II tachycardia and the bradycardia following release of the strain. This is a complex intervention which provokes major alterations of efferent autonomic parasympathetic and sympathetic activity (Eckberg, 1980). The heart rate changes induced by Valsalva maneuver, therefore, are not exclusively vagally mediated, but also dependent on intact sympathetic circulatory control. Our findings suggest preservation of parasympathetic nervous system function in IBS patients in response to more vigorous provocation.

Since IBS patients scored higher on VAS scores for bloating and pain, the test meal was sufficient to cause IBS symptoms. It could be argued that this difference in the VAS scores is produced by the difference between IBS patients and controls in the sympatho-vagal balance during eating. However, if pain were a major factor, one would expect a prompt increase in heart rate, which was not observed, since the increase in heart rate was not significantly different between groups. The finding that there were no significant correlations between the observed changes and the VAS scores suggests that pain was not an important factor in our results.

Some limitations in our study should be noted. First, strict metronome-breathing regimen was not maintained during the meal, making absolute comparison between intra-prandial and baseline LF/HF ratios less reliable. Second, the number of patients recruited was based on power calculations to determine a difference in MSNA (number of bursts) between IBS patients and controls during eating of at least 4 bursts per minute, with an estimated standard deviation of 4 bursts / minute, the same standard deviation we found in previous measurements in our lab. Power calculation showed that 16 patients and 16 controls were needed to find such a difference with a power of 0.8. Significant but smaller differences may very well have been found if more subjects had been included.

This is the first study to directly measure sympathetic activation with MSNA in IBS patients, allowing inferences on parasympathetic and sympathetic outflow individually, in contrast to prior HRV-based studies that were restricted to assessing overall sympatho-vagal balance. Our findings suggest that subtle impairment of parasympathetic tone in patients with IBS may lead to greater activation of sympathetic responses in the absence of any clear increase in sympathetic outflow. The relationship of this finding to IBS symptoms requires further study.

CHAPTER 5

Postprandial hypotension in clinical geriatric patients and healthy elderly Prevalence related to patient selection and diagnostic criteria

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ABSTRACT

BACKGROUND Postprandial hypotension has been reported to occur not only in frail elderly with autonomic failure but also in healthy elderly. There is no consensus on the method to diagnose postprandial hypotension. The aims of this study were to find out whether postprandial hypotension occurs more frequently in patients admitted to a geriatric ward than in healthy elderly individuals, how often it is associated with symptoms and what the optimal interval between blood pressure measurements is in order to diagnose postprandial hypotension.

METHODS Twenty-two consecutive patients (84 ± 5 years) admitted to a geriatric department were compared with 20 healthy elderly individuals (82 ± 4 years). Non-invasive beat-to-beat BP was measured in sitting position, from 15 minutes before until 135 minutes after subjects started to eat a continental breakfast. Mean blood pressure values over 2 minute periods were calculated and detection of postprandial hypotension was evaluated using different intervals.

RESULTS Postprandial hypotension was detected in twenty (91%) patients and 8 (40%) healthy elderly individuals ($P = 0.001$) when blood pressure was measured with 2-minute intervals. In the subjects with postprandial hypotension, the blood pressure fall of 20 mmHg or more, started 34 ± 27 minutes (range 0-104 minutes, in 90% of the patients within 1 hour) after the completion of breakfast and lasted for 42 ± 36 minutes (range 2-108 minutes). When blood pressure was measured with intervals of 6 or 10 minutes, the detection of PPH was reduced by 10 and 15% respectively. The presence of postprandial hypotension and postprandial complaints were positively associated ($P < 0.001$).

CONCLUSIONS Postprandial hypotension is a serious problem in elderly individuals, and the presence of postprandial complaints is a good indicator of the existence of postprandial hypotension. Measuring blood pressure at least every 10 minutes for 60 minutes after breakfast will adequately diagnose postprandial hypotension.

INTRODUCTION

Postprandial hypotension (PPH) is defined as a fall in systolic blood pressure (BP) of ≥ 20 mmHg within 2 hours of eating a meal (Jansen and Lipsitz, 1995). Potential symptoms include dizziness, syncope, and falls. It has been described frequently in elderly individuals, with a higher incidence in certain risk groups, namely, in 24 - 33% of elderly residents of nursing homes (Aronow and Ahn, 1994), in almost 50% of elderly patients with unexplained syncope (Jansen et al., 1995) and in 67% of hospitalized geriatric patients (Vloet et al., 2005). Risk groups are patients with autonomic dysfunction in diabetes mellitus (Sasaki et al., 1992), hypertension (Grodzicki et al., 1998; Mitro et al., 2001), Alzheimer's disease (Idiaquez et al., 1997) and Parkinson's disease (Loew et al., 1995; Mehagnoul-Schipper et al., 2001), although PPH has been reported to occur in 33% of healthy individuals (Jones et al., 1998). In the long term, PPH is associated with an increased incidence of falls, syncope, new coronary events, new stroke, and higher total mortality (Aronow and Ahn, 1997). People who have PPH are at risk of developing cerebral ischemia (Kohara et al., 1999).

The pathophysiology of PPH is probably multifactorial, possibly involving an attenuated baroreflex, an attenuated reflex increase in sympathetic activity by activation of stretch receptors in the stomach (gastrovascular reflex; Van Orshoven et al., 2004), sympathetic dysfunction (e.g., autonomic neuropathy in diabetes mellitus, Parkinson's disease) and patients with an incapability to increase cardiac output due to heart failure or any combination of these factors. Thus, PPH is likely to occur in frail elderly with extensive co-morbidity. There is no consensus on the test conditions that should be used to diagnose postprandial hypotension. The characteristics of the actual decrease in BP in PPH are not clearly defined: when does BP start to decrease after a meal, how long does the decrease last, and what is the magnitude of the decrease in BP? Although a fall in systolic BP of at least 20 mm Hg after a meal, analogous to the definition of orthostatic hypotension, is used in most studies, it is not clear what time interval and which method of BP measurement should be used. In most studies, BP was measured with a sphygmomanometer at intervals varying from 3 up to 60 minutes (Table 1; Aronow and Ahn, 1994; Jansen et al., 1996; Kohara et al., 1998; Puisieux et al., 2000; Schwartz et al., 2001; O'Donovan et al., 2002; Kawaguchi et al., 2002; Vloet et al., 2005). If the frequency of measurements is too high, participants are likely to experience discomfort. Some authors used continuous BP measurements (finger

TABLE 1 Methods used to measure postprandial hypotension

Author (reference)	Subjects (n)	Studygroup	Equipment	Time-interval (min.)	Duration measurement (min.)
Aronow 1994	499	long-term health care facility	Mercury sphygmomanometer	15	120
Jansen 1996	22	nursing home patients	Dinamap sphygmomanometer	5	90
Kohara 1998	121	hospitalized hypertensive patients	ABPM	30	24 hour
Puisieux 2000	120 vs 36	inpatients with syncope or falls vs control patients	Spacelab sphygmomanometer	15	24 hour
O'Donovan 2002	8	healthy elderly	Not mentioned	3	120
Kawaguchi 2002	20 vs 20	healthy elderly vs healthy young individuals	Mercury sphygmomanometer	30	120
Vloet 2005	58	Geriatric patients	Spacelab sphygmomanometer	10	90
Fisher 2005	179	ong-term health care facility	Spacelab and mercury sphygmomanometer	60	60

ABPM: ambulatory blood pressure measurement.
Duration measurement: total time measured after finishing breakfast.

arterial BP; Imai et al., 1998; Maurer et al., 2000; Mehagnoul-Schipper et al., 2001), which might be uncomfortable during prolonged measurements. In most studies standardized test meals were used.

The aims of this study were to establish the prevalence, duration, and association with symptoms of PPH in patients admitted to a geriatric ward, compared with healthy elderly individuals. Patients were provided with a continental breakfast. We measured beat-to-beat BP, and tested different intervals between BP measurements to diagnose postprandial hypotension adequately.

METHODS

SUBJECTS

Consecutive patients admitted to the geriatric ward of the University Medical Center Utrecht over 6 months were eligible for inclusion. This ward serves elderly patients with a wide range of acute and chronic diseases and referrals for diagnostic work-up or therapy. Most patients are referred by general practitioners or geriatricians. Inclusion criteria were being able to give informed consent and to walk (with or without walking aid). Exclusion criteria were myocardial infarction less than 3 months earlier, any acute illness, uncontrolled metabolic disease, resting systolic BP more than 200 mmHg, cardiovascular disease (aortic stenosis, intermittent claudication, angina pectoris), dysphagia, life expectancy less than 3 months, and use of medication that affects BP and which could not be discontinued for 24 hours. All subjects were studied in the week prior to discharge. All participants gave written informed consent prior to the examination.

Healthy elderly individuals, recruited by advertisement in a weekly newspaper, were screened by telephone and underwent a physical examination before being matched for age with the geriatric patients. All subjects were healthy according to modified exclusion criteria defining medically stable elderly subjects for exercise studies (Greig et al., 1994). Subjects who were taking medications that affect BP that could not be withdrawn for at least 24 hours were excluded as well. All subjects gave written informed consent prior to the study. The measurements were carried out in the same period as in the patient group.

INSTRUMENTATION

BP was recorded using Portapres (TNO-TPD Biomedical Instrumentation, Netherlands Organization for Applied Scientific Research). Portapres is a non-invasive method to record beat-to-beat BP alternately from two adjacent fingers and is accurate for measuring changes over time, but not for measuring absolute values (van Lieshout and Karemaker, 2003). The method is based on the volume-clamp method of Peñáz and the Physiocal criteria of Wesseling (Peñáz J., 1973; Wesseling et al., 1995). It also measures heart rate and hydrostatic height of the hand (to correct for pressure changes due to vertical heart – hand distance changes). Beatscope software was used to analyze the measurements (Beatscope 1.0; TNO-TPD Biomedical Instrumentation Netherlands Organization for Applied Scientific Research).

STUDY PROTOCOL

Twenty-four hours before the measurements, all medication that affects BP was withdrawn. After an overnight fast, the subjects were allowed to take their regular medication (with the above exception) with some water 2 hours before breakfast. They could select the ingredients of their continental breakfast, to mimic normal conditions as closely as possible, but were offered weak tea, because caffeine in coffee might affect BP (Heseltine et al., 1991). After breakfast, subjects were not allowed to eat or drink until measurements were completed. BP was measured from 15 minutes before until 135 minutes after the start of breakfast, with subjects in sitting position. To avoid vasoconstriction-related inconsistency in measurements, the hand connected to the Portapres device was placed on a warm cherry stone pillow (Jagomagi et al., 2001). Symptoms or complaints associated with PPH (light-headedness, dizziness, tiredness, and hazy vision) were scored every 15 minutes on a 3-point scale (absent, moderate, or severe). The total intake of calories, carbohydrates, and fat was calculated afterwards. The study protocol was approved by the Medical, Ethical Testing Committee of the University Medical Center Utrecht.

DATA ANALYSIS

Differences between the groups at baseline and maximum changes from baseline were determined with a Mann-Whitney U test. The chi-square and Fisher exact tests were used for comparison of the presence of PPH with PPH-related symptoms.

In this study, PPH was defined as a decline in systolic BP of at least 20 mmHg, determined by calculating the difference between the minimum systolic BP values before and within 120 minutes after the start of breakfast. Because we were not interested in beat-to-beat BP variations, we calculated mean values over 2-minute periods. The start of PPH and the time of maximal fall in BP are expressed in minutes after the completion of breakfast. To determine the minimum interval between BP measurements for diagnosis of PPH, the maximum decrease in BP was calculated for different intervals between measurements, which was done by omitting 1 or more of the 2-minute BP measurements from calculations. The number of patients diagnosed with PPH was calculated, using these different intervals.

Differences were considered significant with a $p < 0.05$. Data are expressed as means ± standard deviation (SD) unless otherwise specified.

RESULTS

PATIENT CHARACTERISTICS

During the study period 101 patients were admitted to the geriatric ward, 68 of whom were excluded because they met one or more of the exclusion criteria (in most cases because they suffered from an acute illness). Five patients did not give consent and five others could not participate because of technical difficulties. The remaining 22 patients (32% male) were studied. They were admitted because of metabolic disorders (4 patients), mobility disorders (4), anemia (3), Parkinsonism (3), delirium (2), syncope (2) or miscellaneous (4). Mean age was 84 years (SD ± 5, range 74-93). Resting BP was 140/65 mm Hg (SD ± 27/15, range 104-200/31-90) and heart rate 67/min (SD ± 10, range 40-80). Of these patients, 45% had a history of cardiovascular disease (other than hypertension), 32% a history of hypertension (partially overlapping the group 45% of subjects with cardiovascular disease), 27% had Parkinson's disease (all were Hoehn and Yahr stage 2 to 3), 18% had a history of cerebrovascular disease, and 14% had diabetes mellitus.

Of the 53 healthy elderly subjects who responded to the advertisement, 20 were excluded because they met one or more of the exclusion criteria, 2 did not give

TABLE 2 Baseline characteristics of patients admitted to a geriatric ward and of healthy elderly individuals

	Patients (n=22)	Healthy elderly (n=20)	p
Male vs Female (n)	7 vs 15	2 vs 18	0.14
Age (yr)	84 ± 5 [74-93]	82 ± 4 [75-88]	0.07
Quetelet index (kg/mμ)	27 ± 7 [18-42]	26 ± 3 [22-33]	0.89
Calorie intake (kcal)	345 ± 118 [185-603]	306 ± 52 [191-367]	0.52
Carbohydrate intake (g)	38 ± 12 [20-70]	35 ± 9 [19-46]	0.67
Fat intake (g)*	16 ± 7 [4-27]	12 ± 3 [7-17]	0.05
Systolic BP (mmHg)**	140 ± 27 [104-204]	136 ± 20 [94-164]	0.48
Diastolic BP (mmHg)**	65 ± 15 [31-90]	65 ± 11 [47-80]	0.99
Heart rate (bpm)**	67 ± 10 [40-80]	68 ± 10 [42-80]	0.53
Vascular resistance (MU)**	1.9 ± 1.4 [0.3-5.1]	2.0 ± 1.8 [0.7-9.1]	0.94

Mean ± standard deviation [range]
* Intake during test meal
** Minimum values in 15 minutes before breakfast
P-values calculated with Mann Whithney U test

consent after reading the patient information, and 11 were excluded because of age mismatching. The remaining 20 healthy elderly were compared with the 22 geriatric patients. There were no differences in baseline characteristics between the two groups (Table 2). Of the healthy elderly individuals, 23% had with hypertension; other diseases were not present.

Postprandial hypotension

Figure 1 shows mean systolic and diastolic BP and heart rate during and after breakfast of individuals. There were no significant differences between the two groups, and BP and heart rate increased in both groups during the meal. The increase in diastolic BP and heart rate tended to be higher in the healthy elderly group ($P = 0.05$ and $P = 0.13$, respectively). After the meal, systolic and diastolic BP decreased below baseline values in both groups, but the fall in systolic BP was significantly greater in the patient group (mean values over period 40-60 minutes after the start of breakfast: 24.1 ± 19.6 versus 9.6 ± 15.8 mmHg; $P = 0.01$). Thus PPH occurred in 20 (91%) of 22 patients and in 8 of 20 (40%) healthy elderly individuals ($P = 0.001$, MWU test). The characteristics of the BP fall in the subjects with PPH are shown in Table 3. There were no statistically significant differences between the 2 groups in the moment of onset and duration of PPH, maximum fall in systolic BP, time between end of breakfast and maximum fall in systolic BP and increase in heart rate, defined as the difference between maximum heart rate in the baseline and in the postprandial period. PPH-related complaints did not differ significantly between the groups. Of the 42 included subjects, 18 developed

TABLE 3 Characteristics of the subjects (elderly patients and healthy elderly individuals) with postprandial hypotension

	Patients (n=20 out of 22)	Healthy elderly (n=8 out of 20)	Range*	P
Start (min)	34 ± 27	34 ± 25	0 - 104	NS
Max fall (mmHg)**	39 ± 19	31 ± 8.3	20-79	NS
Time of max fall (min)**	58 ± 27	48 ± 25	10-110	NS
Duration (min)**	42 ± 36	28 ± 26	2-108	NS
Change in HR (bpm)	8 ± 7	4 ± 5	-11-12	NS
Presence of complaints (n)	13 (= 65%)	4 (= 50%)		NS
Start complaints (min)	33 ± 30	36 ± 27	0-84	NS
Duration complaints (min)	55 ± 29	43 ± 32	14-90	NS

Mean ± standard deviation
*All PPH positives
**Data about the fall in systolic BP
P = statistical difference between the groups
(NS = not significant, when the difference is >0.05)

PPH-related symptoms and 24 had no complaints (Table 4). Seventeen of the 18 subjects (95%) who developed PPH-related symptoms had a simultaneous decrease in BP greater than 20 mmHg, consistent with PPH. Thirteen of the 24 subjects (54%) who did not have PPH-related symptoms did not have a significant decrease in BP. To calculate the sensitivity and specificity of PPH-related symptoms for the diagnosis of PPH, we considered patients to be symptomatic if at least one of the symptoms was scored as 'moderate' during the test. The sensitivity of PPH-related symptoms for a significant decrease in BP was 17/28 = 0.61, the specificity was 13/14= 0.93, the positive predictive value was 17/18 = 0.94, and the negative predictive value was 13/24 = 0.54. There the presence of PPH-related symptoms was strongly associated with a decrease in BP greater than 20 mmHg ($P < 0.001$).

Minimal required interval for blood pressure measurements

We determined the interval between BP measurements that was most appropriate for detecting PPH in the individuals with PPH in the group of patients that were admitted to the geriatric ward. PPH lasted 2 minutes in two patients, 6 minutes in one patient, and 8 minutes in two patients. It lasted between 14 and 108 minutes in the remaining 15 patients (see Figure 2). Eight patients had two periods of PPH. If one of every two 2-minutes values was left out, a maximum of one patient would be missed, depending on which of the first 2-minute values was left out. If BP was measured at 4-minute intervals (one of three 2-minute measurements omitted) at least one patient would be missed. If BP was measured at 6-minute intervals (one of four 2-minute measurements omitted), at least 2 patients (10%) would be missed; with intervals of 10 and 15 minutes, 3 (15%) and 4 (20%) of the patients with PPH would not be detected (Figure 3).

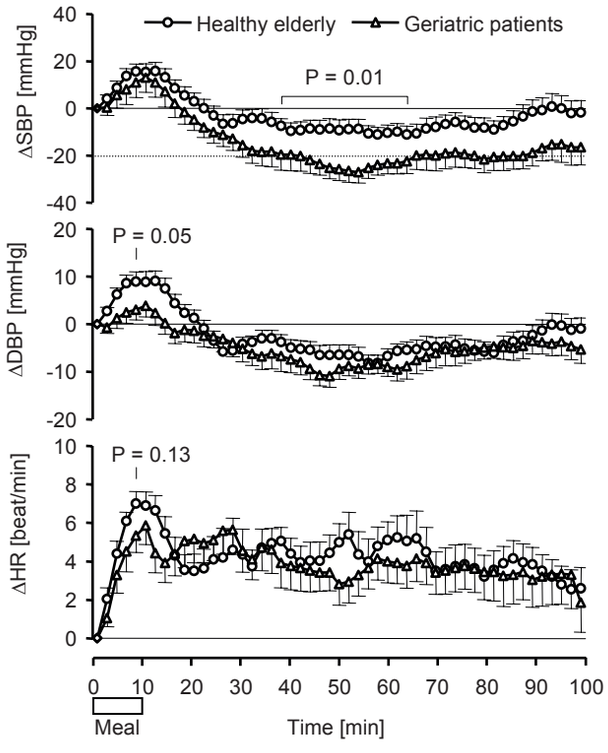
TABLE 4 Test characteristics of 'PPH-related symptoms' for the diagnosis PPH. PPH was defined as a fall in systolic blood pressure of > 20 mmHg within 2 hours of the start of the meal, measured with a Portapres device, using all 2-minute samples. The sensitivity of PPH-related symptoms for a significant fall in blood pressure was 17/28 = 0.61, the specificity was 13/14= 0.93, the positive predictive value was 17/18 = 0.94 and the negative predictive value was 13/24 = 0.54.

	PPH	No PPH	Total
PPH related symptoms present	17	1	18
PPH related symptoms absent	11	13	24
Total	28	14	42

DISCUSSION

We found PPH to be more common among elderly patients (91%), than previously reported (Aronow and Ahn, 1994; Mitro et al., 2001; Rhebergen and Scholzel-Dorenbos, 2002). This difference might be explained by the presence of co-morbidity in the population studied, which might have been more extensive than in other studies, or by a difference in the method used to detect PPH, although we detected PPH in 40% of healthy elderly individuals, as reported earlier (Rhebergen and Scholzel-Dorenbos, 2002). The fall in BP appeared to be clinically relevant because it was significantly associated with symptoms suggestive of hypotension. There was considerable variability in the time of onset, duration, and maximum decrease in BP

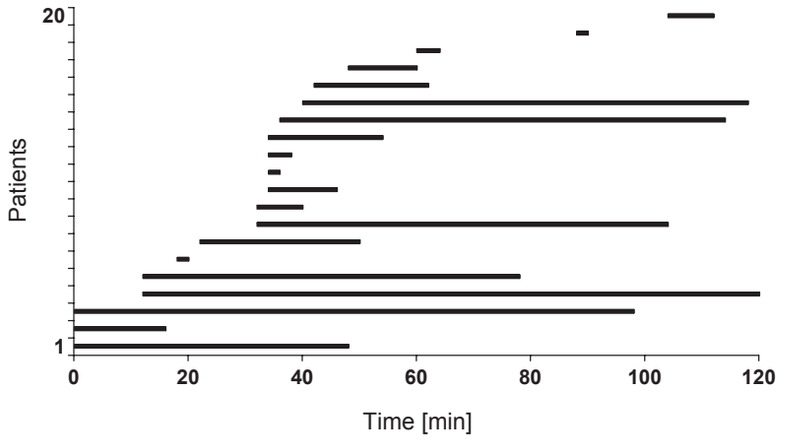
FIG. 1 Mean change in systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in 22 geriatric patients and 20 healthy elderly individuals during and after breakfast (P-values of differences between groups) between groups (P = 0.2).



in the subjects with PPH, which supports the hypothesis that PPH is not caused by the failure of a single mechanism, but rather by the failure of several, and that there is considerable variation between subjects.

Despite this variability, PPH started within an hour of eating breakfast in 18 of 20 patients (90%). Assuming that changes in systolic BP measured with a sphygmomanometric device are similar to the 2-minute mean Portapres values that were used in this study, BP measured at 6-minute intervals would have identified at least 18 patients with PPH (90%); measurement with 10-minute intervals would have identified at least 17 patients (85%) with PPH (Figure 3).

FIG. 2 Start and duration of postprandial hypotension, defined as a decrease in systolic blood pressure of 20 mm Hg, after completion of a meal in 20 geriatric patients.



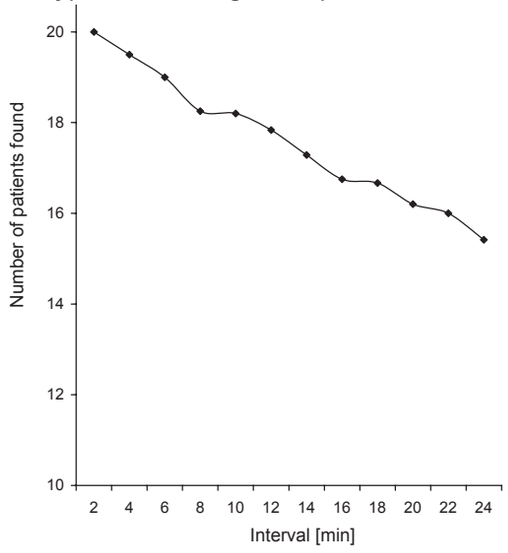
Beat-to-beat measurements will increase the number of short lasting falls in postprandial BP detected relative to the number detected when BP is measured with a sphygmomanometer. However, in general practice this method is not attractive because continuous BP measurements are comprehensive, require skill to get reliable results, and require relatively expensive equipment. For this reason, automatic spyghmomanometer measurements, with devices such as Spacelab, are preferred (Table 1). The results of present study indicate that this method has an acceptable sensitivity for detection of PPH if the interval between measurements is 10 minutes or less.

In this study, the subjects ate a continental breakfast, in which the release of glucose is quite constant, whereas other studies used standardized liquid meals, in which the release of glucose is rapid. We chose a standardized continental breakfast as being more representative of the normal situation. Moreover, we hypothesize that

differences in the severity of PPH observed with high and low carbohydrate-containing meals can be explained by differences in the rate of glucose uptake rather than by differences in the total number of calories consumed, a hypothesis that is supported by the findings of O'Donovan et al. (2002). They found that the fall in systolic BP was greater with a higher rate of glucose delivery into the duodenum. Despite the probably relatively low rate of glucose delivery with our continental breakfast, a large proportion of our subjects developed PPH.

Our study had some potential limitations. We chose to withdraw medication that affects BP for 24 hours to eliminate transient effects of medication that could occur directly after ingestion. However, we cannot rule out the possibility that these medications affect the mechanism of postprandial hypotension. We calculated mean values of BP over 2-minute periods and may thus have missed a short-lasting fall (less than 2 minutes) in BP. However, such a short-lasting fall in BP may not be of clinical relevance. It was our goal to mimic the normal physiological condition by using a continental breakfast instead of standardized meal and to allow subjects to choose their breakfast. Because subjects were not active after they finished their breakfast, we cannot rule out that the fall in BP was the effect of drowsiness or even sleep as might occur during the period of inactivity after the meal, adding to the occurring hypotension, although a researcher was constantly in the room and a symptom-questionnaire was evaluated every 15 minutes, limiting the presence of drowsiness and its potential influence. There is currently no standardized clinically meaningful definition of PPH (Jansen, 2005).

FIG. 3 Time interval of blood pressure beat-to-beat measurements related to the detection of postprandial hypotension in 22 geriatric patients.



From the clinical perspective, it is important to measure BP following a meal in patients who have unexplained syncope and whose orthostatic stress test result is normal. Old age is associated with diminished homeostatic regulation of many physiological functions. Reduced fluid and food intake and common medical conditions in the elderly, such as gastrointestinal disease (malabsorption syndromes), can cause dehydration. Dehydration, and the use of diuretics or other agents may reduce the cardiac output in the elderly. In subjects with PPH, these factors might induce severe hypotension and this is a potential trigger for ischemic stroke or acute myocardial infarction.

In conclusion, PPH is common not only among frail subjects admitted to a geriatric ward but also among healthy elderly individuals. Reducing the frequency of BP measurements from 2-minute intervals to 6-minute or 10-minute intervals causes a failure to detect postprandial hypotension of 10 and 15% respectively. Although measuring BP every 10 minutes with a sphygmomanometer, starting 15 minutes before until 60 minutes after the end of a regular breakfast seems to be a patient-friendly and practical way to evaluate PPH, it will detect only 85% of elderly individuals with PPH.

CHAPTER 6

Is food or alcohol intake a trigger for acute focal cerebral ischemia in patients with a compromised cerebral blood flow? A case crossover study

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ABSTRACT

BACKGROUND While classic vascular risk factors that are associated with an increased risk of stroke on the long-term are well known, little is known about specific activities that could trigger stroke. The aim of this study was to find out whether food or alcohol intake could trigger the occurrence of an ischemic cerebral event in patients with a compromised cerebropetal blood flow.

METHODS A case-crossover design was used to determine the associations between a transient ischemic attack (TIA) or minor disabling ischemic stroke and food or alcohol intake in the two hours preceding the event. The control period was 'a usual day in the three months prior to the ischemic event'. Food intake was defined as any calorie containing solid or high dose glucose containing liquid foodstuff and alcohol intake was defined as the consumption of at least one alcohol containing drink. Consecutive patients with a recent TIA or minor disabling ischemic stroke associated with a unilateral or bilateral internal carotid artery stenosis / occlusion of 70 - 100% were recruited.

RESULTS One hundred and thirty-five ischemic events in 96 subjects (74 males, mean age 64 ± 12) were included. There was no relationship between food intake (odds ratio (OR) 0.71, 95% confidence interval (CI) 0.30 - 1.62), or alcohol intake (OR 0.86, 95% CI 0.29 - 2.55) and the ischemic event.

CONCLUSION Food or alcohol intake did not trigger the onset of a TIA or minor disabling ischemic stroke in patients with unilateral or bilateral >70% internal carotid artery stenosis. This suggests that the hemodynamic changes subsequent to food or alcohol intake do not play a major role in the occurrence of cerebral ischemia in patients with obstructive carotid disease.

INTRODUCTION

Chronic risk factors for cerebral ischemia including hypertension, diabetes mellitus, cardiac diseases, cigarette smoking, and hyperlipidemia, are well established but less is known about specific activities that trigger stroke. Negative emotions, anger, and sudden changes in body posture in response to a startling event may be independent triggers for ischemic stroke (Koton et al., 2004). While eating a heavy meal does not appear to increase the risk of stroke (Koton et al., 2004), it may be a short term risk factor in selected patients with a compromised cerebropetal circulation (Kamata et al., 1994; Isa et al., 2002).

One way to examine the effect of potential triggers is to study the circadian rhythm of an acute disease. If there is a peak at a specific time of the day, an activity preceding this moment may have triggered the onset of disease. Stroke onset has been demonstrated to occur more frequently in the morning, at about 8 a.m. and at about 11 – 12 a.m. (Kelly-Hayes et al., 1995; Gur and Bornstein, 2000; Casetta et al., 2002; Jimenez-Conde et al., 2007), but the mechanism underlying this association with the time of the day is unknown. Blood pressure also shows a circadian rhythm: it is relatively low at night and starts to rise in the morning, on awaking. Some authors hold this rise in morning blood pressure responsible for the increased morning onset of stroke (no authors listed, 2002a; no authors listed, 2002b).

A combination of generalized hypotension and stenosis or occlusion of cerebropetal arteries may lead to focal brain ischemia (Caplan and Sergay, 1976; Barnett, 1978; Bogousslavsky and Regli, 1983; Somerville, 1984; Hankey and Gubbay, 1987; Krajewski et al., 1993; Dobkin, 2001; Altieri et al., 2001; Isa et al., 2002). The importance of hypotension may be underestimated because clinicians often consider emboli from atherosclerotic lesions responsible for ischemic events. The current study focuses on potential triggers that, on theoretical grounds, could lead to acute focal cerebral ischemia as a result of mild hypotension in combination with stenosis or occlusion of the cerebropetal arteries. We investigated the associations between food intake (postprandial blood pressure fall; O'Donovan et al., 2002; Van Orshoven et al., 2008) or alcohol consumption (vasodilatation and hypotension; Chaudhuri et al., 1994)) and focal brain ischemia in patients who experienced a recent transient ischemic attack (TIA) or minor disabling ischemic stroke associated with stenosis or occlusion of the carotid arteries.

METHODS

STUDY DESIGN AND POPULATION

The potential role of 'food intake and 'alcohol consumption' as trigger for a cerebral ischemic event was evaluated using a case-crossover study design (Maclure and Mittleman, 2000). Over a period of 2 years, consecutive patients were included who were treated at the University Medical Center Utrecht, the Netherlands, for a TIA or non-disabling ischemic stroke associated with a carotid stenosis of 70-100% (Nederkoorn et al., 2002) and who were selected for work-up with a view to carotid endarterectomy or extracranial to intracranial (EC-IC) bypass. Patients were excluded if it was not possible to take their history because of aphasia or a linguistic barrier, or if the patient could not remember the details of the event. The study was approved by the ethics committee of our hospital and written informed consent was obtained from all patients.

ASSESSMENTS

Data were collected by one person (NPVO), who interviewed individual patients and their relatives. All patients were asked about the date and exact time of their TIA or minor disabling ischemic stroke. If patients had had recurrent events, the details of all events were collected. Events that occurred during sleep (between 0:00 a.m. and 7:00 a.m.) were excluded from the analysis. Patients were asked about the exact time they last ate food, defined as 'any calorie-containing solid or high-dose glucose-containing liquid foodstuff', before the ischemic event, and about the timing and regularity of food consumption on a 'usual day in the 3 months prior to the ischemic event', defined as the 'control day'. Regularity was defined as 'meal taken at least 4 days a week at the time mentioned, with a variation of time of onset of the meal of 1 hour at most'. Patients were also asked the exact time the last alcohol-containing drink (at least one unit) had been consumed prior to the ischemic event. The control period was again chosen as 'a usual day in the 3 months prior to the ischemic event'. Regularity was defined as 'at least 1 day a week at the time mentioned, with a variation of time of onset of the drink of 1 hour at most'.

The time window for the assumed increased risk associated with the potential trigger 'food intake' was chosen to be 2 hours from the start of the meal, based on the assumption that a postprandial fall in blood pressure occurs within 2 hours from the

start of a meal (Jansen and Lipsitz, 1995). Similarly, for the potential trigger 'alcohol intake', we chose a time window of 2 hours from the start of the drink (Kojima et al., 1993).

POWER AND STATISTICAL ANALYSES

With respect to food intake we estimated the frequency of exposure in the control period at 6/17 (24 minus the excluded 7 at night = 17 hours 'awake'; average number of meals 3, so 3 times 2 hours = 6 hours increased risk time) = 0.343. We calculated that we would need to include at least 135 events in order to detect a statistically significant relative risk of at least 2, with a power of 0.80 and an alpha of 0.05.

We analyzed the association between the occurrence of TIA and the potential triggers 'food intake' and 'alcohol intake' in two different ways. First, we compared the frequency of exposure to food or alcohol consumption in the 2 hours before the onset of ischemic symptoms with the frequency of exposure in the same time window on an average day in the 3 months before the ischemic event and calculated the matched odds ratios. Secondly, we calculated whether the frequency of exposure to food or alcohol consumption in the 2 hours before the ischemic event was higher than would be expected based on the usual frequency of food or alcohol consumption in a control period (case crossover method 'usual frequencies') (Greenland and Robins, 1985; Maclure, 1991). To calculate the relative risk of food consumption, we selected an average day in the 3 months before the ischemic event as control period. Likewise, to calculate the relative risk of alcohol consumption, we selected an average week in the period before the event as control period. As the absolute frequency of the potential trigger in the control period depends on the length of this period (a higher absolute frequency is expected in a control period of a week compared to a control period of 1 day), this calculation contains a correction for the total risk time in the control period.

SUBGROUP ANALYSES

We hypothesized that patients with more severe stenosis or occlusion of the carotid arteries would be more prone to develop cerebral ischemia triggered by food or alcohol intake than patients with a severe stenosis. The patients were divided into two subgroups. Subgroup 1 consisted of patients with a one-sided stenosis of 70-99% in combination with a contralateral stenosis of 0-69%, and subgroup 2 consisted of patients with stenosis of 70 - 100 % in both internal carotid arteries or a one-sided occlusion in combination with a 0 - 69% stenosis on the contralateral side.

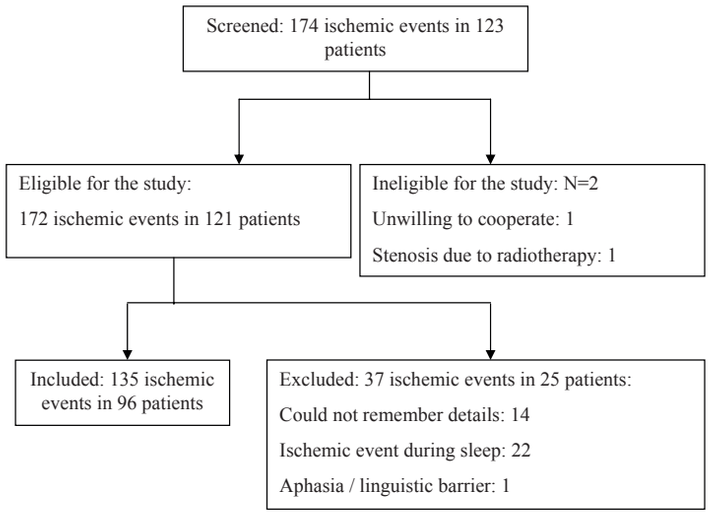
RESULTS

PATIENT POPULATION

From the 174 ischemic events in 123 subjects that were screened, we included 135 ischemic events in 96 subjects. Figure 1 presents a diagram of all subjects that entered the study. Of the included patients, 74 (77%) were male, the age was 64.4 ± 12.4 years (mean ± SD). The time between the ischemic event and the interview was 3.8 [0 – 41] months (median, range).

Of the 135 included ischemic events, 20 events (15%) were minor disabling ischemic strokes, 77 events (57%) were transient ischemic attacks (including five events with hemodynamic characteristics, such as limb shaking), 36 events (27%) were amaurosis fugax and in two events (1%) the classification was unknown. Sixty ischemic events (44%) occurred in the left and 69 (51%) in the right carotid territory and in 6 events (5%) the side was unknown.
Fifty-nine patients (61%) had a left-sided and 61 (64%) had a right-sided stenosis of 70-100%. Fifty patients (52%) had a unilateral stenosis of 70-99% in combination with a contralateral stenosis of 0-69% (subgroup 1), 22 patients (23%) had a unilateral occlusion with a contralateral stenosis of 0-69%, 20 patients (21%) had a unilateral

FIG. 1 **Study population**



occlusion in combination with a contralateral stenosis of 70-99%, 2 patients (2%) had a bilateral stenosis of 70-99%, and 2 patients (2%) had a bilateral occlusion. Of the 135 ischemic events analyzed, 74 (55%) were subgroup 1-type events and 61 (45%) were subgroup 2-type events.

Cardiovascular risk factors are listed in Table 1.

TABLE 1 Cardiovascular risk factors in 96 subjects. Values are percentages

Risk factor	Percentage
Smoking	68
Hypertension	63
Dyslipidemia	53
Diabetes mellitus	22
Overweight	25
Family history of stroke or myocardial infarction	33
Atrial fibrillation	3
Ischemic heart disease	28
Peripheral vascular disease	15

Data about alcohol consumption were collected for 100 ischemic events in 72 patients. Fifteen events were excluded because of occurrence at night (n = 9), no memory about the details about alcohol intake (n = 4), a linguistic barrier (n = 1), or stenosis due to radiotherapy (n = 1). In total, 85 ischemic events in 58 subjects were analyzed.

FREQUENCY DISTRIBUTION OF ISCHEMIC EVENTS

Relatively few ischemic events occurred at night (these were excluded from further analysis, as mentioned in Methods). Most events occurred between 8.00 and 9.00 a.m., between 11.00 and 12.00 a.m., and between 2.00 and 4.00 p.m. (see Figure 2).

FOOD INTAKE AS POTENTIAL TRIGGERS FOR A CEREBRAL ISCHEMIC EVENT

Matched comparisons of 2 hours just before the ischemic event with the same time period on an average day, showed no relationship between food intake and the ischemic event: matched odds ratios (OR) 0.71 [95% confidence interval (CI) 0.30 – 1.62] (Table 2). The secondary case-crossover analysis, using the same data but now comparing exposure to food intake in the 2 hours before the ischemic event with the usual frequency of food intake on an average day, showed a trend toward a significantly increased risk: RR 1.34 [95% CI 0.96 – 1.88, P = 0.09]. Subgroup analysis yielded similar results: the matched odds ratio for an ischemic event after food intake was 1.00 [95% CI 0.35 - 2.85] (24-hour matched method) in subgroup 1 and 0.33 [95% CI 0.07 - 1.65] in subgroup 2.

FIG. 2 Frequency distribution of time of onset of cerebral ischemic events during the day. The night-time period (0 – 7 a.m.) is presented as 1 bar because time of onset during sleep was unknown (those patients were excluded from further analyses).

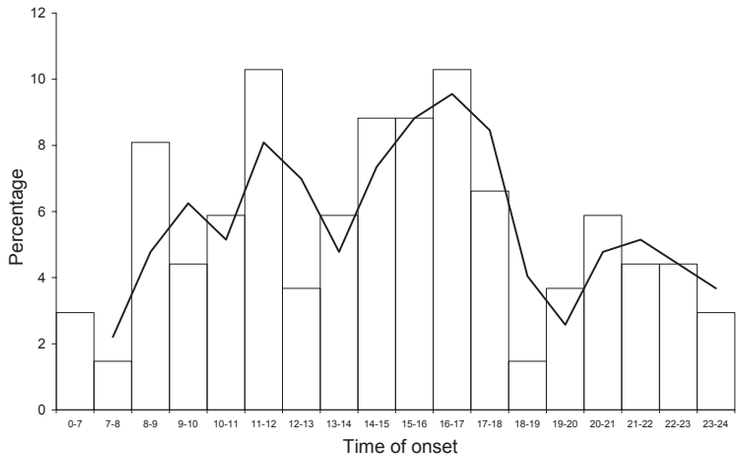


TABLE 2 Association between food or alcohol intake and the development of transient ischemic attacks or minor disabling ischemic stroke. The potential trigger 'food intake' was studied in 135 events and the potential trigger 'alcohol intake' was studied in 85 events. Exposure to the potential trigger in the 2 hours before the ischemic event (day of stroke) was compared with exposure in the same 2 hours on the control day (average day). Each ischemic event was categorized as 'exposure to the potential trigger only on the day of the stroke', 'only on the control day', in both periods or in neither period. Matched odds ratios (ORs) for patients exposed to potential triggers during the 2 hours before the stroke, compared to the same two hours on an average day.

	Exposure to potential trigger				
Potential triggering factor	**Day of the stroke only**	**Average day only**	**Both periods**	**No exposure**	**OR [95%CI]**
Food intake	10	14	43	68	0.71 [0.30 – 1.62]
Alcohol intake	6	7	3	69	0.86 [0.29 – 2.55]

ALCOHOL INTAKE AS POTENTIAL TRIGGERS FOR A CEREBRAL ISCHEMIC EVENT

Analysis of the 24-hour matched data for 'alcohol consumption' did not show there to be a relationship with the ischemic event (matched OR 0.86 [0.29 – 2.55]; Table 2). Similarly, the secondary case – crossover analysis (usual frequency) did not show a significantly increased risk (relative risk: 1.33 [95% CI 0.66-2.69]). Subgroup analyses were not carried out because the subgroups were too small.

DISCUSSION

Food or alcohol consumption was not found to increase the risk of TIA or minor disabling ischemic stroke in patients with unilateral or bilateral obstructive carotid disease. The diurnal distribution of the ischemic events in our study was similar to that reported previously (Jimenez-Conde et al., 2007).

The current study focused on factors that hypothetically could trigger the occurrence of acute, focal cerebral ischemia by mild hypotension. Postprandial hypotension affects 24–36% of elderly individuals and is an important cause of mild or moderate hypotension (Vaitkevicius et al., 1991; Aronow and Ahn, 1994); its prevalence is reported to be as high as 45% in patients with hypertension (Mitro et al., 1999). Postprandial hypotension is usually defined as a fall in systolic blood pressure of at least 20 mmHg or a fall in diastolic blood pressure of at least 10 mmHg within 2 hours of the start of a meal (Jansen and Lipsitz, 1995). The larger the meal and the greater its carbohydrate content, the greater the fall in blood pressure (Puvi-Rajasingham and Mathias, 1996; Vloet et al., 2001). Most studies show a maximal fall between 15 and 90 minutes after the start of the meal (Vaitkevicius et al., 1991; Aronow and Ahn, 1994; Jansen et al., 1996; Mitro et al., 1999; O'Donovan et al., 2002). The decrease in blood pressure is probably caused by insufficient compensation for the meal-induced increase in splanchnic perfusion. A decreased baroreflex sensitivity and failure of the sympathetic response activated by stretch receptors in the stomach, the so-called gastrovascular reflex, probably play an important role in the pathophysiology of postprandial hypotension (Van Orshoven et al., 2004; Van Orshoven et al., 2008).

Postprandial hypotension has been reported to cause hemodynamic focal brain ischemia or amaurosis fugax (Eisenberg and Bental, 1986; Kamata et al., 1994; Obayashi et al., 1995; Levin and Mootha, 1997; Nehmad and Madonna, 1999; Montaner et al., 2000; Isa et al., 2002) and may also be associated with asymptomatic cerebral damage (Kohara et al., 1999). There is more than one explanation for our failure to detect a relationship between food intake and cerebral ischemia in our study. First, the patients studied might not have had postprandial hypotension. Although it frequently occurs in the age group studied and in hypertensive patients, we did not test for the presence of postprandial hypotension. Secondly, food intake could be associated with cerebral ischemia in a subgroup of patients. Our analysis,

which compared the frequency of exposure to a meal before the ischemic event with the usual frequency of meals in the control period, showed a trend toward a significantly increased risk of stroke after meals (RR 1.34 [95% CI 0.96 – 1.88, P = 0.09]. This stimulated us to do a subgroup analysis, hypothesizing that a subgroup of patients could be at increased risk of cerebral ischemia. However, we did not find an increased relative risk in the subgroup of patients with a higher degree of carotid stenosis or occlusion (subgroup 2), but the subgroups were relatively small. Patients with bilateral occlusion of the carotid or vertebral arteries or patients with aberrant transcranial Doppler flow patterns, consistent with hemodynamic failure might be at increased risk of ischemia in the postprandial period. Thirdly, the failure to find such a relation might be due to selection or recall bias. We studied only patients that were selected for work up for carotid surgery. Recall bias might have occurred as a result of the long period between the actual ischemic event and the interview, and because we excluded patients who could not remember details of their TIA or minor disabling ischemic stroke. However, we think that our data are robust because we were often surprised the detail in which patients could remember the day of the event. The event probably made a deep impression on the subjects, who probably repeatedly told the sequence of events of that day to their relatives.

Lastly, the potential risk-increasing effect of postprandial hypotension might be compensated by another risk-decreasing factor, such as the decreased viscosity of blood observed in healthy older subjects after a meal (Coppola et al., 2007). Focal brain ischemia could occur as a consequence of low blood flow due to hyperviscous blood and, inversely, the decrease in blood viscosity elicited by eating a meal might have a preventive effect on stroke onset.

Alcohol intake can induce vasodilatation and hypotension, but in healthy young subjects blood pressure may also increase after alcohol consumption (Iwase et al., 1995).

In conclusion, we found no evidence for a temporal relationship between food intake or alcohol consumption and the onset of TIA or minor disabling ischemic stroke in patients with unilateral- or bilateral obstructive disease of the carotid artery.

CHAPTER 7

General discussion

GENERAL DISCUSSION

In this concluding chapter the strengths and limitations of the main findings of this thesis are discussed. The objectives of the studies were to (1) describe the mechanisms of normal blood pressure regulation in response to food intake, (2) determine the effects of aging on these mechanisms and (3) study potential consequences of failure of these mechanisms.

1 AUTONOMIC NERVOUS SYSTEM REACTIONS TO FOOD INTAKE IN HEALTHY YOUNG INDIVIDUALS

Food intake evoked an increase in sympathetic outflow to muscles (MSNA) in healthy young subjects (chapter 4), as also reported by others (Berne et al., 1989; Fagius and Berne, 1994). However, it was not clear whether the increase was due to the volume of the food bolus, causing gastric distension, or to an interaction of the constituent nutrients with the gut. In the studies described in chapters 2 and 3, we separated the effects of gastric distension from those of the interaction of nutrients with the small bowel. We found that both distension of the stomach and infusion of glucose into the duodenum were associated with an increase in MSNA in healthy young subjects. Gastric distension was associated with an increase in blood pressure, whereas, surprisingly, intraduodenal glucose infusion was associated with a decrease in blood pressure. The finding that MSNA increased but that blood pressure decreased in response to intraduodenal glucose infusion seems to be contradictory: an increase in MSNA would cause an increase in vascular resistance in muscles, which would be expected to increase blood pressure. A possible explanation might be that gastric distension causes an increase in MSNA directly, by a mechanism not involving the baroreflex (see chapter 1), and that intraduodenal glucose causes an increase in MSNA indirectly, by baroreflex activation secondary to a depressor effect on blood pressure. The depressor effect on blood pressure might be related to the decrease in splanchnic arterial resistance in response to a meal.

Several arguments can be put forward to suggest that the MSNA changes were not related to gastric distension (chapter 2) or to intraduodenal glucose infusion (chapter 3). For example, observations indicate that the distress caused by a sustained recording session in a constantly uncomfortable body position might cause an increase in MSNA (Burke et al., 1977). However, in our experiment, the variables measured returned to

baseline values after the end of the barostat procedure (chapter 2, Figure 3) and after the end of intraduodenal glucose infusion (chapter 3), contradicting this hypothesis. Furthermore, in the gastric distension protocol (chapter 2), we increased intragastric pressure by 14 mmHg in 7 steps. These steps in pressure might be greater than the physiological increase in intragastric pressure recorded after the consumption of a heavy meal. Therefore, sympathetic activation might be an indirect effect caused by the pain and discomfort (mainly nausea and fullness) evoked by gastric distension. The subjects indeed tended to feel pain, but only at the higher distension steps (chapter 2), while MSNA started to increase at step 1, before the subjects reported any abdominal discomfort. Furthermore, pain and other stress reactions are characterized by a prompt and immediate increase in heart rate (Freyschuss et al., 1990) and, in our experiment, heart rate did not increase in all subjects.

In our experiments with gastric distension and intraduodenal glucose infusion, the subjects had to sit still for several hours and could not empty their bladder. Bladder distension can also evoke an increase in MSNA and blood pressure (Fagius and Karhuvaara, 1989). However, none of our subjects experienced an urge to void, so this mechanism did not play a major role in our findings.

Some of the subjects found the experimental protocols used in these studies (chapters 2 and 3) to be demanding, which resulted in a number of potential candidates refusing to participate. Thus only a relatively small group of subjects completed the protocols. Therefore, we cannot discount the possibility that the cohorts studied were not representative and that type 2 statistical errors occurred, although the outcome of the majority of the analyses appeared clear-cut.

Suggestions for future research:

- The results of the study of the effects of gastric distension (chapter 2) and intraduodenal glucose infusion (chapter 3) seem contradictory. Suggested reasons for this apparent contradiction included changes in splanchnic blood flow and resistance. Splanchnic blood flow can be measured non-invasively, using Doppler ultrasound. Future studies of the effects of gastric distension and intraduodenal nutrient infusion should therefore include measurement of mesenteric blood flow to determine whether splanchnic blood flow increases, or not, during gastric distension and/or intraduodenal glucose infusion.
- We studied the effects of intraduodenal infusion of glucose only. Future studies of the effects of intraduodenal proteins, fats, or caloric fluids with varying osmolality on MSNA are a logical next step.
- In our studies of the effects of gastric distension and intraduodenal glucose infusion, we tried to isolate the effects of the volume of the food bolus from its

nutrient content. A next step would be to combine the effects again in test meals of different volume and nutrient content, to study the relative contributions of these factors.

2 EFFECTS OF AGING ON THE AUTONOMIC NERVOUS SYSTEM RESPONSES TO FOOD INTAKE

In elderly people, for reasons that are not completely known, blood pressure falls after eating (Jansen and Hoefnagels, 1989; Peitzman and Berger, 1989). The magnitude of the postprandial fall in blood pressure is dependent on the composition of the meal. The depressor effect on blood pressure is greater after the ingestion of carbohydrates, particularly glucose, than after the ingestion of fat, protein, or water (Jansen et al., 1987). In chapters 2 and 3, the effects of healthy aging on the autonomic nervous system responses to food intake were determined, by comparing carefully selected groups of healthy young individuals (age < 40 years) with healthy elderly individuals (age > 70 years). The results of the study described in chapter 2 showed that the reflex increase in MSNA and blood pressure in response to gastric distension, the 'gastrovascular reflex', was attenuated in healthy elderly individuals. The results of the study described in chapter 3 indicated that intraduodenal glucose infusion was associated with a fall in blood pressure that was greater in healthy elderly individuals than in healthy young individuals, despite similar increases in MSNA, which might be explained by reduced baroreflex sensitivity in the elderly. The results of chapter 3 demonstrated that the gastrovascular reflex appears to be of fundamental importance to the regulation of hemodynamics during a meal, in that even young subjects showed a significant fall in blood pressure in response to enteral glucose when the reflex was bypassed.

However, comparison of the responses of healthy young and older subjects (chapters 2 and 3) revealed apparently contradictory results, for which we suggested a number of potential explanations. In the study described in chapter 2, we found that the slope of the regression line of MSNA during the stepwise gastric distension was less steep in elderly individuals than in young individuals, but that total peripheral resistance and blood pressure tended to increase more in the elderly individuals than in the young. These findings seem to be contradictory: a stronger increase in MSNA in the young group would be expected to lead to a stronger increase in total peripheral arterial resistance and subsequently to a greater increase in blood pressure. We suggested that arterial compliance diminishes with age, which limits the ability of arteries to show vasodilatation. The more pronounced vasodilatation in the splanchnic system of the young individuals might explain why total arterial resistance and blood pressure in this group did not increase that much as they did

in the elderly individuals. In the study described in chapter 3, we found that blood pressure decreased more in healthy elderly individuals than in healthy young individuals while there was no difference in MSNA increase. These findings also seem to be contradictory, in that a greater fall in blood pressure would be expected to be associated with an attenuated increase in MSNA. We suggested several, not mutually exclusive, explanations for these findings, and could confirm one of them, namely, that sympathetic baroreflex sensitivity is attenuated in the elderly, meaning that the pressor effect of MSNA is diminished.

Suggestions for future research:

- Studying the effects of gastric distension and intraduodenal glucose infusion in healthy young and elderly individuals, and measuring splanchnic blood flow during the same experimental protocol in a group of patients with postprandial hypotension could provide information about the mechanisms of postprandial hypotension.

3 AUTONOMIC NERVOUS SYSTEM RESPONSES TO FOOD INTAKE IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

Although several authors have suggested that autonomic dysfunction might play a role in the etiology of irritable bowel syndrome (Smart and Atkinson, 1987; Karling et al., 1998; Heitkemper et al., 1998; Elsenbruch and Orr, 2001; Waring et al., 2004), direct measurement of sympathetic outflow by means of MSNA has thus far not been reported in the literature. We carefully selected patient with irritable bowel syndrome who fulfilled the Rome II criteria (Drossman, 1999) and found a similar increase in MSNA and blood pressure in response to food intake in patients with irritable bowel syndrome and healthy young subjects (chapter 4). This suggests that the pressor effect of food intake, which is mainly caused by gastric distension (see chapters 2 and 3), is normal in patients with irritable bowel syndrome, but it does not exclude that a difference in sympathetic outflow plays a role in irritable bowel syndrome. Owing to the extensive and demanding nature of the measurements, we only managed to study a relatively small number of subjects, which limits the power of our negative results. The number of patients recruited was based on a power calculation to determine a difference in MSNA (number of bursts) of at least 4 bursts per minute between patients with irritable bowel syndrome and controls during eating, which means that significant but smaller differences might very well have been found if more subjects had been included. Moreover, sympathetic outflow to different tissues is probably controlled separately (Wallin, 1999; see also chapter 1, Figure 1), and therefore sympathetic outflow to muscles might be different from that to the gastrointestinal system.

Unfortunately, sympathetic outflow to the gastrointestinal system cannot be measured in humans. For this reason, we recorded an indirect measure of sympathetic and parasympathetic activity and found a difference in the LF/HF ratio of spectral analysis of heart rate during eating and a lower deep breathing ratio in the patients with irritable bowel syndrome, suggesting that overall parasympathetic tone is lower in these individuals. However, this conclusion should be considered with caution. First, the interbeat intervals of heart rate, which were used in this calculation, are influenced by the rate of respiration. For this reason, we used a strict metronome breathing pattern, but this could not be maintained during the meal, which made an absolute comparison of intraprandial and baseline LF/HF ratios less reliable. Secondly, spectral analysis of the heart rate is an indirect measure of the balance of activity in the sympathetic and parasympathetic nervous systems (see chapter 1). In a previous study, we found that heart rate variability (HRV), measured as LF/HF ratio using short-term spectral analysis, showed a considerable intraindividual variability (van Schelven et al., 2000). Furthermore, if there is indeed a difference in parasympathetic tone between patients with irritable bowel syndrome and healthy young subjects, our findings do not provide information about the cause of this dysregulation. For example, it might be secondary to psychological factors, because patients with IBS respond with greater negative affect to food intake than healthy controls (Elsenbruch et al., 2001), although studies about the influence of mental stress on MSNA have yielded contradictory results, varying from no influence (Kuipers et al., 2008) to an increase in MSNA (Freyschuss et al., 1990).

Suggestions for future research

- Many studies of indirect measures of the autonomic nervous system in patients with IBS are reported in the literature, with often conflicting results. A meta-analysis of existing data might provide insight into the role of the autonomic nervous system in response to food intake.

4 PATHOPHYSIOLOGICAL MECHANISM OF POSTPRANDIAL HYPOTENSION

The high prevalence of postprandial hypotension reported in the literature (Aronow and Ahn, 1994; Jansen et al., 1995; Vloet et al., 2005) was confirmed by our studies with hospitalized geriatric patients (chapter 5). Despite this high prevalence, we did not manage to include patients suffering from postprandial hypotension in our study of the effects of gastric distension (chapter 2), and only two patients with postprandial hypotension were included in our study of the effects of intraduodenal glucose infusion (chapter 3). The reason for this is that most of the subjects diagnosed with postprandial hypotension met one or more of the study exclusion criteria. These

patients were often very old and frail and suffered from extensive comorbidity and were on many different medications. Most of the patients that could be included did not succeed in completing the demanding protocols. On the basis of data collected from the two subjects and by extrapolating our results from healthy elderly individuals, we can only conclude that the cause of postprandial hypotension is probably multifactorial, with the following factors possibly having a role: (1) an attenuated or absent gastrovascular reflex, as indicated by the responses measured in healthy elderly individuals (chapter 2), (2) a more pronounced decrease in splanchnic arterial resistance in response to food intake, which might be related to 'healthy', compliant arteries (see hypothesis in discussion of chapter 2), (3) a reduced baroreflex sensitivity, as measured in healthy elderly individuals, and (3) a reduced cardiac output, as measured in healthy elderly individuals (chapter 3). Considering these factors, postprandial hypotension is most likely to occur in patients with a strongly diminished capability to increase sympathetic outflow as a result of loss of central autonomic nerves (e.g., as occurs in Parkinson disease) or peripheral autonomic nerves (e.g., autonomic neuropathy in diabetes mellitus) and in patients with an incapacity to increase cardiac output as a result of heart failure.

Suggestions for future research:

- Studies on postprandial hypotension, including ours (chapter 5), report a very high prevalence, but the contribution of drowsiness or even falling asleep to this phenomenon is unclear. A crossover study comparing changes in postprandial blood pressure when healthy elderly, frail elderly individuals and patients with autonomic failure are prevented from becoming drowsy or falling asleep might provide an answer to this question.
- The current definition of postprandial hypotension is very limited: a blood pressure fall of 20 mmHg or more. Criteria including clinically relevant symptoms and studies of how to diagnose postprandial hypotension in a simple and practical way with an acceptable sensitivity and specificity would be helpful in the management of these patients.

5 POTENTIAL CONSEQUENCES OF DISORDERED BLOOD PRESSURE REGULATION AROUND MEALS

Failure of the mechanisms that regulate intraprandial and postprandial hemodynamic changes, as investigated in the studies described in chapters 2, 3 and 4, might lead to a decrease in postprandial blood pressure. Postprandial hypotension, defined as a fall in systolic blood pressure of 20 mmHg or more (Jansen and Lipsitz, 1995), does indeed appear to occur frequently in frail, elderly subjects with major comorbidity that are

admitted to a geriatric ward, and to a lesser degree in healthy elderly individuals (chapter 5). The relevance of this finding is limited, as most subjects only experienced minor symptoms or no symptoms at all. Also, the role of drowsiness, which might increase the fall in blood pressure (Morgan, 2008), is unclear. We did not prevent subjects from becoming drowsy because we tried to simulate a normal, physiological situation as much as possible. This means that the prevalence of clinically relevant postprandial hypotension, occurring because of failure of postprandial hemodynamic mechanisms, might have been lower if we had kept the patients alert. The studies that reported a high prevalence of postprandial hypotension also did not prevent sleepiness or drowsiness (Aronow and Ahn, 1994; Jansen et al., 1995; Vloet et al., 2005).

Although generalized hypotension, like postprandial hypotension, usually does not lead to focal brain ischemia, it might do so in patients with a compromised cerebropetal blood flow. It is not known whether food or alcohol intake could trigger ischemic stroke in patients with a compromised cerebropetal blood flow. In the elderly population, transient ischemic attacks (TIAs) and mild ischemic strokes occur often. Although much is known about chronic risk factors for stroke, such as hypertension, relatively little is known about the acute precipitants of stroke. Studying the acute precipitants, or triggers, of stroke might reveal a short-term increase in the risk of stroke, which in turn might lead to better insight into individual 'stroke prone state' and to temporally focused preventive measures (Elkind, 2007; Krakauer, 2007). The study described in chapter 6 investigated some aspects of this relatively unexplored field. The working hypothesis was that the combination of mild hypotension (postprandial hypotension) and compromised cerebral blood flow, due to vessel stenosis or occlusion, could lead to acute, focal, cerebral ischemia. Food or alcohol intake did not trigger the onset of a TIA or minor disabling ischemic stroke in patients with unilateral or bilateral stenosis (>70%) of the internal carotid artery, suggesting that the hemodynamic consequences of food or alcohol intake are not a causative factor in ischemic stroke (chapter 6). Although we did not find an association between food or alcohol and the occurrence of an acute ischemic cerebral event, our results cannot exclude the existence of this relationship. First, we did not measure whether the subjects suffered from postprandial hypotension, so we do not know whether food or alcohol intake has a role in a subpopulation of patients suffering from postprandial hypotension in addition to a compromised cerebral blood flow. Secondly, we did not investigate whether the occlusive carotid disease in our subjects did lead to a compromised cerebral blood flow with hemodynamic consequences. Thirdly, food or alcohol intake might have increased risk, but another food or alcohol associated factor, such as changes in the blood viscosity, might have had the opposite effect, leading overall to no effect.

Suggestions for future research

- As a next step to our study of the association between food or alcohol intake and focal cerebral ischemia (chapter 6), the same protocol could be used in a subgroup of patients with carotid occlusive disease with proven hemodynamic failure (e.g., those with a reduced CO_2 reactivity, as demonstrated with Doppler ultrasound measurement).

6 CLINICAL IMPLICATIONS

Our observations are likely to be of relevance to the management of patients with postprandial hypotension: measures that increase gastric distension (such as drinking water during a meal), increase cardiac output (light physical activity, pharmacological measures), splanchnic vascular resistance (octreotide, which blocks the release of vasoactive incretin hormones), or baroreflex sensitivity may prove to be effective treatments. Furthermore, in patients with obstructive carotid disease, the absence of an association between food or alcohol intake and the occurrence of an acute, focal cerebral event suggests that temporally focused preventive measures, such as not taking heavy, carbohydrate-rich meals or alcohol, are of no use.

Summary

The studies presented in this thesis focused on the autonomic nervous system mediated interactions between the gastrointestinal and cardiovascular systems in response to food intake and on potential consequences of failure of these interactions.

CHAPTER 1

This introductory chapter provides the background and aims of the studies and a short description of the methods used.

CHAPTER 2

Stretching the stomach wall in young healthy subjects causes an increase in muscle sympathetic nerve activity and in blood pressure, the 'gastrovascular reflex'. The physiological relevance of this reflex is probably to compensate for the shift in blood flow toward the splanchnic system during eating. The aim of the study described in chapter 2 was to determine the effect of aging on the 'gastrovascular reflex'. We compared healthy young individuals with healthy elderly individuals and found that during stepwise gastric distension, elderly individuals had an attenuated increase in muscle sympathetic nerve activity compared with young individuals.

CHAPTER 3

The cardiovascular response to a meal is modulated by gastric distension and by the interaction of nutrients, particularly carbohydrates, with the small intestine. In the study described in chapter 3, the gastrovascular reflex was bypassed by administering glucose by intraduodenal infusion. We tested the hypothesis that the depressor effect of glucose in the small intestine is greater in old subjects than in young subjects because the reflex increase in muscle sympathetic nerve activity is blunted by age. We determined the effect of glucose on heart rate, muscle sympathetic nerve activity, and blood pressure in healthy young and old individuals. We found that in both groups, intraduodenal glucose infusion decreased blood pressure, but the fall in systolic blood pressure was greater in the old subjects. This occurred despite similar increases in muscle sympathetic nerve activity. The old subjects showed a reduced baroreflex

sensitivity, which could partially explain the attenuated responsiveness to the increase in muscle sympathetic nerve activity.

CHAPTER 4

In the study described in this chapter, the autonomic function of patients with irritable bowel syndrome was investigated, focusing on meal-related changes. We measured the effect of a food bolus on heart rate, muscle sympathetic nerve activity, blood pressure, and preprandial- and postprandial autonomic function tests in patients with irritable bowel syndrome. We found that during food intake, the increase in blood pressure, heart rate, and muscle sympathetic nerve activity was equal in patients and controls, but that the increase in the LF/HF ratio of spectral analysis of the heart rate variability was significantly higher in the irritable bowel syndrome - group. Furthermore, patients with irritable bowel syndrome had a lower preprandial- and postprandial RRmax/RRmin ratio during deep breathing (DB-ratio). We concluded that reactivity to food intake, measured as muscle sympathetic nerve activity, is normal in patients with irritable bowel syndrome. Both the lower DB-ratio and higher LF/HF ratio during food intake in patients with irritable bowel syndrome indicate a reduced parasympathetic reactivity. These results suggest that a reduced baseline activity and responsiveness of the parasympathetic system could play a role in the pathogenesis of irritable bowel syndrome.

CHAPTER 5

Postprandial hypotension has been reported to occur not only in frail elderly individuals with autonomic failure but also in healthy elderly individuals. There is no consensus on the method to diagnose postprandial hypotension. The aims of this study were to find out whether postprandial hypotension occurs more frequently in patients admitted to a geriatric ward than in healthy elderly individuals, how often it is associated with symptoms and what the optimal interval between blood pressure measurements is in order to diagnose postprandial hypotension. We detected postprandial hypotension in 91% of the patients and in 40% of the healthy elderly individuals when blood pressure was measured at 2-minute intervals. When blood pressure was measured at 6- and 10-minute intervals, the detection of PPH was reduced by 10% and 15%, respectively. The presence of postprandial hypotension and postprandial complaints were positively associated. We concluded that postprandial hypotension is a serious problem in elderly individuals, and that the presence of post-prandial complaints is a good indicator of the existence of postprandial hypotension. Reducing the frequency of blood pressure measurements from every 2 minutes to every 6 or 10 minutes results in a failure to detect postprandial hypotension in 10% and 15% of patients, respectively.

CHAPTER 6

While classic vascular risk factors associated with an increased risk of stroke in the long-term are well recognized, little is known about specific activities that could trigger stroke. The aim of this study was to find out whether food or alcohol intake could trigger the occurrence of an ischemic cerebral event in patients with compromised cerebropetal blood flow. A case-crossover design was used to determine the associations between a transient ischemic attack (TIA) or minor disabling ischemic stroke and food or alcohol intake in the 2 hours preceding the event. There was no relationship between food or alcohol intake and the ischemic event. We concluded that food or alcohol intake does not trigger a TIA or minor disabling ischemic stroke in patients with unilateral or bilateral stenosis (>70%) of the internal carotid artery. This suggests that the hemodynamic changes subsequent to food or alcohol intake do not play a major role in the occurrence of cerebral ischemia in patients with obstructive carotid disease.

CHAPTER 7

In chapter 7, the strengths and limitations of the studies described in chapters 2 through 6 are discussed and suggestions for future studies are proposed.

Samenvatting

In dit proefschrift worden enkele door het autonome zenuwstelsel tot stand gebrachte effecten van voedselinname op de wisselwerking tussen het gastrointestinale en het cardiovasculaire systeem beschreven, evenals enkele potentiële gevolgen van het falen van deze wisselwerking.

HOOFDSTUK 1

In dit inleidende hoofdstuk wordt achtergrond informatie gegeven en worden de daaruit voortvloeiende vraagstellingen vermeld, die het uitgangspunt vormden voor dit proefschrift. Ook wordt een korte omschrijving gegeven van enkele methoden en technieken, die in de erop volgende hoofdstukken aan de orde komen.

HOOFDSTUK 2

Bij gezonde, jonge proefpersonen, leidt het rekken van de maagwand tot een toename van de muscle sympathetic nerve activity (MSNA, de efferente sympatische activiteit naar arteriolen in spieren) en de bloeddruk, de 'gastrovasculaire reflex'. Deze reflex zou in fysiologische zin een rol kunnen spelen bij het mechanisme dat zorgt voor herverdeling van bloed ten gunste van het maagdarm kanaal tijdens het eten. Het doel van hoofdstuk 2 was om vast te stellen wat het effect van veroudering op de 'gastrovasculaire reflex' is. We vergeleken gezonde jongere met gezonde oudere proefpersonen en vonden dat tijdens het stapsgewijs rekken van de maagwand, de toename van MSNA bij ouderen significant minder sterk was dan bij de jongeren. Wij concludeerden dat de 'gastrovasculaire reflex' minder krachtig wordt met het toenemen van de leeftijd.

HOOFDSTUK 3

De cardiovasculaire reactie op een maaltijd wordt beïnvloed door het rekken van de maagwand en door de interactie van nutriënten, voornamelijk glucose, met de dunne darm. In hoofdstuk 3 wordt het in gang zetten van de gastrovasculaire reflex ontweken door de nutriënt glucose intraduodenaal toe te dienen. Hierdoor kon het effect van glucose worden getest onafhankelijk van de gastrovasculaire reflex. Wij

hebben nagegaan of na intraduodenale toediening van glucose, de toename van MSNA kleiner, en dientengevolge het bloeddrukverlagende effect groter is bij oudere dan bij jonge proefpersonen. Wij vonden dat intraduodenale glucose infusie zowel bij oudere als bij jonge proefpersonen leidt tot een daling van de systolische bloeddruk, waarbij de bloeddrukdaling groter was bij de ouderen, ondanks een gelijke toename van MSNA in beide groepen. De oudere proefpersonen hadden een verlaagde baroreflex gevoeligheid, wat de verminderde toename van de bloeddruk op de toename van MSNA zou kunnen verklaren.

HOOFDSTUK 4

In deze studie werden de door het autonome zenuwstelsel tot stand gebrachte reacties op voedselinname getest bij patiënten met het spastisch colon syndroom. Het doel van de studie was om het effect van voedselinname op de hartfrequentie, MSNA en bloeddruk te meten en daarnaast gestandaardiseerde autonome functietesten te verrichten in de pre- en postprandiale fase bij deze patiënten. We vonden dat de toename van MSNA en bloeddruk tijdens voedselinname bij patiënten met het spastisch colon syndroom en gezonden identiek was, maar de toename van de LF/HF ratio van de hartslag (een maat voor het evenwicht tussen sympathicus en parasympathicus) significant hoger was bij de patiëntgroep. Tevens hadden de patiënten een significant lagere RRmax/RRmin ratio van de hartslag tijdens diep zuchten (een maat voor parasympathische activiteit). We concludeerden dat de reactiviteit van het sympathische systeem op eten normaal was bij patiënten met het spastisch colon syndroom, maar de lagere RRmax/RRmin ratio tijdens diep zuchten en de hogere LF/HF ratio van de hartslag tijdens eten suggereren een verminderde parasympathische reactiviteit bij deze groep patiënten. Deze resultaten suggereren een verlaagde rust activiteit en ook een verlaagde reactiviteit van het parasympathische systeem bij patiënten met het spastisch colon syndroom.

HOOFDSTUK 5

In de literatuur wordt postprandiale hypotensie niet alleen beschreven bij fragiele, oudere patiënten met te kort schieten van het autonome zenuwstelsel, maar ook bij gezonde ouderen. Er bestaat geen consensus over de methode om postprandiale hypotensie te diagnosticeren. Het doel van dit hoofdstuk was om uit te vinden of postprandiale hypotensie vaker voorkomt bij patiënten die zijn opgenomen op een afdeling geriatrie dan bij gezonde oudere individuen en hoe vaak dit samenging met het optreden van symptomen en wat het optimale interval is tussen bloeddrukmetingen om PPH te diagnosticeren. Wij vonden postprandiale hypotensie bij 91% van de opgenomen patiënten en 40% van de gezonde ouderen als bloeddruk

gemeten werd met tussentijden van 2 minuten. Als bloeddruk met tussentijden van 6 of 10 minuten werd gemeten, werd de sensitiviteit van de diagnostische methode resp. 10 en 15 % minder. De aanwezigheid van PPH bleek samen te gaan met symptomen, die suggestief waren voor hypotensie. Wij concludeerden dat postprandiale hypotensie een groot probleem is bij ouderen en dat de aanwezigheid van hierop wijzende klachten een goede indicator zijn voor het bestaan van postprandiale hypotensie. Als de bloeddruk minder frequent dan eens per 2 min. wordt gemeten, zal de sensitiviteit van de diagnostische methode afnemen.

HOOFDSTUK 6

De klassieke vasculaire risicofactoren die op lange termijn samenhangen met een verhoogd risico op het optreden van herseninfarcten zijn goed bekend, maar de kennis van specifieke activiteiten met een acuut risicoverhogend effect is beperkt. Het doel van deze studie was om te bepalen of voedsel of alcohol inname het optreden van acute focale hersenischemie kan provoceren bij patiënten met een bedreigde cerebrale doorbloeding.

De samenhang tussen het optreden van de acute focale hersenischemie en voedsel- of alcohol inname in de twee uur hieraan voorafgaand werd bepaald met behulp van een ‘case crossover design’. Er werd geen relatie gevonden tussen voedsel - of alcohol inname en acute focale hersenischemie. Wij concludeerden dat voedsel noch alcohol inname een acuut risicoverhogend effect heeft op het optreden van een TIA of licht/matig herseninfarct, voorkomend bij een enkel- of dubbelzijdige carotis-stenose van >70%. Dit suggereert dat hemodynamische veranderingen als gevolg van voedsel of alcohol inname hierbij geen grote rol spelen.

HOOFDSTUK 7

In hoofdstuk 7 worden de sterke en zwakke punten van de hoofdstukken 2 tot en met 6 nagegaan en worden suggesties gedaan voor toekomstige studies.

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als sumoworstelaar sta je je mannetje. Het gevreesde 'rode potloodje' waar je in het verleden menig manuscript mee hebt neergesabeld en waar mijn voorgangers, jouw eerdere promovendi, met ontzag over spraken, is mij erg meegevallen. Wellicht kwam dit doordat je bent overgegaan op een digitaal rood potloodje, gebruikt met de wat bewerkelijke 2-vinger typtechniek, of doordat ik juist blij was met iemand die in staat was mijn meestal veel te lange manuscripten in te korten zonder inhoudelijk iets in te leveren. Ik denk dat laatste. Veel dank voor je uitstekende begeleiding.

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LIST OF ABBREVIATIONS

ABM	Area of sympathetic bursts per minute
ABR	area under the curve of muscle sympathetic bursts per 100 heart beats
ABR	Area of sympathetic bursts per 100 heart beats
ANOVA	analysis of variance
ANS	autonomic nervous system
BMI	body mass index
BP	blood pressure
BPM	beats per minute
CO	cardiac output
CPT	cold pressor test
CVLM	caudal ventrolateral medulla
DB	deep breathing
DBP	diastolic blood pressure
EC-IC bypass	extracranial to intracranial bypass
ECG	electrocardiogram
HF power	spectral power in high range of heart rate
HR	heart rate
HRV	heart rate variability
IBI	Interbeat interval; interval between R peaks in ECG
IBS	irritable bowel syndrome
IDGI	intraduodenal glucose infusion
ILC	intermediolateral collum
LF power	spectral power in low frequency range of heart rate
MAP	mean arterial pressure
MDP	minimal distension pressure; minimal pressure inside intragastric bag to overcome the intra-abdominal pressure
MSNA	Muscle Sympathetic Nerve Activity
MWU test	Mann-Whitney U test
NBM	Number of sympathetic bursts per minute
NBR	Number of sympathetic bursts per 100 heart beats
NTS	nucleus tractus solitarii
PPH	postprandial hypotension
RVLM	rostral ventrolateral medulla
S.E.M.	standard error of the mean
SBP	systolic blood pressure
SD	standard deviation
SV	stroke volume
TIA	transient ischemic attack
TMPD	transmucosal potential difference
TPR	total peripheral resistance
VAS	visual analogue scale

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 21 december 1972 te Assendelft. Na de middelbare school (VWO, Sint Michaël college in Zaandam) begon hij in 1991 aan de studie geneeskunde aan de Universiteit van Amsterdam (UvA).

Tijdens zijn geneeskunde studie deed hij een wetenschappelijke stage van 4 maanden in het Academisch Medisch Centrum (AMC) in Amsterdam, bij de onderzoeksgroep 'replication strategies of human retroviruses'. Tijdens deze periode voerde hij onder leiding van K. Verhoef en B. Berkhout moleculair biologisch werk uit met als doel de structuur en functie van het HIV-1 Tat eiwit te bepalen. Het doctoraal examen geneeskunde werd behaald in 1996.

Voor aanvang van de co-schappen volgde hij een extracurriculair co-schap in het 'Guifree Guinlee hospital' in Rio de Janeiro, Brazilië. Hij volgde ook een keuze co-schap neurologie in het 'Groote Schuur hospital' in Kaapstad, Zuid-Afrika (prof. dr. R. Eastman) en keuze co-schappen op de afdelingen neuro-radiodiagnostiek (dr. F.J. Hulsmans) en klinische neurofysiologie (dr. J.H.T.M. Koelman) van het AMC in Amsterdam.

In 1999 behaalde hij zijn artsexamen (cum laude) en werd hij achtereenvolgens assistent niet-in-opleiding op de afdeling neurologie van het AMC (prof. dr. J. Stam) en de afdeling neurologie van het Sint Lucas Andreas ziekenhuis (dr. J.A.L. Vanneste).

Van 2001 tot 2004 werkte hij op de afdeling klinische neurofysiologie van het Academisch Medisch Centrum Utrecht (UMC Utrecht, prof. dr. A.C. van Huffelen en dr. P. Liam Oey) als arts onderzoeker en voerde hij het onderzoek uit dat uiteindelijk leidde tot dit proefschrift.

Van 2004 tot heden is hij in opleiding tot neuroloog (AIOS) op de afdeling neurologie van het Sint Lucas Andreas ziekenhuis in Amsterdam (dr. J.A.L. Vanneste, dr. H.C. Weinstein en dr. D.M. Laman).

De auteur is getrouwd met Renate de Jeu en is vader van Juna.

LIST OF PUBLICATIONS

1 Vogels, R.L., **Van Orshoven, N.P.**, Koning-Tijssen, M.A., Wouda, E.J. 'Stiff-person'-syndrome. Nederlands Tijdschrift voor Geneeskunde 2003; 147(25):1228-1232.

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3 **Van Orshoven, N.P.**, Andriesse, G.I., Van Schelven, L.J., Smout, A.J., Akkermans, L.M.A., Oey, P.L. Subtle involvement of the parasympathetic nervous system in patients with irritable bowel syndrome. Clinical Autonomic Research 2006; 16(1):33-39.

4 **Van Orshoven, N.P.**, Van Schelven, L.J., Akkermans, L.M.A., Jansen, P.A.F., Horowitz, M., Feinle-Bisset, C., van Huffelen, A.C., Oey, P.L. The effect of intraduodenal glucose on muscle sympathetic nerve activity in healthy young and older subjects. Clinical Autonomic Research 2008; 18(1):28-35.

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