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Vagale Stimulation über das äußere Ohr

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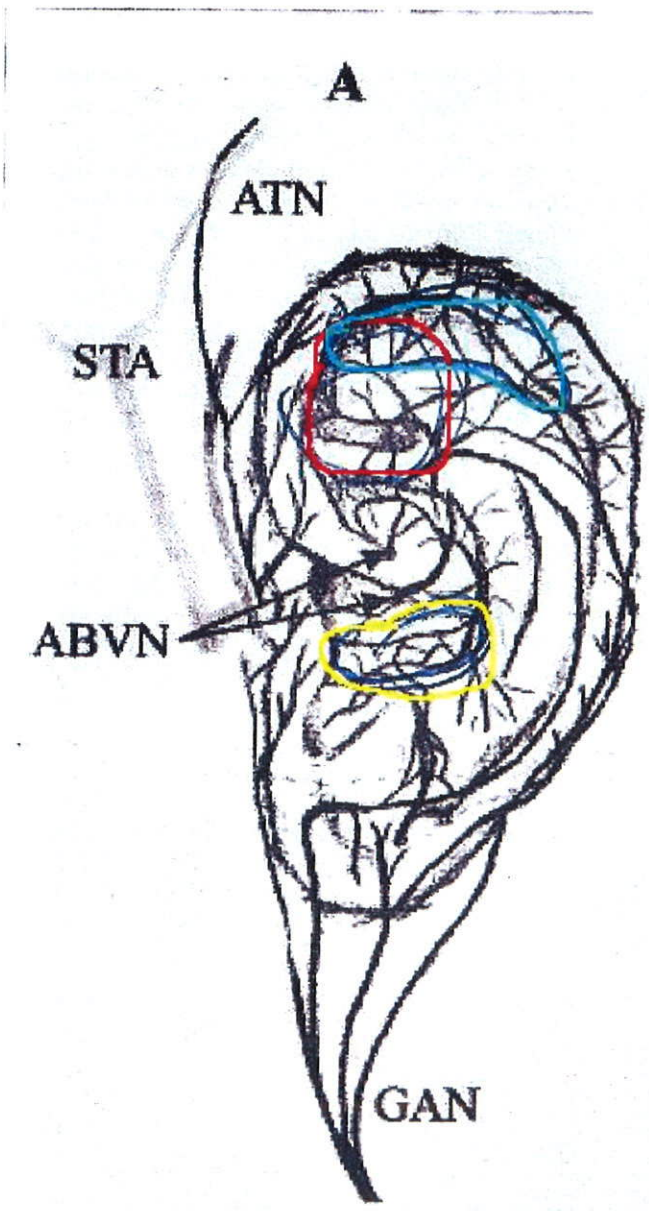
Darstellung des Nervus Vagus am äußeren Ohr

Fig. 2. **A.** Lateral surface of the external ear with corresponding scheme. ABVN = auricular branch of vagus nerve; GAN = great auricular nerve; ATN = auriculotemporal nerve; STA = superficial temporal artery. **B.** Medial surface of the external ear with corresponding scheme. ABVN = auricular branch of vagus nerve; LON = lesser occipital nerve; V = vessels.



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The Nerve Supply of the Human Auricle

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Knowledge of the innervation of the outer ear is crucial for surgery in this region. The aim of this study was to describe the system of the auricular nerve supply. On 14 ears of seven cadavers the complete course of the nerve supply was exposed and categorized. A heterogeneous distribution of two cranial branchial nerves and two somatic cervical nerves was found. At the lateral as well as the medial surface the great auricular nerve prevails. No region with triple innervation was found. Clin. Anat. 15:35–37, 2002. © 2002 Wiley-Liss, Inc.

Key words: innervation; external ear; cadaveric study; variation

INTRODUCTION

A detailed knowledge on vascularization and innervation of the outer ear is crucial for reconstructive and plastic surgery in this region. Moreover, the innervation of the auricle is the theoretical basis of different reflex therapies (e.g., ear acupuncture). However, data on the innervation as provided by scientific publications are scarce, incomplete, and inconsistent. The aim of this study is to describe the system of the auricular nerve supply.

MATERIALS AND METHODS

On 14 ears of seven cadavers the complete course of nerve supply was exposed under magnifying glasses. Each branch was defined by identifying its origin. The bodies (both sexes, age between 68 and 84 years) donated to the Institute of Anatomy had been embalmed with a mixture of formaldehyde, chloral hydrate, and sorbitum solution. Ramifications were coated with a water-soluble dye and photographically documented. The results were transferred to a scheme of the external ear and classified.

RESULTS

A heterogeneous distribution of cranial branchial nerves and somatic cervical nerves was found.

At the lateral surface the GAN (great auricular nerve) prevails. In 73% of cases the ABVN (auricular branch of vagus nerve) and in 18% the GAN was found on the antihelix solely, and 9% showed a double innervation. The lobule and the antitragus were supplied by the GAN in all cases. The tragus was innervated by GAN in 45% solely, in 9% by the ATN (auriculotemporal nerve), and in all other cases by both of them. The tail of helix and the scapha were always supplied by the GAN, the spine of helix in 91% by the ATN (9% GAN). The ATN was found in 80% at the crus helicis; in 20% the ABVN branched on this part. In 9% the ABVN provided ramification for the crura antihelices (91% GAN), in 45% of the specimen for the cavity of conchae, and in 100% for the cymba conchae. In 55% two nerves were found on the cavity of conchae (GAN and ABVN). No region with triple innervation was found. For an overview see Table 1 and Figure 1A.

At the medial surface of the auricle the LON (lesser occipital nerve) participated in 55% of the innervation of the upper third (in 37% solely). The GAN participated in 63% (in 27% solely); in 36% double innervation was found. The supply of the middle third was provided in 64% by the GAN (18% solely), in 73% by the ABVN (27% solely), and in 18% by the LON (in no case solely). Double innervation was seen in 55% of the middle third. At the lower third, in 91% of the cases GAN was found (73% solely), and in 27% the ABVN (9% solely). No region with triple innervation was found at the medial surface of the auricle as

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TABLE 1. Overview of the Innervation Pattern of the Lateral Surface of the Auricle

	ABVN	GAN	ATN
Crus of helix	20%		80%
Spine of helix		9%	91%
Tail of helix		100%	
Scapha		100%	
Crura of anthelix	9%	91%	
Antihelix	73%	9%	18%
Antitragus		100%	
Tragus	45%	46%	9%
Cymba conchae	100%		
Cavity of concha	45%	55%	
Lobule of auricle		100%	

ABVN = auricle branch of the vagus nerve; GAN = great auricular nerve; ATN = auriculotemporal nerve.

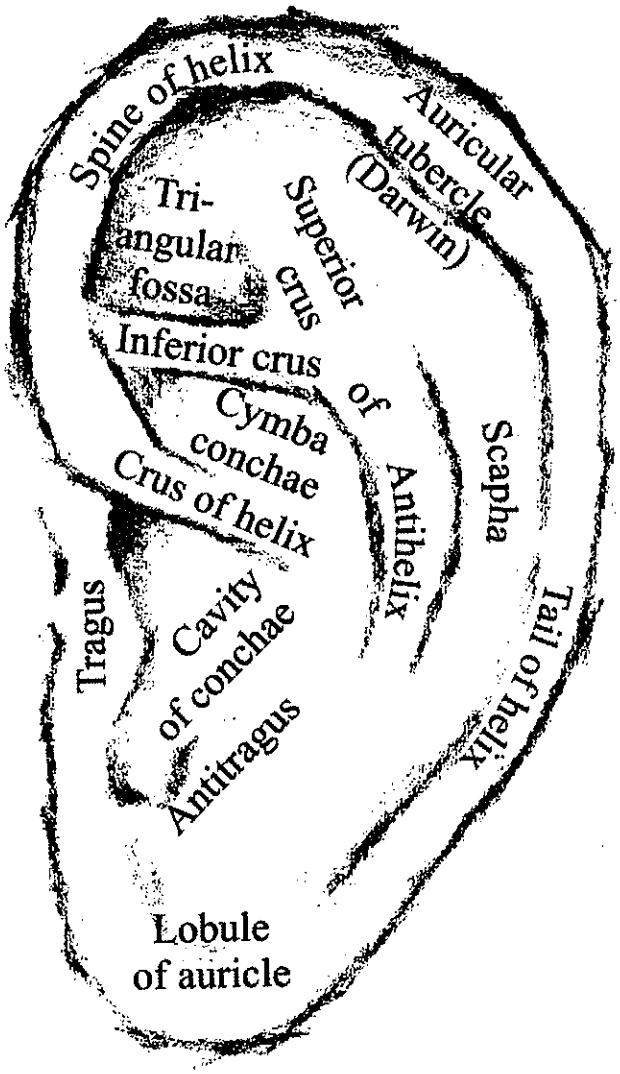

Fig. 1. Scheme of left auricle, lateral aspect.

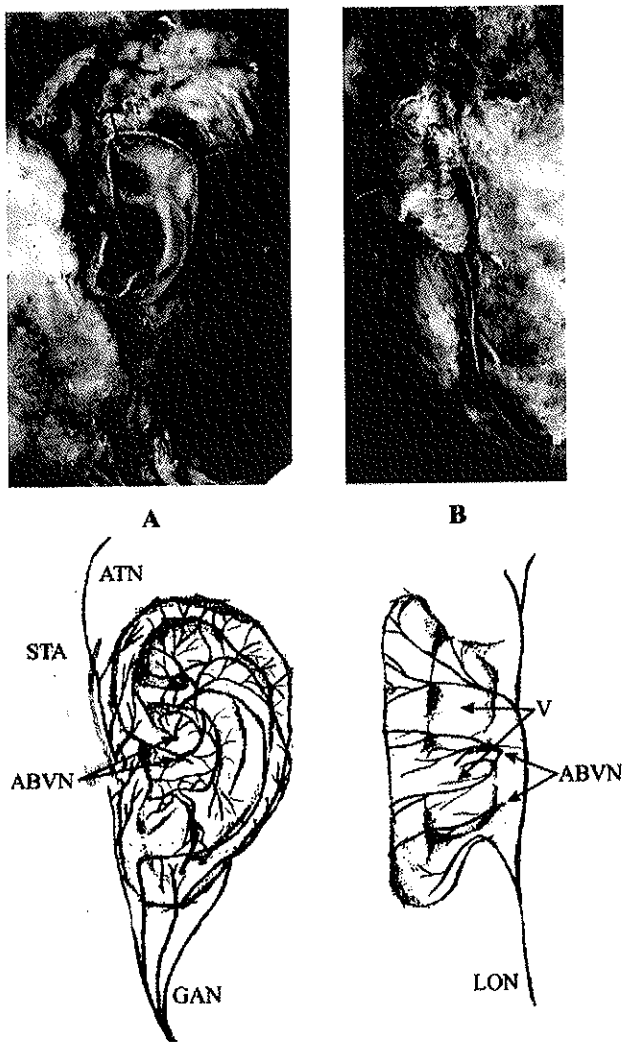

Fig. 2. **A.** Lateral surface of the external ear with corresponding scheme. ABVN = auricular branch of vagus nerve; GAN = great auricular nerve; ATN = auriculotemporal nerve; STA = superficial temporal artery. **B.** Medial surface of the external ear with corresponding scheme. ABVN = auricular branch of vagus nerve; LON = lesser occipital nerve; V = vessels.

well. For details on double innervation see Figures 1B and 2.

DISCUSSION

The external ear appears only in mammals. The density of nerve fibers in the human auricle compared to other regions of the head seems rather high. In addition, four different nerves are distributed to the external ear. They are partly branchiogenic and somatogenic. Concerning the sensory innervation, there is a gap in the origin between the first and third branchiogenic nerves on the upper side and on the

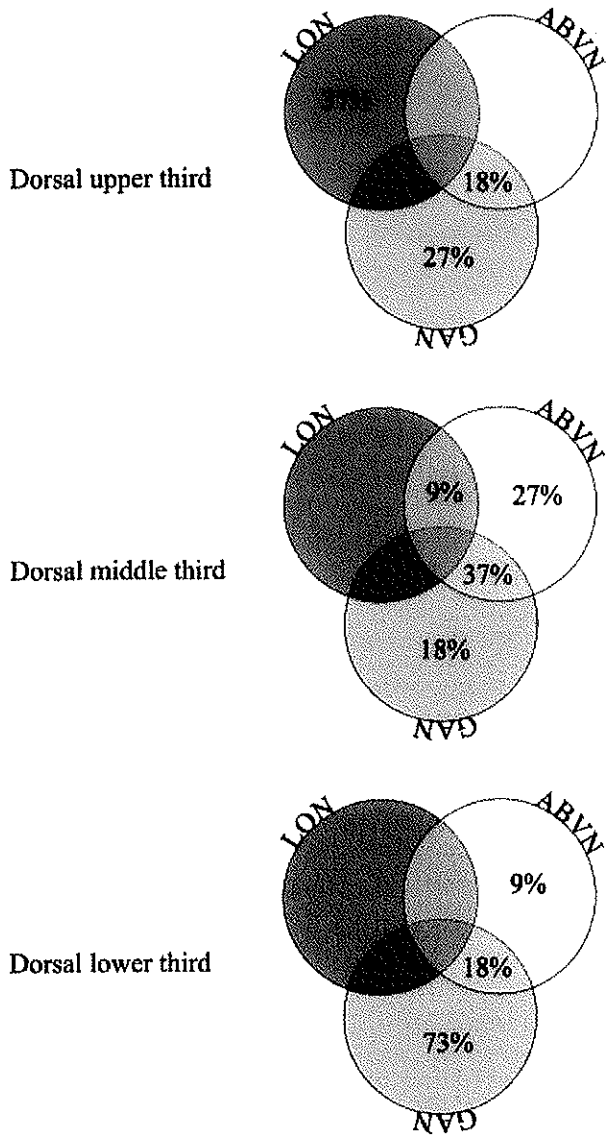

Fig. 3. Scheme of the distribution patterns of the medial surface of the auricle. ABVN = auricular branch of vagus nerve; GAN = great auricular nerve; LON = lesser occipital nerve.

third to fourth spinal nerves from the lower side. No overlapping of the branchial branches was noted, whereas the somatogenic nerves showed double innervation with both origins of the nerves. The overlapping was seen on the lateral site only within the middle third (tragus, inferior concha, and antihelix), whereas the medial surface revealed overlapping in all parts.

Other studies imply that the sensory innervation is provided by the cranial and cervical nerves (Satomi and Takahashi, 1991). Labeling of the central projections shows a remarkable ipsilateral distribution (Nomura and Mizuno, 1984). However, the respective studies have been performed mainly on cat auricles, and no suggestions were made on the function of this extensive innervation. Regulation of temperature might be a possible explanation, control of ear formation could be another. Nonetheless, to our knowledge, there are no related studies available.

Depending on the technique, complications of otoplasty and auricular reconstruction are quite common. Cutaneous problems, residual pain, hypesthesia, sensitivity to cold or touch, and delayed wound healing are mentioned in scientific publications (Calder and Naasan, 1994; Caouette-Laberge et al., 2000; Weerda and Siegert, 1994). Most of the surgical techniques do not consider the innervation pattern (Heppt and Trautmann, 1999). However, disturbances of the nerve supply of the vessels and therefore of the tissue nutrition may be responsible for the considerable postoperative problems.

REFERENCES

Calder JC, Naasan A. 1994. Morbidity of otoplasty: a review of 562 consecutive cases. Br J Plast Surg 47:170–174.

Caouette-Laberge L, Guay N, Bortoluzzi P, Belleville C. 2000. Otoplasty: anterior scoring technique and results in 500 cases. Plast Reconstr Surg 105:504–515.

Heppt W, Trautmann Y. 1999. Otoplastic techniques for the correction of protruding ears. HNO 47:688–694.

Nomura S, Mizuno N. 1984. Central distribution of primary afferent fibers in the Arnold's nerve (the auricular branch of the vagus nerve): a transganglionic HRP study in the cat. Brain Res 292:199–205.

Satomi H, Takahashi K. 1991. Distribution of the cells of primary afferent fibers to the cat auricle in relation to the innervated region. Anat Anz 173:107–112.

Weerda H, Siegert R. 1994. Complications after otoplasty and their treatment. Laryngorhinootologie 73:394–399.

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ADAPTIVE AURICULAR ELECTRICAL STIMULATION CONTROLLED BY VITAL BIOSIGNALS

Transition from Fixed to Adaptive and Synchronized Electrical Stimulation Controlled by Heart Rate Variability and Blood Perfusion

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Keywords: Electrical stimulation, heart rate variability, physiological sensors, adaptive stimulation, ear.

Abstract: The auricular electrical punctual stimulation is usually applied for pain relief. The common application involves fixed stimulation parameters, which makes the simulation insensitive to prevailing pain or stress level and may lead to a disadvantageous over-stimulation. In order to address this issue, the given position paper presents an experimental background leading to a conceptual design of an adaptive and synchronized stimulation technique. Here parameters of the heart rate variability are used as stimulation biofeedback, while the stimulating signal is synchronized with cardiac or respiratory activity to boost stimulation effects.

1 INTRODUCTION

The auricular electrical punctual stimulation (P-Stim) is an electrical nerve stimulation technique, newly introduced by Dr. Szeles (Szeles, 2001a). The P-Stim is usually applied for acute and chronic pain relief. A reduction of pain perception and pain-relieving medications is attained (Szeles, 2001b; Sator-Katzenschlager, 2006; Likar, 2007), even with an induction of anaesthesia state (Litscher, 2007). Furthermore, reduction of body mass index (BMI) in obese patients (Szeles, 2001b), increase of blood flow velocity and oxygenation (Szeles, 2004) were reported during the P-Stim application. The advantages of the electrical stimulation over conventional (manual) acupuncture with respect to pain relief, well-being and sleep quality were documented in (Sator-Katzenschlager, 2004) for extended periods of time up to 3 months.

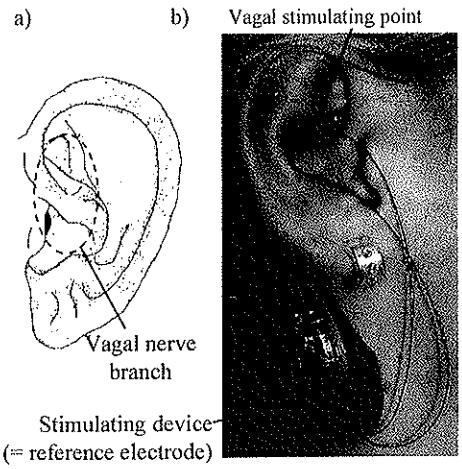

Figure 1: a) Ear with indicated approximate auricular branch of vagus nerve according to (Peuker, 2002; Gao, 2008). b) Electrical punctual stimulation of the auricular vagus nerve (P-Stim).

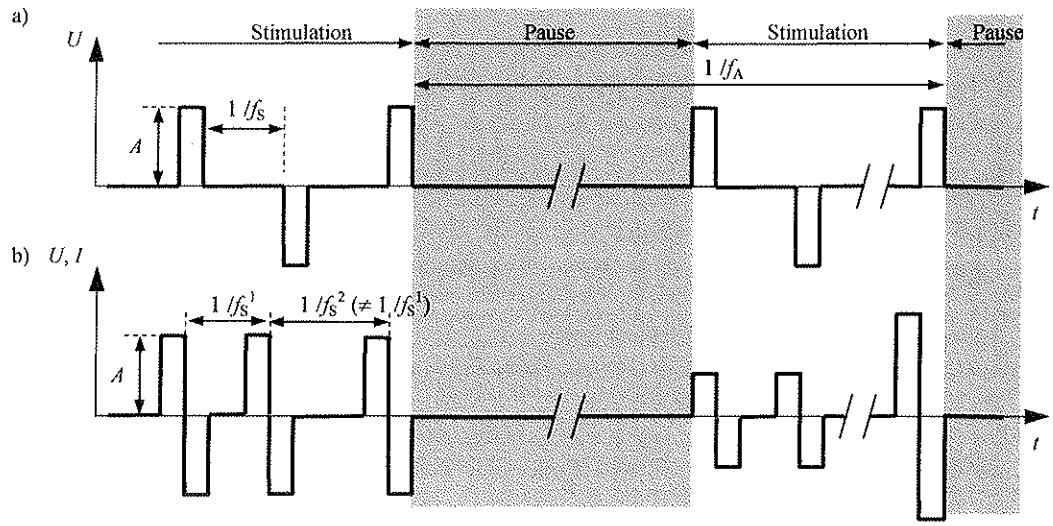

Figure 2: Stimulation waveforms of a fixed (a) and (b) adaptive electrical punctual stimulation.

The particular beneficial effects of the P-Stim are still under discussion, whereas a number of the following mechanisms seem to be involved. The electrical stimulation of the afferent nerve receptors may influence gate mechanisms in the central nervous system (CNS), preventing pain-related action impulses from reaching the CNS and avoiding the person's perception of pain. Furthermore, an indirect stimulation of pain receptors and activation of inhibitory pain control systems may be involved, as well as a stimulated release of neurotransmitters, e.g., endorphins and other endogenous opioids.

Though the efficiency of the P-Stim was subjectively proved in many cases and the P-Stim is already in clinical use, only recently some objective and statistical evidence was established on the stimulation effects. Given that an auricular branch of vagal nerve (Fig. 1a) is electrically stimulated by the P-Stim device (Fig. 1b), effects on the heart rate variability (HRV) were assessed in the time and spectral domain (Kaniusas, 2008; Gbaoui, 2008a) and in the state space (Gbaoui, 2008b) by our group. In addition, blood perfusion (BP) changes during stimulation were investigated (Kaniusas, 2008). In the latter studies optical plethysmography (OPG) served as biofeedback to derive the HRV and BP.

Here the suitability of the HRV and BP analysis is given by the fact that the stimulated afferent vagal nerve goes to the nucleus solitarius in the CNS, whereas the sinus node of the heart is controlled by the efferent vagus nerve from the nucleus ambiguous in the CNS. The node initiates heart contractions with particular rate dynamic and ejection strength, thus the HRV and BP being the appropriate parameters to register the stimulation effects.

The given position paper is intended to introduce a novel technology for an adaptive and synchronous P-Stim controlled by the HRV and BP. As a starting point, technical data and new experimental results concerning parasympathetic/sympathetic power in the HRV from the standard P-Stim are presented, which yield a substantial basis and arguments for the introduction of the adaptive stimulation.

2 ESTABLISHED STIMULATION

2.1 Methodology

The P-Stim was applied in supine position of three healthy volunteers: two men aged 41/29 with BMI 25/23 kg/m^2 and one female aged 19 with BMI of 20 kg/m^2. A precise positioning of the needle in the vicinity of the vagal nerve (Fig. 1) was facilitated by local conductivity measurements, for the local conductivity increases in the region of the nerve and its supporting blood vessels.

As demonstrated in Fig. 2a, the voltage U of the electrical stimulation comprises monophasic impulses with changing polarity, stimulation (=repetition) rate f_S of 1 Hz, amplitude A of 4 V and impulse duration of about 1 ms.

The duration of the recordings was about 15 min before, during, and after the stimulation, respectively. At least two recordings were performed per volunteer with a time-lag in-between of more

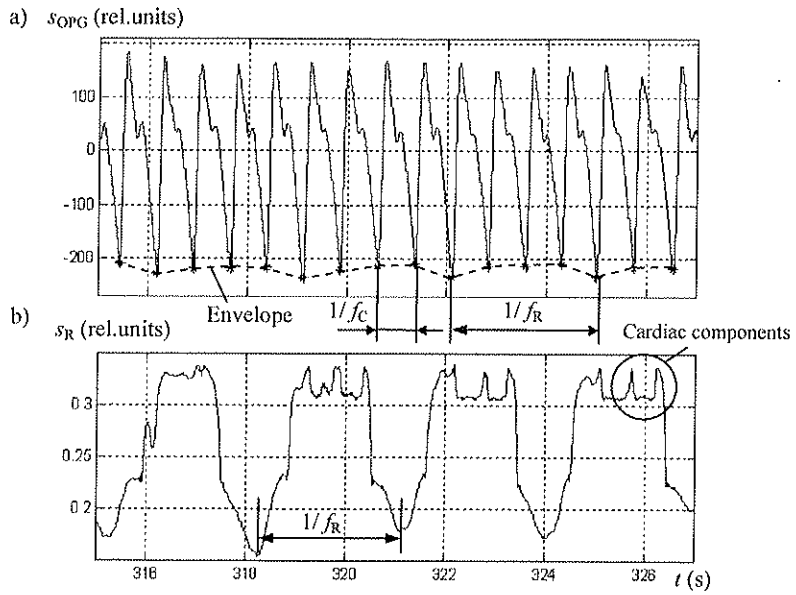

Figure 3: a) Optical plethysmography signal s_{OPG} with an estimated cardiac rate f_C from indicated systolic onset points (*) and an estimated respiratory rate f_R from the envelope. b) The corresponding respiration signal s_R from the chest skin curvature sensor.

than 10 days. It should be noted that the needles for stimulation were inserted about 5 min before the recording to avoid needle's positioning effects, i.e., to avoid temporal effects of manual acupuncture.

In parallel, the OPG signal s_{OPG} from the finger was assessed as biofeedback. Here the relatively high sampling rate of 2 kHz is needed for an accurate HRV analysis (Guidelines, 1996). A typical course of s_{OPG} is depicted in Fig. 3a.

The instantaneous heart rate f_C for the HRV analysis was estimated from s_{OPG}, as demonstrated in Fig. 3a, with artefacts and noisy segments being manually removed. The prominent minima in s_{OPG}, which correspond to the onset of the systole or blood ejection, were detected as fiducial points for the calculation of the instantaneous f_C.

The investigation of the resulting f_C sequence in the spectral domain comprised power in the established frequency ranges (Guidelines, 1996): low frequency range 0.04-0.15 Hz corresponding to sympathetic power P_{SYM} and high frequency range 0.15-0.4 Hz corresponding to parasympathetic power P_{PAR}. Both P_{SYM} and P_{PAR} were estimated for sequence windows of 300 s with 50 % overlap. It should be noted that there are controversial indications that P_{PAR} is also present in the low frequency range.

The BP is given by the course of s_{OPG} (Fig. 3a). In particular, the amplitude deflection of s_{OPG} within a single heart cycle corresponds approximately to both amount of blood ejected (=left ventricular stroke volume) and vesicular compliance.

The respiration reference s_R (Fig. 3b) was established by a skin curvature sensor on the chest, as described in (Pfützner, 2006; Kaniusas, 2004).

2.2 Results

2.2.1 Heart Rate Variability

Fig. 4b and Fig. 5b demonstrate a temporal increase of P_{PAR} during stimulation, which temporal activation is given in Fig. 4a and Fig. 5a. The relative increase of P_{PAR} among volunteers was about 20 %, which was observed in all sessions but one, probably because of a relatively high initial value of P_{PAR}. A temporal dip of P_{PAR} was often observed during the stimulation.

No unique tendencies were registered in the behaviour of P_{SYM}, as demonstrated in Fig. 4c and Fig. 5c. However, stress relaxation effects could be observed in some cases even in healthy volunteers. In Fig. 4b,c and Fig. 5b,c dashed ellipses mark the corresponding time intervals, where P_{PAR} increases and P_{SYM} concurrently decreases. In general, such changes of P_{PAR} and P_{SYM} tend to indicate ongoing restorative effects.

The stimulation effects on P_{PAR} were discussed in a wider context in (Kaniusas, 2008; Gbaoui, 2008a), considering additionally parameters in the

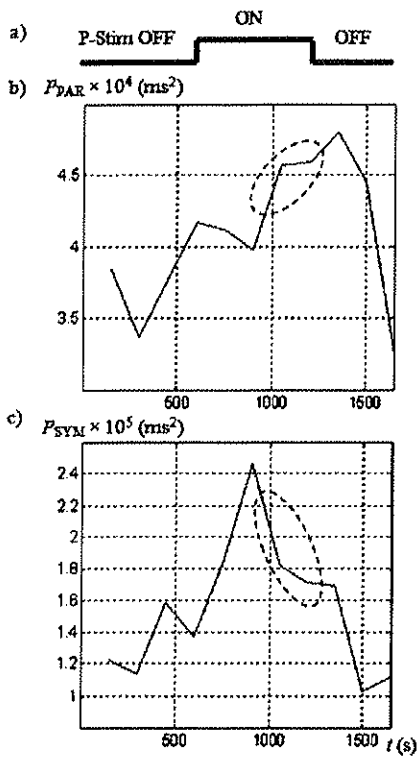

Figure 4: Effects on heart rate variability in the female subject. a) Temporal activation of the electrical stimulation (P-Stim OFF or P-Stim ON). b) The corresponding parasympathetic power P_{PAR}. c) The corresponding sympathetic power P_{SYM}.

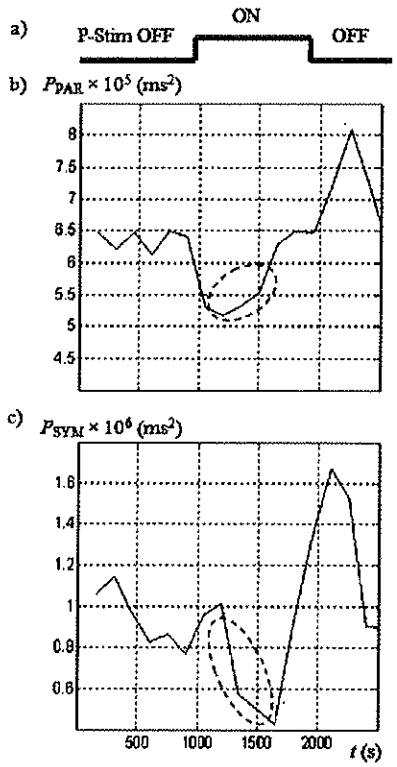

Figure 5: Effects on heart rate variability in a male subject. a) Temporal activation of the electrical stimulation (P-Stim OFF or P-Stim ON). b) The corresponding parasympathetic power P_{PAR}. c) The corresponding sympathetic power P_{SYM}.

time domain and state space. Aforementioned tendencies of P_{PAR} were also found in (Haker, 2000), even during non-electrical auricular stimulation by acupuncture needle.

In contrast to P_{PAR}, none of the mentioned studies indicate clear tendencies of P_{SYM}. This is likely to be attributed to the study enrolment of only healthy unstressed pain-free individuals in resting state, where potential changes or improvements of P_{SYM} are strongly restricted.

2.2.2 Blood Perfusion

The BP is given by the course of s_{OPG}, as show in Fig. 3a. It is important to observe that not only the instantaneous cardiac activity but also the respiration can be derived from s_{OPG}.

In particular, the systolic onset points, as marked by asterisks in Figure 3a, give a useful reference to heart excitation. These points are delayed by about 200 ms from the actual excitation of the heart ventricles (= R peaks in electrocardiography (ECG)) with the delay being nearly constant.

The respiratory cycle can be derived from s_{OPG}, as indicated by the envelope in Fig. 3a. Here the amplitude modulation of s_{OPG} results from the respiratory induced modulation of the left ventricular stroke volume which temporally increases during expiration. The simultaneously recorded respiration reference s_R (Fig. 3b) proves the respiratory related modulation of the s_{OPG} deflection.

3 PROPOSED STIMULATION

3.1 Rationale

Since the spectral HRV parameters are specifically influenced by the standard P-Stim application and the instant cardio-respiratory data can be derived from the BP, as shown above, a novel adaptive and synchronized P-Stim could be established.

A targeted control of the stimulation waveform (compare Fig. 2) is highly reasonable for avoiding over-stimulation and realising stimulation on-

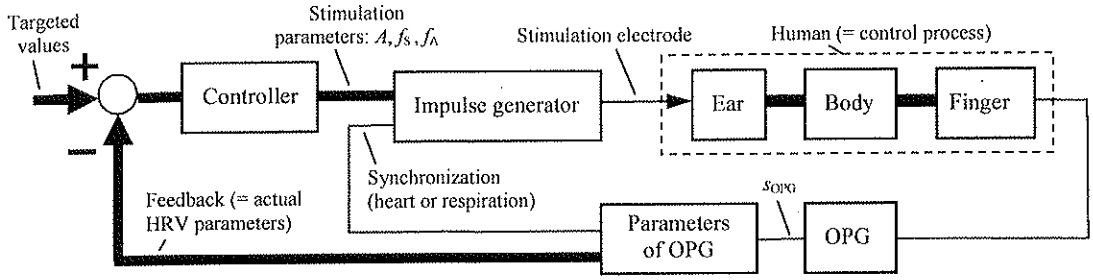

Figure 6: Control loop of the adaptive auricular stimulation with OPG as the optical plethysmography.

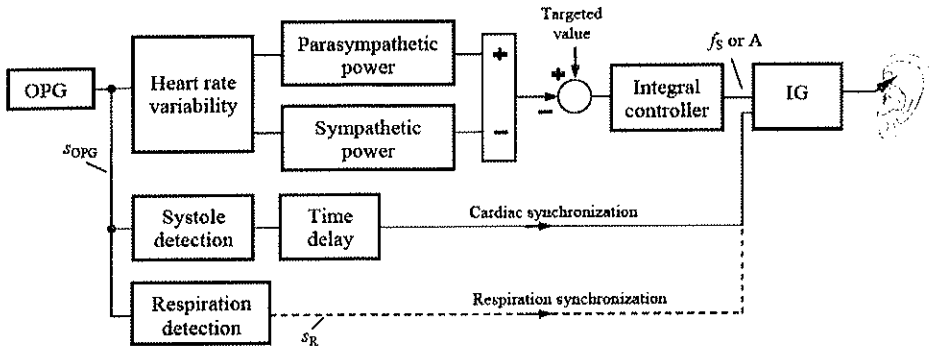

Figure 7: Establishment of biofeedback for controlling and synchronization purposes with IG as the impulse generator.

demand controlled by HRV parameters. In other words, if pain perception is already reduced, as detected by e.g., reduced stress and diminished P_{SYM}, then A, f_S (Fig. 2b) could be reduced as well. In addition, efficient energy use in the stimulation would be facilitated.

The synchronization of the stimulation waveform with the cardio-respiratory activity would allow a constructive interference of the stimulated pain-relieving effects and the residual body attempts. In particular, the cardiac synchronization would allow a timely activation of the gate mechanisms in the CNS or a timely and indirect stimulation of receptors (e.g., blood pressure), regulating vital body functions. The respiratory synchronization would help to interfere with body phenomena like respiratory sinus arrhythmia, yielding a forced increase of P_{PAR} in the expiration phase.

3.2 Realization

The proposed set-up is shown in Fig. 6. The input parameters A, f_S and the activation rate f_A of the impulse generator are adaptively adjusted according to the HRV parameters via a control loop. The cardio-respiratory synchronization signal for the impulse generator is also derived from s_{OPG}.

In particular, Fig. 7 suggests the difference P_{PAR} - P_{SYM} as a possible realization of the stimulation feedback, while the targeted value could be the pain intensity to be reduced. That is, the higher P_{PAR} and the lower P_{SYM} get in the course of the stimulation, the more strongly the pain has already been reduced. Similar behaviour of P_{PAR} and P_{SYM} during stimulation was already observed in Fig. 4 and Fig. 5. Obviously the ratio P_{PAR}/P_{SYM} could be used instead of the difference.

According to Fig. 7 an adaptive control of A and f_S is established, assuming that these parameters are directly interrelated with the stimulation strength. In an analogous way, a composition of bursts by controlling of f_A could be attained (compare Fig. 2). Here a proportional-integral controller or integral controller could be applied, for the human (Fig. 6) can be roughly approximated as a proportional control process with a single time constant (compare Fig. 4b). The time delay in Fig. 7 may be needed for synchronizing the stimulation pulses with a particular time instant in the heart cycle.

Fig. 2b exemplifies a possible adaptive controlling of the stimulation curve, while more efficient biphasic impulses are used (compare Fig. 2a). In addition, constant current stimulation would be preferred over voltage application, for the skin impedance is relatively low with electrode needles inserted and thus the risk of local tissue damage

though locally increased current density is low.

4 DISCUSSION

It is worth to note that the HRV is usually derived from the ECG (Guidelines, 1996). However, the P-Stim induced very strong artefacts in the ECG since the stimulation and the ECG have the same electrical origin. In contrast, the OPG with optical origin serves as a reliable biosignal, being independent of the P-Stim activation. However, the OPG conveys mechanical information on the systole-diastole cycle rather than electrical on the heart excitation (= origin for the HRV). In addition, the OPG exhibits relatively slow changes if compared to the ECG, for the pulse waves are much more inert than electrical heart excitation. The use of the OPG may have reduced an effective time resolution of f_C.

The time delay of about 200 ms between the systolic onset in the OPG and the R peak in the ECG depends on the speed of the heart excitation and mechanical vessel properties. Nevertheless, the delay can be assumed to be constant, if the respiratory induced blood pressure changes and thus arterial distension and stiffness changes can be neglected.

Lastly, the limitations of the presented experimental results should be mentioned. The observed effects, especially concerning P_{SYM}, are restricted by the fact that all volunteers were young pain-free healthy persons. Furthermore, the stimulation duration was relatively short: 15 min versus 4 hours (with 4 hours pause in-between) over at least seven days, as clinically applied and subjectively verified for being effective. The initial state of the volunteers, as their possible excitation at the beginning of the recording, and their mental activity changes during the investigation - both influencing the HRV - may have limited the range of potential changes or improvements of HRV parameters during the stimulation.

However, the provided experimental background leads to a comprehensible design of an adaptive and synchronized stimulation technique. This would allow a pain sensitive adjustment of the stimulating parameters avoiding over-stimulation and comforting the patients.

REFERENCES

Gao, X.Y., Zhang S.P., Zhu, B., Zhang H.Q., 2008. Investigation of specificity of auricular acupuncture points in regulation of autonomic function in anesthetized rats. *Autonomic Neuroscience: Basic and Clinical*, 138, 50-56.

Gbaoui, L., Kaniusas, E., Szeles, J.C., Materna, T., Varoneckas, G., 2008a. Heart rate variability during electrostimulation on ear: spectral domain versus state space (in german). *Proceedings of XXII Symposium on Measuring Technique*, 230-238.

Gbaoui, L., Kaniusas, E., Szeles, J.C., Materna, T., Varoneckas, G., 2008b. Effects of the auricular electrical stimulation on heart rate variability assessed in phase space: pilot study. Accepted for *IEEE Sensors 2008* in Lecce, Italy.

Haker, E., Egekvist, H., Bjerring, P., 2000. Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. *Journal of the Autonomic Nervous System*, 79, 52-59.

Kaniusas, E., Gbaoui, L., Szeles, J.C., Materna, T., Varoneckas, G., 2008. Validation of auricular electrostimulation by heart rate variability and blood perfusion: possibilities and restrictions. *Proceedings of Microelectronics Conference 2008*, 180-184.

Kaniusas, E., Pfützner, H. et al., 2004. Magnetoelastic skin curvature sensor for biomedical applications. *Proceedings of IEEE Sensors*, 1484-1487.

Likar, R., Jabarzadeh, H. et al., 2007. Auricular electrical punctual stimulation (P-STIM): a randomized, double-blind, controlled pilot study in laparoscopic nephrectomy (in german). *Schmerz*, 21(2), 154-159.

Litscher, G., Wang, L., Gaischek, I., 2007. Electroencephalographic responses to laserneedle and punctual stimulation quantified by bispectral (BIS) monitoring: a pilot study to evaluate methods and instrumentation. *Internet Journal of Laserneedle Medicine*, 1(1).

Peuker, E.T., Filler, T.J., 2002. The nerve supply of the human auricle. *Clinical Anatomy*, 15, 35-37.

Pfützner, H., Kaniusas, E. et al., 2006. Magnetostrictive bilayers for multi-functional sensor families. *Sensors and Actuators A: Physical*, 129, 154-158.

Sator-Katzenschlager, S.M., Scharbert, G. et al., 2004. The short and long term benefit in chronic low back pain through adjuvant electrical versus manual auricular acupuncture. *Anesthesia & Analgesia*, 98, 1359-1364.

Sator-Katzenschlager, S.M., Wölfler, M.M. et al., 2006. Auricular electro-acupuncture as an additional perioperative analgesic method during oocyte aspiration in IVF treatment. *Human reproduction*, 21(8), 2114-2120.

Szeles, J.C., 2001a. Therapy appliance for punctual stimulation. *Patents* WO 01/35897, US7336993.

Szeles, J.C., Hoda, M.R., Polterauer, P, 2001b. Application of electrostimulation acupuncture (P-Stim) in clinical practice. *Schmerznachrichten from Austrian Pain Association*, 1.

Szeles, J.C., Litscher, G., 2004. Objectivation of cerebral effects with a new continuous electrical auricular stimulation technique for pain management. *Neurological Research*, 26(7), 797-800.

Guidelines, 1996. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation*, 93(5), 1043-1065.

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Cytokines and the immunomodulatory function of the vagus nerve

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Cytokine synthesis and release is an essential component of the innate immune system, but inappropriate, excessive production results in a generalized systemic inflammatory response which damages distant organs. Recent research has identified an immunomodulatory function of the vagus nerve whereby activation of the efferent arm results in regulation of cytokine production. Termed the 'cholinergic anti-inflammatory pathway', this neuro-immune communication provides the host with a fast, discrete, and localized means of controlling the immune response and preventing excessive inflammation. Stimulation of the vagus nerve attenuates cytokine production and improves survival in experimental sepsis, haemorrhagic shock, ischaemia–reperfusion injury, and other conditions of cytokine excess and research is now underway in developing new and novel therapeutics aimed at stimulating the vagus nerve either directly or targeting specific components of the pathway.

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Survival depends on the immune system's ability to defend the body against attack from invading pathogens and injury. However, the extent of such a response is of critical importance; deficient responses may result in secondary infections from immunosuppression and excessive responses can be more harmful than the original insult.[76 81] The immune response can be divided into two different types of response (Table 1). The innate (non-specific) response is rapid, does not distinguish between foreign substances, and does not improve with repeated exposure to the same antigen. It uses natural killer cells, cells that release inflammatory mediators, and phagocytic cells such as neutrophils, monocytes, and macrophages. In contrast, the acquired (specific) immune response is induced by exposure, is specific for each antigen encountered, and improves with repeated exposure to that antigen. It uses antigen-specific B and T lymphocytes to recognize the antigen and initiate responses to it.[23 51 72]

Cytokines

A fundamental feature of the innate immune system is the production and release of cytokines. Cytokines are low-molecular-weight proteins which after binding to specific receptors affect immune cell differentiation, proliferation, and activity. They are not stored, but are newly synthesized and released during activation of the inflammatory cascade. They are multi-functional but in essence direct the inflammatory response to sites of infection and injury and enhance wound healing. Broadly speaking, cytokines can be divided into those with predominantly pro-inflammatory actions and those with anti-inflammatory actions. Pro-inflammatory cytokines include tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and IL-8. Anti-inflammatory cytokines include IL-1 receptor antagonist, IL-10, IL-13, and TNF-binding proteins 1 and 2.[11 14 17 49 51]

Tumour necrosis factor-α

During infection or after injury, TNF-α is a primary and potent mediator of inflammation. It is synthesized mainly by monocytes/macrophages and T cells and has a half-life within the circulation of <20 min. TNF-α has a critical role in coordinating the inflammatory response and activating mediators distally in the cytokine cascade. It is also a potent inducer of other pro-inflammatory cytokines. TNF-α elicits considerable metabolic and haemodynamic changes and is capable of causing end-organ dysfunction. Other functions include activation of coagulation, muscle catabolism, and cachexia.[11 49]

Interleukin-1

There are two forms of IL-1 (IL-α and IL-β) which recognize the same cell surface receptors and therefore share various biological activities. IL-1 is synthesized by monocytes and leucocytes and other cell types and has a half-life of ~6 min. Both IL-α and IL-β evoke metabolic and haemodynamic changes similar to those of TNF-α,

Table 1 Components of the immune system

Innate	Acquired
Primary defence mechanism	Second line of defence
Immediate response time	Time to maximal response delayed
Non-specific response—activated by the presence of differing foreign substances	Specific response—antigen- and pathogen-specific response induced by previous exposure
No immunological memory	Immunological memory allows improved response to repeated exposure to a specific antigen
Uses natural killer cells, mast cells, eosinophils, basophils, and phagocytic cells	Uses antigen-specific B and T lymphocytes

activate production of other cytokines, and attenuate pain perception by promoting the release of β-endorphins.[11 25 49 71]

Interleukin-6

IL-6 synthesis is induced by TNF-α and IL-1 from many cell types including lymphocytes, fibroblasts, and monocytes. It can be detected for up to 10 days after injury and it has a number of biological effects including neutrophil activation, induction of the hepatic acute-phase response, and activation of coagulation.[11 49]

Interleukin 8

IL-8 is a chemokine produced by a variety of cells including monocytes, leucocytes, and endothelial cells in response to numerous stimuli including TNF-α, IL-1, and endotoxin. IL-8 does not produce the haemodynamic decompensation seen with TNF-α and IL-1 but activates and attracts neutrophils to sites of inflammation.[11 49]

Interleukin 10

With predominantly counter-inflammatory actions, IL-10 profoundly suppresses activation of macrophages and inhibits their ability to produce and secrete pro-inflammatory cytokines. *In vitro*, IL-10 can inhibit production of TNF-α, IL-1, IL-6, and IL-8 and thus has been described as a cytokine synthesis inhibitory factor.[11 72]

Interleukin 13

Similar to IL-10, IL-13 is regarded as a potent anti-inflammatory cytokine that inhibits macrophage activation with resultant suppression of pro-inflammatory cytokine production and expression. In addition, IL-13 can inhibit nitric oxide (NO) production and can stimulate neutrophils to produce IL-1 receptor antagonist.[49 72]

Effects of pro-inflammatory cytokines

In health and in disease, there is a sensitive balance between pro- and anti-inflammatory cytokines—this has been termed the cytokine balance. Pro-inflammatory cytokines operate close to their site of release, but if the inflammatory response escapes local control, it elicits a generalized systemic response. To keep this to a minimum, TNF-α and IL-1 have short half-lives, a system of membrane reservoirs hold the cytokines close to the site of release and the anti-inflammatory cytokines modify the host inflammatory response.[51]

Cardiac

TNF-α can produce immediate and delayed negative inotropic effects on myocardial tissue and has been shown to cause left ventricular dysfunction.[56 57] TNF-α, IL-1β, and IL-6 have also been implicated in causing myocardial depression by direct actions on the myocytes[14 41] (Table 2).

Vascular

Pro-inflammatory cytokines have significant effects on vascular tone, mainly mediated through the NO pathway, with resultant vasodilatation. TNF-α and IL-1β have both been observed to increase NO production, and failure to respond to vasoconstrictors after prolonged exposure to these cytokines has been reported.[14]

Respiratory

Inflammatory lung injury occurs when activated neutrophils and macrophages migrate from the pulmonary vasculature into the alveolar and interstitial spaces. Macrophages secrete TNF-α, IL-1, and IL-8 which in turn stimulate further cytokine production by lung epithelial and mesenchymal cells.[14 50] IL-8 has been shown to have a pathogenic role in the establishment of acute respiratory

Table 2 Effects of pro-inflammatory cytokines on organ systems

Cardiac	Negative inotropic effect on myocardium
	Left ventricular dysfunction
Vascular	Vasodilatation
Respiratory	Acute lung injury
	Pathogenic role in ARDS
Renal	Glomerular injury
	Tubular cell damage
Hepatic	Increased synthesis of acute phase proteins
Coagulation	Modulation of extrinsic and Protein C pathways
	Inhibition of fibrinolysis
	Role in development of DIC and thrombosis

distress syndrome (ARDS) by causing neutrophil-mediated lung injury.[53]

Renal

In animal studies, TNF-α has been shown to induce glomerular injury in kidneys without pre-existing renal disease. In response to TNF-α and IL-1, glomerular cells produce oxygen free radicals, complement, arachidonic acid derivatives, and NO which further escalates local inflammation with resultant additional glomerular and tubular cell damage.[14]

Hepatic

Predominantly IL-6, but also TNF-α and IL-1β stimulate the liver to alter its synthetic function and increase the synthesis of acute phase proteins such as serum amyloid A, α2-macroglobulins, and C-reactive protein (CRP). The substrates for this increase in production are provided by cytokine-mediated skeletal muscle breakdown, hence the alternative name for TNF-α; cachectin.[14]

Coagulation

Cytokines are not directly involved in the coagulation pathways, but TNF-α and IL-1 have been shown to modulate the extrinsic pathway of coagulation and the protein C pathway. Evidence suggests important roles for these cytokines in disseminated intravascular coagulation (DIC) and thrombosis.[27] IL-1 has also been shown to inhibit fibrinolysis.[72]

Elderly

The consequences of ageing on the immune system are thought to contribute considerably to morbidity and mortality in the elderly.[63] TNF-α and IL-6 concentrations are raised in the elderly and studies have shown that, in response to surgical trauma, the elderly have a magnified and late inflammatory cytokine response.[45]

Conditions of cytokine excess

Inappropriate synthesis of cytokines occurs with excessive or persistent activation of macrophages and neutrophils. If this escapes local control, cytokines enter the systemic circulation resulting in widespread activation of inflammatory cascades and the systemic inflammatory response syndrome (SIRS) (Fig. 1). This evokes further release of inflammatory cytokines resulting in a downward spiral of organ dysfunction and ultimately multiple organ dysfunction syndrome (MODS). Mortality from MODS is high. In patients with ARDS alone, mortality is around 50% and with each additional organ failing, this risk increases in a multiplicative fashion.

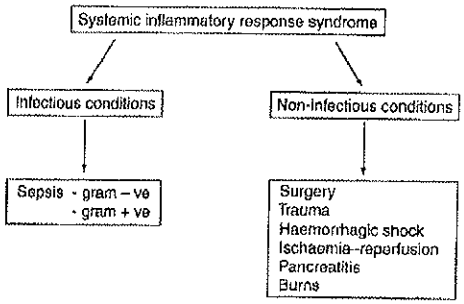

Fig 1 Conditions of cytokine excess. SIRS can be classified as resulting from either infectious or non-infectious conditions.

TNF-α, IL-1β, IL-6, and IL-8 have been strongly implicated as mediators of sepsis and studies of sepsis have shown elevated circulating levels of these cytokines.[11 14 16 21] Furthermore, raised levels of pro-inflammatory cytokines generally appear to correlate with severity of illness and outcome[11 14 21 83] with IL-6 most closely associated with mortality.[11 16] Increased plasma concentrations of pro-inflammatory cytokines have been demonstrated after major surgery[40 51 68] and the magnitude of the cytokine-mediated inflammatory response appears to be related to the extent of the surgical insult.[68] In particular, high plasma concentrations of IL-6 in response to major surgery appear to be associated with postoperative mortality.[51] After severe trauma, serum levels of TNF-α, IL-6, and IL-8 are significantly elevated and there appears to be a close relationship between the extent of pro-inflammatory cytokine release and the severity of injury and hospital mortality.[15 35 39] Haemorrhage results in a markedly increased production of pro-inflammatory cytokines in the lungs and is associated with the onset of ARDS with TNF-α playing a central role in the pathogenesis of acute lung injury after haemorrhage, even after adequate resuscitation.[1–4 12 48 73]

Anti-inflammatory mechanisms

To avoid an inappropriate, excessive inflammatory response, there exist a number of anti-inflammatory mechanisms to prevent inflammatory mediators entering the circulation. Anti-inflammatory cytokines such as IL-10, TNF-binding protein, IL-receptor antagonist (IL-1ra), and transforming growth factor-β (TGF-β), which are produced through a normal immune response, can inhibit the release of TNF-α and other pro-inflammatory cytokines.[20 76] TNF-binding protein interferes with the binding of TNF to its receptor and thus inhibiting its actions.[46] 'Stress' hormones such as glucocorticoids, epinephrine, norepinephrine, and α-melanocyte-stimulating hormone inhibit cytokine production.[76] In addition to suppressing TNF-α production, β-adrenergic receptor stimulation has also

been shown to up-regulate IL-10 production thereby enhancing the anti-inflammatory action.[79] Other local effectors such as prostaglandin E2, acute phase proteins, heat-shock proteins, spermine, and feutin all have additional roles in limiting the immune response. Impairment or loss of any of these endogenous anti-inflammatory pathways can turn a normally self-limiting response into an excessive and potentially damaging one.[76]

Neural regulation of the immune response

Blalock suggested that the immune system also functions as a sensory organ. A sixth sense that detects bacteria, viruses, and other potentially deleterious cells that we cannot otherwise see, hear, taste, touch, or smell. This information would then be relayed to the central nervous system (CNS) to bring about a favourable physiological response.[12]

Surprisingly, recent research has revealed an autonomic neural pathway that monitors and adjusts the inflammatory response. Termed 'the inflammatory reflex', this pathway has both immunosensing and immunosuppressing functions. Compared with the humoral anti-inflammatory mechanisms which are slow, diffuse, and dependent on concentration gradients, the inflammatory reflex pathway is fast, localized, and integrated[5 76] (Fig. 2).

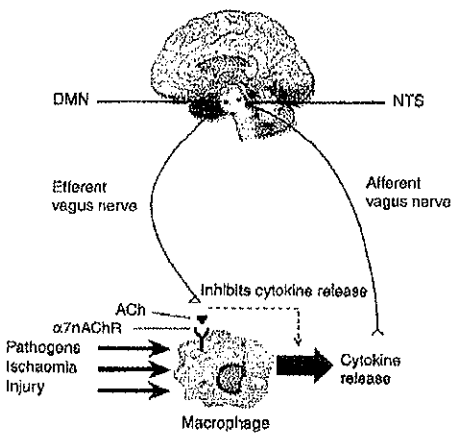

Fig 2 The inflammatory reflex. Pathogens, ischaemia, and other forms of injury result in cytokine release from immune cells which is sensed by the afferent vagus nerve. The information is relayed to the nucleus tractus solitarius (NTS) and subsequently to the dorsal motor nucleus (DMN) resulting in activation of the efferent vagus nerve—termed the cholinergic anti-inflammatory pathway. Efferent vagus nerve activity inhibits cytokine production via the α7 nicotinic acetylcholine receptor (α7nAchR) located on macrophages and other immune cells.

Derived from Latin meaning 'wandering', the vagus nerve originates in the medulla oblongata of the brainstem and innervates visceral organs including those of the reticuloendothelial system such as the liver and spleen and, as such, is well positioned to relay information between the immune system and the CNS. It contains both sensory (afferent) and motor (efferent) components. Sensory fibres relay information to the brain that results in the development of fever and other signs of illness and the efferent arm of the vagus nerve is traditionally associated with physiological responses such as bradycardia, bronchoconstriction, increased gastric motility, and myosis. It appears that the vagus nerve also has an important immunomodulatory role in the detection and inhibition of the inflammatory response.[20 29 76 77]

Immunosensing function of the afferent vagus nerve

There is evidence that pro-inflammatory cytokines send signals to the brain,[87] and several mechanisms may be involved. There are a number of humoral mechanisms of immune-to-brain communication. Studies have shown that TNF-α, IL-1α, IL-1β, and IL-6 are transported across the blood–brain barrier (BBB) by saturable transport systems.[6 33 65 87] Cytokines can also enter the brain through circumventricular organs such as the area postrema where the BBB is absent and here they have been shown to trigger the production and release of prostaglandin E2 (PGE2) which can subsequently activate the hypothalamic–pitultary–adrenal axis.[20 87] Pro-inflammatory cytokines, in particular the interleukins, can also bind to cerebral vascular endothelium and alter endothelial cell metabolism resulting in the release of neuroactive substances on the brain side of the BBB.

However, all the above mechanisms require the pro-inflammatory cytokines to be present in the circulation in order to reach the brain. But, it has been shown that inflammatory responses can occur in the absence of detectable blood cytokine levels.[87]

Sensory vagal afferent fibres, however, can detect low levels of cytokines and other inflammatory mediators and, via the ascending fibres, inform the brain of inflammation in the periphery.[76 87] Indeed, local tissue concentrations of cytokines may be sufficient to activate sensory vagal afferents without ever requiring an elevation in systemic cytokine levels. Evidence demonstrates that subdiaphragmatic vagotomy disrupts the development of fever in animals exposed to low intra-abdominal doses of IL-1 or endotoxin because the brain simply does not receive the message that there is inflammation present.[76 86] It has also been demonstrated that vagal sensory neurones express IL-1 and PGE2 receptor mRNA and IL-1 binding sites on glomus cells located within vagus nerve-associated paraganglia.[26 30]

Central integration of the afferent and efferent components of the inflammatory reflex occurs in the medullary nucleus tractus solitaries (NTS) which is the major site for

termination of vagal afferents. NTS neurones then project to the dorsal motor nucleus of the vagus (DMN) where most of the efferent vagal preganglionic fibres originate.[61,62]

Immunosuppressing function of the efferent vagus nerve

The efferent arm of the inflammatory reflex has been termed the 'cholinergic anti-inflammatory pathway' because acetylcholine (ACh) is the principle vagus nerve neurotransmitter.[13] Macrophages and other cytokine-producing cells express ACh receptors and when exposed to ACh are inactivated. Studies indicate that ACh post-transcriptionally suppresses TNF synthesis and inhibits the release of IL-1β, IL-6, and IL-8 without preventing the release of the anti-inflammatory cytokine IL-10.[13]

ACh receptors are divided into two classes: muscarinic and nicotinic. Nicotinic receptors can be further subdivided into α-bungarotoxin sensitive (α1, α7, and α9 subunit-containing) and non-sensitive.

Both muscarinic and nicotinic receptors are distributed in the CNS and periphery but with different synaptic locations and varied functions in cholinergic transmission.[61] Initial studies indicated that ACh acts on the α-bungarotoxin-sensitive nicotinic receptors on human macrophages, but the specific receptor subunit was until recently unknown.[13] Further research has, however, identified this receptor subunit as the α7 nicotinic ACh receptor (α7nAChR). Electrical stimulation of the vagus nerve was shown to inhibit macrophage TNF synthesis in wild-type mice but not in α7-deficient mice, thus demonstrating the α7nAChR as an essential peripheral component of the cholinergic anti-inflammatory pathway.[85]

Although muscarinic ACh receptors are also expressed on macrophages and other cytokine-producing cells, block of peripheral muscarinic receptors does not stop vagus nerve anti-inflammatory signalling.[59] It therefore appears that peripheral muscarinic receptors are not required for the cytokine modulating actions of the cholinergic anti-inflammatory pathway. Recently, a role for central muscarinic receptors in inhibiting systemic inflammation has been described. Central cholinergic activation by muscarinic receptor ligands was shown to significantly inhibit systemic TNF in endotoxaemic rats and also activate efferent vagus nerve activity.[59] Bacteria and endotoxin localize to macrophages primarily in the spleen and liver and studies have shown the spleen to be the major source of systemic TNF production during endotoxaemia. Recent research indicates that the cholinergic anti-inflammatory pathway is functionally hard-wired to the spleen via the common coeliac branch of the vagus nerve. This α7nAChR-dependent vagus nerve pathway to the spleen can inhibit pro-inflammatory cytokine production during lethal endotoxaemia and polymicrobial sepsis.[38]

Vagus nerve activity can also trigger the systemic humoral anti-inflammatory response by transmitting information to the medullary reticular formation, the hypothalamus, and the locus ceruleus resulting in the release of adrenocorticotrophin (ACTH) from the anterior pituitary.[76]

Evidence for immunomodulation by the vagus nerve

The most compelling evidence for the role of the vagus nerve in modulating the inflammatory response comes from studies of rats challenged with lethal doses of i.v. endotoxin where electrical stimulation of the efferent vagus nerve significantly decreased serum TNF-α, hepatic TNF-α synthesis, and considerably attenuated the development of endotoxic shock. In contrast, vagotomy led to substantially increased serum and hepatic TNF-α levels and accelerated the development of shock.[13] Research into the role of the cholinergic anti-inflammatory pathway during infection rather than sterile inflammation was determined by inducing abdominal sepsis in mice with an intraperitoneal injection of live *Escherichia coli*. In this study, cervical vagotomy resulted in enhanced cytokine release, an increase in inflammatory cells recruited to the peritoneum, and the development of extensive liver damage. Activation of the peripheral component of the cholinergic anti-inflammatory pathway by nicotine resulted in decreased cytokine production, reduced influx of inflammatory cells, and the absence of liver damage.[81] Other studies indicate that vagus nerve stimulation (VNS) in rat models of endotoxaemia inhibits activation of coagulation and fibrinolysis,[82] and during inflammation, cholinergic stimulation suppresses leucocyte recruitment and endothelial cell activation.[66]

The role of the vagus nerve in modulating inflammation during haemorrhagic shock has also been studied. VNS of rats during lethal haemorrhagic shock significantly increased survival rate, protected against the development of hypotension, and reduced TNF-α plasma concentrations.[31] ACTH activates the cholinergic anti-inflammatory pathway and has been shown to improve cardiovascular and respiratory functions, reverse shock condition, and increase survival rate in further animal studies of haemorrhagic shock.[32]

VNS in animals subjected to ischaemia/reperfusion by transient aortic occlusion significantly attenuated TNF-α synthesis and the development of shock.[8] In an experimental model of ischaemic heart disease, stimulation of the vagus nerve during myocardial ischaemia/reperfusion resulted in a reduction in free radical levels and significantly reduced the incidence of severe arrhythmias and lethality.[52]

The cholinergic anti-inflammatory pathway also has a role in specifically inhibiting localized inflammation. In an experimental model of local inflammation, VNS attenuated the inflammatory response and suppressed the development of paw swelling in murine arthritis.[76]

Potential therapeutic implications of vagus nerve immunomodulation

With the discovery of the cholinergic anti-inflammatory pathway comes the possibility of developing therapies aimed at controlling the inflammatory response. Suppression of pro-inflammatory cytokine production may be possible by either altering vagus nerve activity or targeting specific components of the pathway.

Vagus nerve stimulation

Direct electrical stimulation of the vagus nerve attenuates TNF-α production during experimental models of endotoxaemia, haemorrhagic shock, and other conditions of cytokine excess.[8 13 31 38 85] The voltage and frequency of the stimulation required to activate the cholinergic anti-inflammatory pathway is below the threshold required to activate cardiac vagal fibres and so no significant effects on heart rate have been observed.[20 37] VNS has been a United States FDA approved treatment for refractory epilepsy for more than 10 yr[70] and is undergoing clinical trials for resistant depression.[18] VNS requires implantation of a small, pacemaker-like device and has been shown to be safe, effective, and well tolerated.[69] The VNS device is inserted through an incision in the neck with placement of wires on the vagus nerve and implantation of an electrical pack inferior to the clavicle.[70] Complications from the implantation procedure itself are uncommon and the most commonly reported side-effects are hoarseness, voice alteration, throat or neck pain, cough, headache, and dyspnoea. These side-effects occur mainly during stimulation, can be reduced by altering the stimulation, and tend to lessen over time.[69 70] Unexpectedly, in a study of VNS in patients with resistant depression, circulating levels of both pro- and anti-inflammatory cytokines were found to be markedly raised. Furthermore, as reflected by CRP levels, the increase was unlikely to be a non-specific inflammatory reaction.[18]

Transcutaneous non-electrical VNS can attenuate serum TNF-α levels and improve survival in murine sepsis. Transcutaneous stimulation in this study was achieved in conscious mice by alternating direct pressure applied perpendicularly and directly adjacent to the border of the trachea. Critically ill patients tend to be poor surgical candidates and the ability to stimulate the vagus nerve via the trancutaneous route may have potentially important clinical implications.[37] Carotid sinus massage can be used to terminate supraventricular tachyarrhythmias through reflex activation of the efferent vagus nerve and further data are needed to assess for any immunological effects of this approach to VNS.[7]

In addition, it is possible that acupuncture techniques may be used to stimulate immunomodulatory effects through the vagus nerve. Studies have shown that acupuncture can evoke measurable increases in vagus nerve activity, so it is at least theoretically possible that acupuncture could also activate the cholinergic anti-inflammatory pathway and regulate the immune response.[34 36 54 77] Studies have also demonstrated that various forms of meditation can alter the parasympathetic component of heart rate variability and can have a positive effect on cardiac autonomic tone.[44 58 64] Similarly, relaxation therapy and biofeedback can significantly increase the parasympathetic component of heart rate variability reflecting an increase in vagus nerve activity.[19 55 67 75 80]

Pharmacological agents

There has been some development of potential therapeutic agents based on targeting the cholinergic anti-inflammatory pathway through action on central muscarinic receptors or α7nACh receptors in the periphery.

CNI-1493

It has been shown that CNI-1493, a tetravalent guanylhydrazone compound and potent anti-inflammatory agent, is a central activator of the cholinergic anti-inflammatory pathway[9] through interaction with central muscarinic receptors.[59] CNI-1493 suppresses pro-inflammatory cytokine release from monocytes and macrophages and systemic administration is effective in the treatment of experimental, animal models of endotoxaemia, and sepsis.[9 10 59] Furthermore, an intact CNS-vagus nerve pathway is required for the anti-inflammatory effects of CNI-1493 and increased efferent vagus nerve activity has been observed after its administration.[59]

Nicotine

Nicotine is a relatively non-specific α7nAChR agonist and has been shown to significantly inhibit TNF-α and other pro-inflammatory cytokines from endotoxin-stimulated human macrophages.[20] In addition, nicotine also suppresses high mobility group box 1 (HMGB1) release from human macrophages. HMGB1 protein is a late mediator of lethal systemic inflammation in sepsis and in animal models of experimental sepsis. Treatment with nicotine attenuated serum HMGB1 levels, decreased the clinical manifestations of sepsis, provided significant protection against death and improved survival in 'established' sepsis.[84] Additionally, nicotine treatment was not started until 24 h after the induction of lethal peritonitis in mice indicating that the cholinergic anti-inflammatory pathway can modulate the inflammatory response even in established sepsis.[77] Administration of nicotine also appeared to offer lasting protection as indicated by the absence of any late animal deaths.[84] However, the development of nicotine as a therapeutic intervention has its limitations due to toxicity related side-effects and pharmacological non-specificity. A study of nicotine replacement therapy in smokers admitted to a medical intensive care unit found nicotine replacement to be associated with a higher mortality rate.[47] What these findings do indicate is the potential role of α7nAChR agonists as a new class of anti-inflammatory drugs.[60]

GTS-21

Also known as DMXB or DMXBA, GTS-21 is a selective α7nAChR agonist and unlike nicotine is well tolerated with no clinically significant adverse effects in healthy male volunteers.[42] GTS-21 has been shown to suppress serum levels of both TNF-α and HMGB1 and significantly improves survival in septic mice.[60]

Ghrelin

Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHSR) and the biological effects of ghrelin are thought to be mediated through GHSR.[88] GHSR has wide central and peripheral distribution and is present in afferent neurones of the nodose ganglion, suggesting ghrelin signals are relayed to the brain via vagal afferent fibres.[22] In animal models of sepsis, ghrelin is decreased in early and late sepsis, although its receptor is markedly elevated in early sepsis. It has also been demonstrated in animals that vascular sensitivity to ghrelin stimulation is increased during the hyperdynamic phase of sepsis.[89] In a study using a rat model of sepsis, ghrelin treatment was shown to significantly decrease serum and peritoneal fluid levels of TNF-α and IL-6 with additional findings that an intact vagus nerve was essential for the inhibitory effect of ghrelin on the release of pro-inflammatory cytokines.[88]

Although activation of the cholinergic anti-inflammatory pathway to suppress inflammation may be of benefit in conditions of cytokine excess, there may also be a role in manipulation of the pathway in patients with traumatic brain injury (TBI). Infection is a prevalent and serious complication after TBI and the phenomenon post-traumatic immune paralysis is thought to be responsible for the increased infection rate seen in these patients. In the acute phase after TBI, studies have demonstrated severe impairment of the immune response and an increase in vagal activity, possibly as a result of raised intracranial pressure. These observations have led to the hypothesis that the increased risk of developing an infection after TBI results from a vagal-mediated suppression of the immune response and that intervening in the cholinergic anti-inflammatory pathway may have a potential role in benefiting these patients.[43]

Conclusion

Despite recent advances in intensive care treatment, MODS resulting from excessive and prolonged pro-inflammatory cytokine release in conditions such as sepsis, ischaemia/reperfusion, and haemorrhagic shock remains associated with a high mortality rate. Pro-inflammatory cytokines such as TNF-α and IL-1β have been identified as 'early' mediators of the inflammatory response and when neutralized using specific antibodies can prevent the development of septic shock in animal models.[76 84] Unfortunately, these strategies have not been translated into effective treatments for critically ill patients[1 4 28] and studies have shown that TNF-α antibodies are ineffective if treatment is commenced after serum TNF-α has been cleared.[78] The identification of HMGB1 as a 'late' inflammatory mediator may allow therapeutic development of agents to specifically target HMGB1 and expand the therapeutic time window.[84]

With the discovery that the vagus nerve has an immunomodulatory function comes the possibility of developing novel therapeutic agents for the treatment of inflammatory disease. Knowledge of the immunosuppressing function of the efferent vagus nerve, termed the 'cholinergic anti-inflammatory pathway', represents a new approach to targeting the regulation of pro-inflammatory cytokine production. VNS has been shown to decrease cytokine production, attenuate the development of shock, and increase survival in animal models of sepsis, haemorrhagic shock, and ischaemia/reperfusion.[8 13 31 32 38 81 88]

The vagus nerve can be stimulated directly and is already for treatment of refractory epilepsy, but it is an invasive technique and other methods such as transcutaneous stimulation have been shown to improve survival in murine sepsis. It remains to be seen if these strategies can be translated into treatment of SIRS and MODS in humans. Acupuncture, meditation, biofeedback, and other forms of alternative therapies have all been shown to increase vagus nerve activity and it will be interesting to reveal if these approaches can activate the cholinergic anti-inflammatory pathway.[19 34 36 44 54 55 58 64 67 75 80]

The vagus nerve can be stimulated chemically and the identification of specific components of the cholinergic anti-inflammatory pathway has paved the way for the development of promising new therapeutic approaches such as agents acting on central muscarinic receptors and specific α7nAChR agonists. It has been suggested that a new class of anti-inflammatory compounds could be synthesized based on the GTS-21 molecule. It is also possible that commonly used drugs such as cholinergics, anticholinergics, non-steroidal anti-inflammatory drugs, and amiodarone, all of which have an effect on vagus nerve activity, may exert previously unrecognized effects on the cholinergic anti-inflammatory pathway.[24 77 78]

The finding that the vagus nerve has an immunomodulatory function represents an exciting opportunity to develop new and novel therapeutics aimed at regulating the immune response and although it is unlikely that VNS will replace standard intensive care therapy, there may be a role as an adjunct treatment to benefit future patients.

References

1 Abraham E, Anzueto A, Gutierrez G, *et al.* Double-blind randomized controlled trial of monoclonal antibody to tumour necrosis factor in treatment of septic shock. *Lancet* 1998; **351**: 929–33

2 Abraham E, Bursten S, Shenkar R, *et al.* Phosphatidic acid signaling mediates lung cytokine expression and lung inflammatory injury after hemorrhage in mice. *J Exp Med* 1995; **181**: 569–75

3 Abraham E, Jesmok G, Tuder R, Allbee J, Chang Y. Contribution of tumor necrosis factor-alpha to pulmonary cytokine expression and lung injury after hemorrhage and resuscitation. *Crit Care Med* 1995; **23**: 1319–26

4 Abraham E, Laterre P, Garbino J, *et al.* Lenercept (p55 tumor necrosis factor receptor fusion protein) in severe sepsis and early septic shock: a randomized, double-blind, placebo-controlled, multicenter phase III trial with 1,342 patients. *Crit Care Med* 2001; **29**: 503–10

5 Andersson J. The inflammatory reflex—introduction. *J Intern Med* 2005; **257**: 112–25

6 Banks WA, Kastin AJ. Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci* 1991; **48**: 117–21

7 Bauhofer A, Torossian A. Mechanical vagus nerve stimulation—a new adjunct in sepsis prophylaxis and treatment. *Crit Care Med* 2007; **35**: 2868–9

8 Bernik TR, Friedman SG, Ochani M, *et al.* Cholinergic anti-inflammatory pathway inhibition of tumor necrosis factor during ischemia reperfusion. *J Vasc Surg* 2002; **36**: 1231–6

9 Bernik TR, Friedman SG, Ochani M, *et al.* Pharmacological stimulation of the cholinergic anti-inflammatory pathway. *J Exp Med* 2002; **195**: 781–8

10 Bianchi M, Bloom O, Raabe T, *et al.* Suppression of pro-inflammatory cytokines in monocytes by a tetravalent guanylhydrazone. *J Exp Med* 1996; **183**: 927–36

11 Blackwell TS, Christman JW. Sepsis and cytokines: current status. *Br J Anaesth* 1996; **77**: 110–17

12 Blalock JE. The immune system as a sixth sense. *J Intern Med* 2005; **257**: 126–38

13 Borovikova LV, Lyudmila V, Ivanova S, *et al.* Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000; **405**: 458–62

14 Bown MJ, Nicholson ML, Bell PRF, Sayers RD. Cytokines and inflammatory pathways in the pathogenesis of multiple organ failure following abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg* 2001; **22**: 485–95

15 Carlstedt F, Lind L, Lindahl B. Pro-inflammatory cytokines, measured in a mixed population on arrival in the emergency department, are related to mortality and severity of disease. *J Intern Med* 1997; **242**: 361–5

16 Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann Intern Med* 1993; **119**: 771–8

17 Chachkhiani I, Gurlich R, Maruna P, Frasko R, Lindner J. The postoperative stress response and its reflection in cytokine network and leptin plasma levels. *Physiol Res* 2005; **54**: 279–85

18 Corcoran C, Connor TJ, O'Keane V, Garland MR. The effects of vagus nerve stimulation on pro- and anti-inflammatory cytokines in humans: a preliminary report. *Neuroimmunomodulation* 2005; **12**: 307–9

19 Cowan MJ, Kogan H, Burr R, Hendershot S, Buchanan L. Power spectral analysis of heart rate variability after biofeedback training. *J Electrocardiol* 1990; **23**: 85–94

20 Czura CJ, Tracey KJ. Autonomic neural regulation of immunity. *J Intern Med* 2005; **257**: 156–66

21 Damas P, Canivet J, De Groote D, *et al.* Sepsis and serum cytokine concentrations. *Crit Care Med* 1997; **25**: 405–12

22 Date Y, Murakami N, Toshinai K, *et al.* The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120–8

23 Delves PJ, Roitt IM. The immune system: first of two parts. *N Engl J Med* 2000; **343**: 37–49

24 Dias Da Silva VJ, Gnecchi-Ruscone T, Lavelli B, *et al.* Opposite effects of iv amiodarone on cardiovascular vagal and sympathetic efferent activities in rats. *Am J Physiol* 2002; **283**: R543–8

25 Dinarello CA. Interleukin-1 and interleukin-1 antagonism. *Blood* 1991; **77**: 1627–52

26 Ek M, Kurosawa M, Lundeberg T, Ericsson A. Activation of vagal afferents after intravenous injection of interleukin-1β: role of endogenous prostaglandins. *J Neurosci* 1998; **18**: 9471–9

27 Esmon CT. Possible involvement of cytokines in diffuse intravascular coagulation and thrombosis. *Baillieres Clin Haem* 1994; **7**: 453–66

28 Fisher CJ, Dhainaut JA, Opal SM, *et al.* Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome: results from a randomized, double-blind, placebo-controlled trial. *J Am Med Assoc* 1994; **271**: 1836–43

29 Gallowitsch-Puerta M, Pavlov VA. Neuro-immune interactions via the cholinergic anti-inflammatory pathway. *Life Sci* 2007; **80**: 2325–9

30 Goehler LE, Relton JK, Dripps D, *et al.* Vagal paraganglia bind interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull* 1997; **43**: 357–64

31 Guarini S, Altavilla D, Cainazzo M, *et al.* Efferent vagal fibre stimulation blunts nuclear factor κB activation and protects against hypovolemic hemorrhagic shock. *Circulation* 2003; **107**: 1189–94

32 Guarini S, Cainazzo M, Giuliani D, *et al.* Adrenocorticotropin reverses hemorrhagic shock in anesthetized rats through rapid activation of a vagal anti-inflammatory pathway. *Cardiovasc Res* 2004; **63**: 357–65

33 Gutierrez EG, Banks WA, Kastin AJ. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J Neuroimmunol* 1993; **47**: 169–76

34 Haker E, Egekvist H, Bjerring P. Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. *J Auton Nerv Syst* 2000; **79**: 52–9

35 Hoch RC, Rodriguez R, Manning T, *et al.* Effects of accidental trauma on cytokine and endotoxin production. *Crit Care Med* 1993; **21**: 839–45

36 Huang ST, Chen GY, Lo HM, Lin JG, Lee YS, Kuo CD. Increase in the vagal modulation by acupuncture at neiguan point in healthy subjects. *Am J Chin Med* 2005; **1**: 157–64

37 Huston JM, Gallowitsch-Puerta M, Ochani M, *et al.* Transcutaneous vagus nerve stimulation reduces high mobility group box 1 levels and improves survival in murine sepsis. *Crit Care Med* 2007; **35**: 2762–8

38 Huston JM, Ochani M, Rosas-Ballina M, *et al.* Splenectomy inactivates the cholinergic anti-inflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 2006; **203**: 1623–8

39 Jiang JX, Tian KL, Chen HS, Zhu PF, Wang ZG. Plasma cytokines and endotoxin levels in patients with severe injury and their relationship with organ damage. *Injury* 1997; **28**: 509–13

40 Kato M, Suzuki H, Murakami M, Akama M, Matsukawa S, Hashimoto Y. Elevated plasma levels of interleukin-6, interleukin-8, and granulocyte colony-stimulating factor during and after major surgery. *J Clin Anesth* 1997; **9**: 293–8

41 Kelly RA, Smith TW. Cytokines and cardiac contractile function. *Circulation* 1997; **95**: 778–81

42 Kitagawa H, Tekenouchi T, Azuma R, *et al.* Safety, pharmacokinetics and effects of cognitive function of multiple doses of GTS-21 in healthy male volunteers. *Neuropsychopharmacology* 2003; **28**: 542–51

43 Kox M, Pompe JC, Pickkers P, Hoedemaekers CW, van Vugt AB, van der Hoeven JG. Increased vagal tone accounts for the

observed immune paralysis in patients with traumatic brain injury. *Neurology* 2008; **70**: 480–5

44 Kubota Y, Sato W, Toichi M, *et al.* Frontal midline theta rhythm is correlated with cardiac autonomic activities during performance of an attention demanding meditation procedure. *Cogn Brain Res* 2001; **11**: 281–7

45 Kudoh A, Katagai H, Takazawa T, Matsuki A. Plasma proinflammatory cytokine response to surgical stress in elderly patients. *Cytokine* 2001; **15**: 270–3

46 Lantz M, Guilberg U, Nilsson E, Olsson I. Characterization in vitro of a human tumor necrosis factor-binding protein. A soluble form of a tumor necrosis factor receptor. *J Clin Invest* 1990; **86**: 1396–402

47 Lee AH, Afessa B. The association of nicotine replacement therapy with mortality in a medical intensive care unit. *Crit Care Med* 2007; **35**: 1517–21

48 Le Tulzo Y, Shenkar R, Kaneko D, *et al.* Hemorrhage increases cytokine expression in lung mononuclear cells in mice: involvement of catecholamines in nuclear factor-kappa B regulation and cytokine expression. *J Clin Invest* 1997; **99**: 1516–24

49 Lin E, Calvano SE, Lowry SF. Inflammatory cytokines and cell response in surgery. *Surgery* 2000; **127**: 117–6

50 Martin TE. Lung cytokines and ARDS: Roger S. Mitchell lecture. *Chest* 1999; **116**: 2S–8S

51 McBride WT, Armstrong MA, McBride SJ. Immunomodulation: an important concept in modern anaesthesia. *Anaesthesia* 1996; **51**: 465–73

52 Mioni C, Bazzani C, Giuliani D, *et al.* Activation of an efferent cholinergic pathway produces strong protection against myocardial ischemia/reperfusion injury in rats. *Crit Care Med* 2005; **33**: 2621–8

53 Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. *Cytokine Growth Factor Rev* 1998; **9**: 9–23

54 Nishijo K, Mori H, Yosikawa K, Yazawa K. Decreased heart rate by acupuncture stimulation in humans via facilitation of cardiac vagal activity and suppression of cardiac sympathetic nerve. *Neurosci Lett* 1997; **227**: 165–8

55 Nolan RP, Kamath MV, Floras JS, *et al.* Heart rate variability biofeedback as a behavioral neurocardiac intervention to enhance vagal heart rate control. *Am Heart J* 2005; **149**: 1137

56 Oral H, Dorn GW, Mann DL. Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-α in the adult mammalian cardiac myocyte. *J Biol Chem* 1997; **272**: 4836–42

57 Pagani FD, Baker LS, Hsi C, Knox M, Fink MP, Visner MS. Left ventricular systolic and diastolic dysfunction after infusion of tumor necrosis factor-α in conscious dogs. *J Clin Invest* 1992; **90**: 389–98

58 Paul-Labrador M, Polk D, Dwyer JH, *et al.* Effects of a randomized controlled trial of transcendental meditation on components of the metabolic syndrome in subjects with coronary heart disease. *Arch Intern Med* 2006; **166**: 1218–24

59 Pavlov VA, Ochani M, Gallowitsch-Puerta M, *et al.* Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc Natl Acad Sci USA* 2006; **103**: 5219–23

60 Pavlov VA, Ochani M, Yang LH, *et al.* Selective α7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis. *Crit Care Med* 2007; **35**: 1139–44

61 Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. *Brain Behav Immun* 2005; **19**: 493–9

62 Pavlov VA, Tracey KJ. Controlling inflammation: the cholinergic anti-inflammatory pathway. *Biochem Soc Trans* 2006; **34**: 1037–40

63 Pawelec G, Adibzadeh M, Pohla H, Schaudt K. Immunosenescence: ageing of the immune system. *Immunol Today* 1995; **16**: 420–2

64 Peng CK, Henry IC, Mietus JE, *et al.* Heart rate dynamics during three forms of meditation. *Int J Cardiol* 2004; **95**: 19–27

65 Pluckin SR, Banks WA, Kastin AJ. Comparison of saturable transport and extracellular pathways in the passage of interleukin-1α across the blood–brain barrier. *J Neuroimmunol* 1996; **67**: 41–7

66 Saeed RW, Varma S, Peng-Nemeroff T, *et al.* Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *J Exp Med* 2005; **201**: 1113–23

67 Sakakibara M, Takeuchi S, Hayano J. Effect of relaxation training on cardiac parasympathetic tone. *Psychophysiology* 1994; **31**: 223–8

68 Salo M. Cytokines and attenuation of responses to surgery. *Acta Anaesthesiol Scand* 1996; **40**: 141–2

69 Schachter SC. Vagus nerve stimulation: where are we? *Curr Opin Neurol* 2002; **15**: 201–6

70 Schachter SC. Vagus nerve stimulation therapy summary. Five years after FDA approval. *Neurology* 2002; **59**: S15–20

71 Schafer M, Carter L, Stein C. Interleukin 1β and corticotrophin-releasing factor inhibit pain by releasing opioids from immune cells in inflamed tissue. *Proc Natl Acad Sci USA* 1994; **91**: 4219–23

72 Sheoran P, Hall GM. Cytokines in anaesthesia. *Br J Anaesth* 1997; **78**: 201–19

73 Shenkar R, Coulson WF, Abraham E. Hemorrhage and resuscitation induce alterations in cytokine expression and the development of acute lung injury. *Am J Respir Cell Mol Biol* 1994; **10**: 290–7

74 Sierpina VS, Frenkel MA. Acupuncture: a clinical review. *South Med J* 2005; **98**: 330–7

75 Teradhongkum S, Pickler RH. Relationships among heart rate variability, hypertension and relaxation techniques. *J Vasc Nurs* 2004; **22**: 78–82

76 Tracey KJ. The inflammatory reflex. *Nature* 2002; **420**: 853–9

77 Tracey KJ. Physiology and immunology of the cholinergic anti-inflammatory pathway. *J Clin Invest* 2007; **117**: 289–96

78 Tracey KJ, Fong Y, Hesse DG, *et al.* Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 1987; **330**: 662–4

79 van der Poll T, Coyle SM, Barbosa K, Braxton CC, Lowry SF. Epinephrine inhibits tumor necrosis factor-α and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest* 1996; **97**: 713–9

80 van Dixhoorn J, White A. Relaxation therapy for rehabilitation and prevention in ischaemic heart disease: a systematic review and meta-analysis. *Eur J Cardiovasc Prev Rehabil* 2005; **12**: 193–202

81 van Westerloo DJ, Giebelen IAJ, Florquin S, *et al.* The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis* 2005; **191**: 2138–48

82 van Westerloo DJ, Giebelen IAJ, Meijers JCM, *et al.* Vagus nerve stimulation inhibits activation of coagulation and fibrinolysis during endotoxemia in rats. *J Thromb Haemost* 2006; **4**: 1997–2002

83 Wakefield CH, Barclay GR, Fearon KCH, *et al.* Proinflammatory mediator activity, endogenous antagonists and the systemic inflammatory response in intra-abdominal sepsis. *Br J Surg* 1998; **85**: 818–25

84 Wang H, Liao H, Ochani M, *et al.* Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med* 2004; **10**: 1216–21

85 Wang H, Yu M, Ochani M, *et al.* Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature* 2003; **421**: 384–8

86 Watkins LR, Goehler LE, Relton JK, *et al.* Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 1995; **183**: 27–31
87 Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: a review and analysis of alternative mechanisms. *Life Sci* 1995; **57**: 1011–26
88 Wu R, Dong W, Cui X, *et al.* Ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve. *Ann Surg* 2007; **245**: 480–6
89 Wu R, Zhou M, Cui X, Simms H, Wang P. Upregulation of cardiovascular ghrelin receptor occurs in the hyperdynamic phase of sepsis. *Am J Physiol* 2004; **287**: H1296–302

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Relationship between Heart Rate Variability, Interleukin-6, and Soluble Tissue Factor in Healthy Subjects

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Abstract

Decreased heart rate variability (HRV) has been associated with an increased risk of atherosclerosis. We hypothesized that a decrease in frequency domains of resting HRV would be associated with elevated plasma levels of interleukin (IL)-6 and soluble tissue factor (sTF) both previously shown to prospectively predict atherothrombotic events in healthy subjects. Subjects were 102 healthy and unmedicated black and white middle-aged men and women. We determined IL-6 and sTF antigen in plasma and HRV measures from surface electrocardiogram data using spectral analysis. All statistical analyses controlled for age, gender, ethnicity, smoking status, blood pressure, and body mass index. Low amounts of low frequency (LF) power (β=-0.31, p=0.007) and high frequency (HF) power (β=-0.36, p=0.002) were associated with increased amounts of IL-6, explaining 7% and 9% of the variance, respectively. Interactions between LF power and IL-6 (p=0.002) and between HF power and IL-6 (p=0.012) explained 8% and 5%, respectively, of the variance in sTF. Post hoc analyses showed associations between IL-6 and sTF when LF power (β=0.51, p<0.001) and HF power (β=0.48, p<0.001) were low but not when LF power and high HF power were high. The findings suggest that systemic low-grade inflammatory activity is associated with a decrense in HRV. Furthermore, there was a positive relationship between plasma levels of IL-6 and sTF antigen when HRV was low. Inflammation and related hypercoagulability might particularly contribute to atherothrombotic events in a setting of decreased HRV.

Keywords

Cardiovascular disease; coagulation; cytokines; heart rate variability; inflammation; vagus nerve

Introduction

An imbalance in the autonomic nervous system (ANS) as reflected by reduced heart rate variability (HRV) has been associated with atherosclerotic diseases (Thayer and Lane, 2007). Decreased HRV predicted progression of coronary atherosclerosis (Huikuri et al., 1999), increased risk of acute coronary events (Tsuji et al., 1996), and death from coronary artery

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disease (CAD) (Tsuji et al., 1996). Atherosclerosis is predominately an inflammatory disease (Ross, 1999). Because the vagus nerve inhibits production of proinflammatory cytokines in peripheral tissues via a cholinergic anti-inflammatory pathway (Tracey, 2002), one mechanism that could link decreased HRV with atherosclerosis is by kindling low-grade systemic inflammation (Gidron et al., in press; Thayer and Lane, 2007). In accordance with this notion, HRV was inversely associated with circulating levels of the proinflammatory cytokine interleukin (IL)-6 in patients with chronic kidney disease (Psychari et al., 2005), CAD (Janszky et al., 2004), and decompensated chronic heart failure (Aronson et al., 2001). IL-6 plays a central role in the pathogenesis of CAD morbidity and mortality by promoting atherosclerotic plaque proliferation and instability, thereby ultimately increasing the risk of plaque rupture (Lobbes et al., 2006; Ridker et al., 2000). Elevated IL-6 levels predicted future myocardial infarction in healthy individuals (Ridker et al., 2000) and coronary mortality in patients with unstable angina (Koukkunen et al., 2001).

Inflammation and coagulation processes closely intertwine in the development of atherothrombotic diseases (Croce and Libby, 2007; Esmon, 2004). For instance, IL-6 has been associated with soluble tissue factor (sTF) in the circulation of patients with diabetes (Conway et al., 2004) and with chronic atrial fibrillation (Lim et al., 2004). IL-6 may influence the level of sTF by stimulating its release from endothelial cells into the circulation (Szotowski et al., 2005). Blood-borne or sTF is mainly associated with circulating procoagulant cellular microparticles (Eilertsen and Osterud, 2004; Rauch and Nemerson, 2000). Soluble TF plays a major role in atherothrombosis (Giesen and Nemerson, 2000; Moons et al., 2002) by virtue of exerting procoagulant activity in the blood and thrombus propagation after rupture of an atherosclerotic plaque (Steffel et al., 2006). Accordingly, sTF was increased in patients with acute coronary syndromes (Misumi et al., 1998; Suefuji et al., 1997) and predicted poor prognosis in patients with unstable angina (Soejiama et al., 1999) and the risk of future CAD in apparently healthy individuals (Keller et al., 2006).

This study had two aims. First, we sought to replicate in a sample of reasonably healthy subjects an inverse relationship between decreased HRV and IL-6 levels previously observed in patients with CAD (Janszky et al., 2004). We postulated that this relationship would be independent of a variety of atherosclerotic risk factors known to be associated with HRV. Specifically, decreased HRV has been associated with older age, male sex, hypertension, elevated blood glucose and cholesterol levels, smoking, physical inactivity, and overweight (Snieder et al., 2007; Thayer and Lane, 2007). Ethnic differences have also been found with white individuals generally showing lower HRV than their black counterparts (Liao et al., 1995; Wang et al., 2005). These associations could reflect that HRV is mostly driven by breathing induced changes in blood pressure (BP) where sympathetic tone and parasympathetic tone to the heart primarily reflect baroreflex driven variation in heart rate. In atherosclerotic vessels, even at pre-clinical stages, the baroreceptors do not stretch well and there is little input to the brain to drive the baroreflex such that HRV is relatively depressed (Nasr et al., 2005). Second, we hypothesized a direct relationship between IL-6 and sTF, which would be moderated by HRV. A moderator effect statistically defines under what circumstances two variables are associated with one another (Kraemer et al., 2002). We specifically hypothesized that a significant association between plasma levels of IL-6 and sTF antigen would exist when HRV is low (i.e. greater inflammatory activity and greater associated procoagulability in the presence of reduced vagal tone) but not when HRV is high. To our knowledge, these relationships have not been investigated in healthy populations.

Methods

Study participants

All subjects provided informed written consent to participate in a study on cardiovascular physiology. The study protocol was approved by the University of California San Diego (UCSD) Institutional Review Board. The study design intended to recruit a similar proportion of men and women and black and white subjects. Recruitment of subjects was by advertisement, by word-of-mouth, or by referral from local medical practitioners. Data in terms of demographic and metabolic characteristics, HRV measures and IL-6 and sTF levels were complete in 106 subjects allowing for a full linear regression approach. Four subjects were additionally excluded because their IL-6 and sTF levels were outliers even after log transformation (i.e. mean values >3SD above or below the log transformed mean) such that the final sample reported here comprises 102 subjects.

In addition to major medical problems, the following conditions were specific exclusion criteria: congestive heart failure, symptomatic obstructive pulmonary, CAD, cerebrovascular disease, history of life-threatening arrhythmias, cardiomyopathy, severe asthma, diabetes, fasting glucose >120 mg/dl, cancer, liver or renal disease, creatinine >1.4 mg/dl, bruit on physical examination, known sleep disorders, history of psychosis, current drug or alcohol abuse. Women were excluded if they were postmenopausal, diagnosed with premenstrual syndrome, taking oral contraceptives, or pregnant. These exclusion criteria for women were designed to minimize hormonal influences on inflammation and coagulation. Many "healthy" subjects in industrialized countries have cardiovascular risk factors and, as a consequence, have silent atherosclerosis of their blood vessels or rigid blood vessels. No attempt was made to exclude such subjects with e.g. high normal blood pressure (BP), obesity, and smoking.

Demographic factors

Ethnicity was determined via self-report. Subjects who currently smoked ≥1 cigarette per day were termed smokers and all others were termed non-smokers. Resting systolic/diastolic BP was required to be <180/110 mm Hg at screening. Screening BP was defined as the average of three seated readings. Mean arterial BP (MAP) was used for statistical analyses. No subjects took any medications on a regular basis. Body mass index (BMI) was computed as the ratio of body weight in kilograms divided by the square of height in metres (kg/m^2). All subjects with BMI ≥ 40 kg/m^2 (i.e. those with morbid obesity) were excluded from the present study.

Heart rate variability

Subjects were tested between 9:00 am and 11:00 am. They were equipped with an electrocardiogram (ECG) (model 78352C; Hewlett-Packard, Andover, MA) that relayed ECG data to an analogue-to-digital converter (DT2801; Data Translation Inc., Marlboro, MA) sampling at 1 kHz. After they sat quietly for 30 minutes, 3-min data were collected during resting seated baseline condition using Global Lab software (Data Translation). Data were stored in a computer for subsequent review, artifact rejection, and calculation. A previously developed program was used to perform review and calculation of data (Nagel et al., 1993). Spectral power analyses were performed in accordance with previously published standards yielding the two frequency domain measures low frequency (LF) power (0.04–0.15 Hz) and high frequency (HF) power (>0.15 Hz) of HRV (Task Force, 1996). Power frequency (Hz) was converted to $msec^2$ by fast fourier transformation applying customized software (Nagel et al., 1993). Subjects were excluded from the analysis a priori if values were ≥40 $msec^2$ for LF power and ≥25 $msec^2$ for HF power because both values indicated clinical outliers to our subject population. We also calculated the ratio of LF to HF power.

Biochemical measures

Fasting venous blood samples were drawn at 6:00 am and preserved with 3.8% sodium citrate (ratio 9:1) for the analysis of sTF or with EDTA for the analysis of IL-6. Samples were spun in a refrigerated centrifuge. Obtained plasma was immediately frozen in polypropylene tubes at −80°C until further analyzed. Plasma levels of sTF antigen (Imubind Tissue Factor, American Diagnostica, Stamford, CT) and of high-sensitive IL-6 (Quantikine, R&D Systems, Minneapolis, MN) were determined by commercial enzyme-linked immunosorbent assays. The sTF antigen assay recognizes Apo-TF, TF, and TF/factor VII complexes and is not interfered with by other coagulation factors or inhibitors of procoagulant activity. All inter- and intra-assay coefficients of variation were <10%.

Statistical analyses

Data were analyzed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) and are presented as means±SE or as percentages in tables and as geometric means±SE in the text. The level of significance was set at $p \leq 0.05$ (two-tailed). Before analyses, raw values of all variables were examined for deviations from normality by the Kolmogorov-Smirnov test. The distribution of values for BMI, the three HRV measures, IL-6, and sTF deviated from normality and were log-transformed yielding normal distributions. All analyses were performed using the log transformed data. Pearson correlation coefficients were estimated to compute bivariate relationships between variables.

We computed multiple linear regression analyses using forced entry to investigate whether a) HRV measures would predict IL-6 levels, and b) whether the interaction between HRV measures and IL-6 levels would predict sTF antigen levels, independent of demographic covariates. In order to reduce problems with multicollinearity, all independent variables were centered to the mean, and centered values of HRV measures and of IL-6 levels were multiplied to obtain interaction terms (Kraemer et al., 2002). A significant interaction would mean that the slope of the relationship between IL-6 and sTF is different with high HRV (mean centered HRV measure minus one SD) compared to low HRV (mean centered HRV measure plus one SD) (Kraemer et al., 2002). Holmbeck's method was applied for post-hoc analyses on interaction terms to determine the significance of the relationship between IL-6 and sTF for high and low HRV (Holmbeck, 2002). This test particularly allows an assessment of significance within groups (e.g. in a group with lower levels of HRV vs. in a group with higher levels of HRV) and is not solely an indication of the presence or absence of statistical interaction. Leverage statistics identified no cases which influenced these regression models more than others.

We also computed effect size measure (Cohen's f^2) for the main and interaction effects in regression analysis. By convention, f^2 effect sizes of 0.02, 0.15, and 0.35 are considered small, medium, and large, respectively (Cohen, 1988). We did not correct the p-values by the number of statistical tests performed because the three HRV measures differ in their indicative value of autonomic function (Task Force, 1996). Moreover, the two hypotheses tested, namely that HRV predicts IL-6 and that the level of HRV moderates the relationship between IL-6 and sTF antigen are conceptually different. In this case of specific pre-established hypotheses, some authors do not recommend statistical adjustment for multiple tests as this might render truly important associations insignificant (Perneger, 1998).

Results

Subject characteristics

Table 1 shows the distribution of demographic and medical factors as well as of HRV measures and plasma levels of IL-6 and sTF in the 102 subjects studied. The mean plasma concentrations

of IL-6 and sTF antigen were similar to those measured in other healthy populations applying the same assays (Naumnik et al., 2003;Ridker et al., 2000). Also, mean levels of HRV measures were largely identical with previously obtained results in similarly healthy subjects (Minami et al., 1999).

Bivariate relationships with heart rate variability measures

There was a direct correlation between LF and HF components of HRV (r=0.57, p<0.001). Table 2 shows that HF power correlated inversely with IL-6 (p<0.001) and sTF (p=0.025). Also, LF power showed an inverse association with IL-6 (p<0.001) and the LF/HF power ratio was directly related to sTF (p=0.034). There were also several significant relationships between the HRV measures and virtually all subject characteristics. The directions of the bivariate relationships suggested that a decrease in LF power and/or in HF power were associated with older age, black ethnicity, current smoking, higher MAP, and higher BMI. In addition, the sympathovagal balance (i.e. sympathetic preponderance) was greater in men than in women and in whites than in blacks, respectively. Altogether, these significant bivariate relationships justified adjustment for age, gender, ethnicity, smoking, MBP, and BMI in the subsequent regression equations allowing us to test for an independent relationship between HRV and IL-6 on the one hand and between HRV and IL-6 in determining sTF on the other.

Heart rate variability predicting interleukin-6

In linear regression analyses, we controlled for age, gender, ethnicity, smoking status, MAP and BMI. After these covariates had been entered, LF power independently predicted 7% of the variance in IL-6 (Table 3a) with this association showing a small-to-medium effect size (f^2=0.07). HF power was also an independent predictor of IL-6 explaining 9% of the variance in IL-6 (Table 3b) with a small-to-medium effect size (f^2=0.09). IL-6 was not independently predicted by the LF/HF power ratio (p=0.82).

Smoking status also emerged as an independent predictor of plasma IL-6 levels. As compared to non-smokers, current smokers had significantly higher levels of IL-6 in the model with LF power (Table 3a) and, with borderline significance, in the model with HF power (Table 3b).

Interaction between heart rate variability and interleukin-6 predicting tissue factor

In separate models, we regressed each HRV measure, IL-6 levels, and their interaction on levels of sTF antigen after controlling for age, gender, ethnicity, smoking status, MAP, and BMI. There was a significant interaction between LF power and IL-6 (Table 4a) and between HF power and IL-6 (Table 4b) explaining 8% (f^2=0.08) and 5% (f^2=0.06), respectively, of the variance in sTF. The interaction between LF/HF power ratio and IL-6 was not significant (p=0.35).

These interactions suggested that the slopes of the relationship between IL-6 and sTF would be different with different levels of LF power and HF power. Post hoc analyses controlled for age, gender, ethnicity, smoking status, MAP, and BMI. They revealed a positive association between IL-6 and sTF when LF power was low (β=0.51, p<0.001; Figure 1a) but not when LF power was high (β=0.03, p=0.80; Figure 1b). Also, when HF power was low, there was a positive association between IL-6 and sTF (β=0.48, p<0.001; Figure 2a) that was not observed when HF power was high (β=0.02, p=0.86; Figure 2b).

Ethnicity emerged as a significant predictor of sTF with whites having higher geometric mean ±SE levels of sTF than blacks in the models with LF power (184.1±11.8 vs. 147.6±10.9 pg/mL; p=0.030) and HF power (185.8±11.9 vs. 146.2±11.2 pg/mL; p=0.021). In a complementary analysis, we regressed the interaction between HRV measures and IL-6 on sTF in the subsamples of black and white subjects to explore whether the interaction would be

similar in the two ethnic groups. The interaction between LF power and IL-6 for sTF was significant in blacks (β=-0.44, p=0.025) and whites (β=-0.30, p=0.034). The interaction between HF power and IL-6 for sTF showed borderline significance in blacks (β=-0.34, p=0.071) but was not significant in whites (β=-0.12, p=0.39). Also, higher MBP significantly predicted higher sTF levels in the model with HF power (Table 4b) and, with borderline significance, in the model with LF power (Table 4a).

Discussion

The HRV literature suggests that one issue associated with the use of HRV as a research tool with regard to cardiovascular disease is that there are a number of variability indexes, and it currently remains unclear which measures may be the best (Task Force, 1996; Thayer and Lane, 2007). We selected three HRV indexes for which there is general consent with regard to their autonomic meaning. The HF component primarily reflects the variability of vagal outflow (i.e. parasympathetic activity) to the heart, whereas the LF component refers to both sympathetic and parasympathetic influences. However, LF power often contents a substantial amount of parasympathetic influence. This may explain why HF power and LF power are frequently directly correlated with each other (such as seen in our subjects) and why decreased levels in HF power and LF power both predict cardiovascular outcome in the same direction (Thayer and Lane, 2007). The LF to HF power ratio quantifies the sympathovagal balance with relatively higher ratios indicating relatively more sympathetic than parasympathetic modulation of the heart rhythm (Task Force, 1996).

We found in a middle-aged sample of reasonably healthy subjects that LF power and HF power were both inversely associated with plasma IL-6 concentration independent of covariates. This finding is in line with previous studies showing that decreased HRV was associated with elevated IL-6 levels in patient populations (Aronson et al., 2001; Janszky et al., 2004; Psychari et al., 2005). Particularly, the bivariate association between LF power and IL-6 levels (r=-0.35 vs. r=-0.27) and between HF power and IL-6 levels (r=-0.39 vs. r=-0.16) seemed somewhat stronger in our healthy subjects than in a previous study on women with CAD (Janszky et al., 2004). This suggests that the interplay between depressed HRV and inflammation could be important across a range of preclinical and clinical disease states (Thayer and Lane, 2007).

The association between reduced HF power and increased IL-6 suggests that plasma IL-6 levels could be under tonic vagal control because HF power predominantly denotes parasympathetic activity (Task Force, 1996). This reasoning seems intriguing because it is in agreement with the recently proposed cholinergic anti-inflammatory pathway as mediated by the vagus nerve postulating that vagal withdrawal disinhibits suppression of proinflammatory cytokine production in peripheral tissue (Tracey, 2002). The observation that decreased LF power was also associated with elevated IL-6 could be expected given that the LF and HF component of HRV were so highly correlated. We may assume that parasympathetic modulation of the LF component was substantial because we investigated our subjects at rest when cardiac autonomic balance favors energy conservation through parasympathetic dominance over sympathetic influences (Thayer and Lane, 2007). Therefore, in the context of our analysis, LF power and HF power may reflect similar than different aspects of HRV. The sympathovagal balance – as expressed by the LF to HF power ratio – was unrelated to IL-6 levels. We may speculate that, under resting conditions, the parasympathetic influence on cardiac autonomic control and related HRV indexes (i.e. LF power and HF power) was more important as a predictor of plasma IL-6 levels than sympathetic modulation as reflected by the LF to HF ratio. On the whole, the finding of an inverse relationship between IL-6 and HF power and LF power concurs with previous studies in which decreases in both HF and LF power predicted atherosclerosis progression, incident CAD, and cardiac mortality after myocardial infarction (Bigger et al., 1992; Huikuri et al., 1999; Liao et al., 2002; Tsuji et al., 1996).

Plasma IL-6 levels were significantly associated with sTF antigen levels independent of covariates, corroborating similar findings from patient populations (Conway et al., 2004; Lim et al., 2004). This observation was also in agreement with previous observations on inflammation processes interacting with thrombogenic changes in general (Croce and Libby, 2007; Esmon, 2004) and with the effect of IL-6 on release of sTF from endothelial cells in particular (Szotowski et al., 2005). However, our finding that HRV was a moderator of the association between IL-6 and sTF antigen levels is novel. IL-6 and sTF were only directly related to one other when HRV was low but not when HRV was high. Again, this was observed for both HF power and LF power but not for the LF to HF power ratio. We therefore propose that the direct relationship between levels of IL-6 and sTF is particularly relevant in a setting of decreased HRV.

Atherosclerotic risk factors related to demographics (age, gender, ethnicity) and metabolic disturbance (MBP, BMI) were variably associated with the HRV measures in similar directions as previously observed such that decreased HRV was generally associated with greater atherosclerotic risk (Snieder et al., 2007; Thayer and Lane, 2007). This may suggest that atherosclerotic risk factors should be controlled for in analyses investigating an independent relationship between HRV and inflammation, even in healthy populations. This seems particularly important in a context of decreased baroreflex sensitivity with greater atherosclerosis lowering HRV (Nasr et al., 2005). Regression analyses yielded smoking status was an independent predictor of IL-6 and MAP as an independent predictor of sTF. Both these associations concur with previous studies (Ridker et al., 2000; Sommeijer et al., 2006). In terms of ethnicity, white subjects had higher sTF antigen levels than blacks. To the best of our knowledge, this finding has previously not been reported and seems contradictory to the much higher CAD risk in African Americans than in whites (Clark, 2005). In contrast and compatible with their increased cardiovascular risk, blacks had lower levels of LF power than whites (Task Force, 1996). However, the moderating effect of LF power for the relationship between IL-6 and sTF was quite similar in blacks and whites. The respective moderating effect of HF power seemed somewhat stronger in whites than in blacks, although it did not reach statistical significance in both groups. Parsimoniously interpreted, our data do not provide a strong argument for ethnic differences in vagal function as a moderator of the association between inflammation and coagulation activity in the circulation.

Under steady state conditions, even in reasonably healthy subjects, decreased HRV in both the LF and HF spectra could reflect a low-grade inflammatory state and related procoagulant activity. Such a mechanism could provide a unifying theory for why decreased HRV and elevated circulating levels of both IL-6 and TF may all predict atherothrombotic events. With regard to the potential clinical consequences of such a theory, it has been shown that decreased HRV and vagal tone are favourably restored by physical exercise and other life style modifications, including stress management (Blumenthal et al., 2005; Thayer and Lane, 2007; Tracey, 2007). It therefore seems warranted to investigate whether increasing HRV by way of behavioural interventions may alter inflammation and associated coagulation processes and ultimately cardiovascular outcome (Edwards et al., in press).

Our study has several limitations. First, its cross-sectional nature does not allow us making an inference on a causal mechanism leading from decreased HRV to elevated IL-6, and from elevated IL-6 to an increase in sTF. Second, we did not control for psychosocial stressors and related changes in hypothalamic-pituitary adrenal (HPA) axis function, all of which could account for some of the associations observed in our study. Psychosocial factors have been associated with reduced HRV in several studies (Hemingway et al., 2001) and generally healthy individuals with low cortisol responsivity showed less flexibility in HRV with acute mental stress compared to cortisol responders (Kunz-Ebrecht et al., 2003). Acute and chronic stressors particularly increase IL-6 and endogenous cortisol surge may dampen this stress-induced IL-6

increase (Kunz-Ebrecht et al., 2003; Ranjit et al., 2007; von Känel et al., 2006). In turn, low HRV by its own right may lead to HPA axis dysregulation or sympathetic hyperactivity both favoring proinflammatory cytokine production (Thayer and Sternberg, 2006). Third, our assay to determine sTF antigen levels did not specifically assess for a recently detected alternatively spliced form of sTF in plasma that exerts particular prothrombotic potential (Bogdanov et al., 2003). However, previous studies showing that sTF antigen levels were related to CAD risk also employed earlier assay techniques (Misumi et al., 1998; Soejima et al., 1999; Suefuji et al., 1997). There was a time lag of three to five hours between the blood draw and assessment of HRV. Given the diurnal variation in cytokines, this could also have impacted on the results. Fourth, our subjects were reasonably healthy and, therefore, it is unclear whether the relationship between IL-6 and sTF is similarly moderated by HRV in CAD populations. Also, exclusion criteria for women were rather rigorous, thereby limiting inference from our data to the general population of women. For instance, our men and women did not differ in IL-6 levels independent of covariates, whereas recent data suggests that greater IL-6 expression in women than men could be explained by gender differences in HRV (O'Connor et al., 2007). Fifth, although significant with our ample sample size, effect sizes of relationships between HRV, IL-6, and sTF were at best medium. Hence, it remains to be seen whether these associations have prognostic value in terms of future acute coronary syndromes (i.e. unstable angina and myocardial infarction) and death from CAD in populations with and without established CAD.

Taken together, we showed that decreased HRV is independently associated with elevated plasma IL-6 levels in reasonably healthy subjects as has been shown in patient populations (Aronson et al., 2001; Janszky et al., 2004; Psychari et al., 2005). We further found an independent relationship between plasma levels of IL-6 and sTF which have previously predicted an increased risk of future atherothrombotic events in apparently healthy individuals (Keller et al., 2006; Ridker et al., 2000). Most intriguingly, the relationship between elevated IL-6 and sTF was only significant when HRV was lowered but not when it was increased.

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References

Aronson D, Mittleman MA, Burger AJ. Interleukin-6 levels are inversely correlated with heart rate variability in patients with decompensated heart failure. J Cardiovasc Electrophysiol 2001;12:294–300. [PubMed: 11291801]

Bigger JT Jr, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. Circulation 1992;85:164–171. [PubMed: 1728446]

Blumenthal JA, Sherwood A, Babyak MA, Watkins LL, Waugh R, Georgiades A, Bacon SL, Hayano J, Coleman RE, Hinderliter A. Effects of exercise and stress management training on markers of cardiovascular risk in patients with ischemic heart disease: a randomized controlled trial. JAMA 2005;293:1626–1634. [PubMed: 15811982]

Bogdanov VY, Balasubramanian V, Hathcock J, Vele O, Lieb M, Nemerson Y. Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. Nat Med 2003;9:458–462. [PubMed: 12652293]

Clark LT. Issues in minority health: atherosclerosis and coronary heart disease in African Americans. Med Clin North Am 2005;89:977–1001. [PubMed: 16129108]

Cohen, J. Statistical power analysis for the behavioral sciences. 2nd. Hillsdale, NJ: Erlbaum; 1988.

Conway DS, Buggins P, Hughes E, Lip GY. Relationship of interleukin-6 and C-reactive protein to the prothrombotic state in chronic atrial fibrillation. J Am Coll Cardiol 2004;43:2075–2082. [PubMed: 15172416]

Croce K, Libby P. Intertwining of thrombosis and inflammation in atherosclerosis. Curr Opin Hematol 2007;14:55–61. [PubMed: 17133101]

Edwards KM, Ziegler MG, Mills PJ. The potential anti-inflammatory benefits of improving physical fitness in hypertension. J Hypertens. in press

Eilertsen KE, Osterud B. Tissue factor: (patho)physiology and cellular biology. Blood Coagul Fibrinolysis 2004;15:521–538. [PubMed: 15389118]

Esmon CT. The impact of the inflammatory response on coagulation. Thromb Res 2004;114:321–327. [PubMed: 15507261]

Gidron Y, Kupper N, Kwaijtaal M, Winter J, Denollet J. Vagus-brain communication inatherosclerosis-related inflammation: A neuroimmunomodulation perspective of CAD. Atherosclerosis. in press

Giesen PL, Nemerson Y. Tissue factor on the loose. Semin Thromb Hemost 2000;26:379–384. [PubMed: 11092212]

Hemingway H, Malik M, Marmot M. Social and psychosocial influences on sudden cardiac death, ventricular arrhythmia and cardiac autonomic function. Eur Heart J 2001;22:1082–1101. [PubMed: 11428849]

Holmbeck GN. Post-hoc probing of significant moderational and mediational effects in studies of pediatric populations. J Pediatr Psychol 2002;27:87–96. [PubMed: 11726683]

Huikuri HV, Jokinen V, Syvanne M, Nieminen MS, Airaksinen KE, Ikaheimo MJ, Koistinen JM, Kauma H, Kesaniemi AY, Majahalme S, Niemela KO, Frick MH. Heart rate variability and progression of coronary atherosclerosis. Arterioscler Thromb Vasc Biol 1999;19:1979–1985. [PubMed: 10446081]

Janszky I, Ericson M, Lekander M, Blom M, Buhlin K, Georgiades A, Ahnve S. Inflammatory markers and heart rate variability in women with coronary heart disease. J Intern Med 2004;256:421–428. [PubMed: 15485478]

Keller TT, Choi D, Nagel C, Te Velthuis H, Gerdes VE, Wareham NJ, Bingham SA, Luben R, Hack CE, Reitsma PH, Levi M, Khaw KT, Boekholdt SM. Tissue factor serum levels and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. J Thromb Haemost 2006;4:2391–2396. [PubMed: 16938131]

Koukkunen H, Penttila K, Kemppainen A, Halinen M, Penttila I, Rantanen T, Pyorala K. C-reactive protein, fibrinogen, interleukin-6 and tumour necrosis factor-alpha in theprognostic classification of unstable angina pectoris. Ann Med 2001;33:37–47. [PubMed: 11310937]

Kunz-Ebrecht SR, Mohamed-Ali V, Feldman PJ, Kirschbaum C, Steptoe A. Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav Immun 2003;17:373–383. [PubMed: 12946659]

Kraemer HC, Wilson GT, Fairburn CG, Agras WS. Mediators and moderators of treatment effects in randomized clinical trials. Arch Gen Psychiatry 2002;59:877–883. [PubMed: 12365874]

Liao D, Carnethon M, Evans GW, Cascio WE, Heiss G. Lower heart rate variability is associated with the development of coronary heart disease in individuals with diabetes: the atherosclerosis risk in communities (ARIC) study. Diabetes 2002;51:3524–3531. [PubMed: 12453910]

Liao D, Barnes RW, Chambless LE, Simpson RJ Jr, Sorlie P, Heiss G. Age, race, and sex differences in autonomic cardiac function measured by spectral analysis of heart rate variability--the ARIC study. Atherosclerosis Risk in Communities. Am J Cardiol 1995;76:906–912. [PubMed: 7484830]

Lim HS, Blann AD, Lip GY. Soluble CD40 ligand, soluble P-selectin, interleukin-6, and tissue factor in diabetes mellitus: relationships to cardiovascular disease and risk factor intervention. Circulation 2004;109:2524–2528. [PubMed: 15136493]

Lobbes MB, Lutgens E, Heeneman S, Cleutjens KB, Kooi ME, van Engelshoven JM, Daemen MJ, Nelemans PJ. Is there more than C-reactive protein and fibrinogen? The prognostic value of soluble CD40 ligand, interleukin-6 and oxidized low-density lipoprotein with respect to coronary and cerebral vascular disease. Atherosclerosis 2006;187:18–25. [PubMed: 16360159]

Minami J, Ishimitsu T, Matsuoka H. Effects of smoking cessation on blood pressure and heart rate variability in habitual smokers. Hypertension 1999;33:586–590. [PubMed: 9931170]

Misumi K, Ogawa H, Yasue H, Soejima H, Suefuji H, Nishiyama K, Takazoe K, Kugiyama K, Tsuji I, Kumeda K, Nakamura S. Comparison of plasma tissue factor levels in unstable and stable angina pectoris. Am J Cardiol 1998;81:22–26. [PubMed: 9462600]

Moons AH, Levi M, Peters RJ. Tissue factor and coronary artery disease. Cardiovasc Res 2002;53:313–325. [PubMed: 11827681]

Nagel JH, Han K, Hurwitz BE, Schneiderman N. Assessment and diagnostic applications of heart rate variability. Biomed Eng Appl Basis Commun 1993;5:147–158.

Naumnik B, Borawski J, Mysliwiec M. Different effects of enoxaparin and unfractionated heparin on extrinsic blood coagulation during haemodialysis: a prospective study. Nephrol Dial Transplant 2003;18:1376–1382. [PubMed: 12808176]

Nasr N, Pavy-Le Traon A, Larrue V. Baroreflex sensitivity is impaired in bilateral carotid atherosclerosis. Stroke 2005;36:1891–1895. [PubMed: 16100025]

O'Connor MF, Motivala SJ, Valladares EM, Olmstead R, Irwin MR. Sex differences in monocyte expression of IL-6: role of autonomic mechanisms. Am J Physiol Integr Comp Physiol 2007;293:R145–R151.

Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998;316:1236–1238. [PubMed: 9553006]

Psychari SN, Sinos L, Iatrou C, Liakos G, Apostolou TS. Relations of inflammatory markers to lipid levels and autonomic tone in patients with moderate and severe chronic kidney disease and in patients under maintenance hemodialysis. Clin Nephrol 2005;64:419–427. [PubMed: 16370154]

Ranjit N, Diez-Roux AV, Shea S, Cushman M, Seeman T, Jackson SA, Ni H. Psychosocial factors and inflammation in the multi-ethnic study of atherosclerosis. Arch Intern Med 2007;167:174–181. [PubMed: 17242319]

Rauch U, Nemerson Y. Circulating tissue factor and thrombosis. Curr Opin Hematol 2000;7:273–277. [PubMed: 10961576]

Ridker P, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767–1772. [PubMed: 10769275]

Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115–126. [PubMed: 9887164]

Snieder H, van Doornen LJ, Boomsma DI, Thayer JF. Sex differences and heritability of two indices of heart rate dynamics: a twin study. Twin Res Hum Genet 2007;10:364–372. [PubMed: 17564526]

Soejima H, Ogawa H, Yasue H, Kaikita K, Nishiyama K, Misumim K, Takazoe K, Miyao Y, Yoshimura M, Kugiyama K, Nakamura S, Tsuji I, Kumeda K. Heightened tissue factor associated with tissue factor pathway inhibitor and prognosis in patients with unstable angina. Circulation 1999;99:2908–2913. [PubMed: 10359735]

Sommeijer DW, Hansen HR, van Oerle R, Hamulyak K, van Zanten AP, Meesters E, Spronk HM, ten Cate H. Soluble tissue factor is a candidate marker for progression of microvascular disease in patients with Type 2 diabetes. J Thromb Haemost 2006;4:574–580. [PubMed: 16371118]

Steffel J, Luscher TF, Tanner FC. Tissue factor in cardiovascular diseases: molecular mechanisms and clinical implications. Circulation 2006;113:722–731. [PubMed: 16461845]

Suefuji H, Ogawa H, Yasue H, Kaikita K, Soejima H, Motoyama T, Mizuno Y, Oshima S, Saito T, Tsuji I, Kumeda K, Kamikubo Y, Nakamura S. Increased plasma tissue factor levels in acute myocardial infarction. Am Heart J 1997;134:253–259. [PubMed: 9313605]

Szotowski B, Antoniak S, Poller W, Schultheiss HP, Rauch U. Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines. Circ Res 2005;96:1233–1239. [PubMed: 15920023]

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 1996;93:1043–1065. [PubMed: 8598068]

Thayer JF, Lane RD. The role of vagal function in the risk for cardiovascular disease and mortality. Biol Psychol 2007;74:224–242. [PubMed: 17182165]

Thayer JF, Sternberg E. Beyond heart rate variability. Vagal regulation of allostatic systems. Ann N Y Acad Sci 2006;1088:361–372. [PubMed: 17192580]

Tracey KJ. The inflammatory reflex. Nature 2002;420:853–859. [PubMed: 12490958]

Tracey KJ. Physiology and immunology of the cholinergic anti-inflammatory pathway. J Clin Invest 2007;117:289–296. [PubMed: 17273548]

Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation 1996;94:2850–2855. [PubMed: 8941112]

von Känel R, Kudielka BM, Preckel D, Hanebuth D, Fischer JE. Delayed response and lack of habituation in plasma interleukin-6 to acute mental stress in men. Brain Behav Immun 2006;20:40–48. [PubMed: 15890495]

Wang X, Thayer JF, Treiber F, Snieder H. Ethnic differences and heritability of heart rate variability in African- and European American youth. Am J Cardiol 2005;96:1166–1172. [PubMed: 16214458]

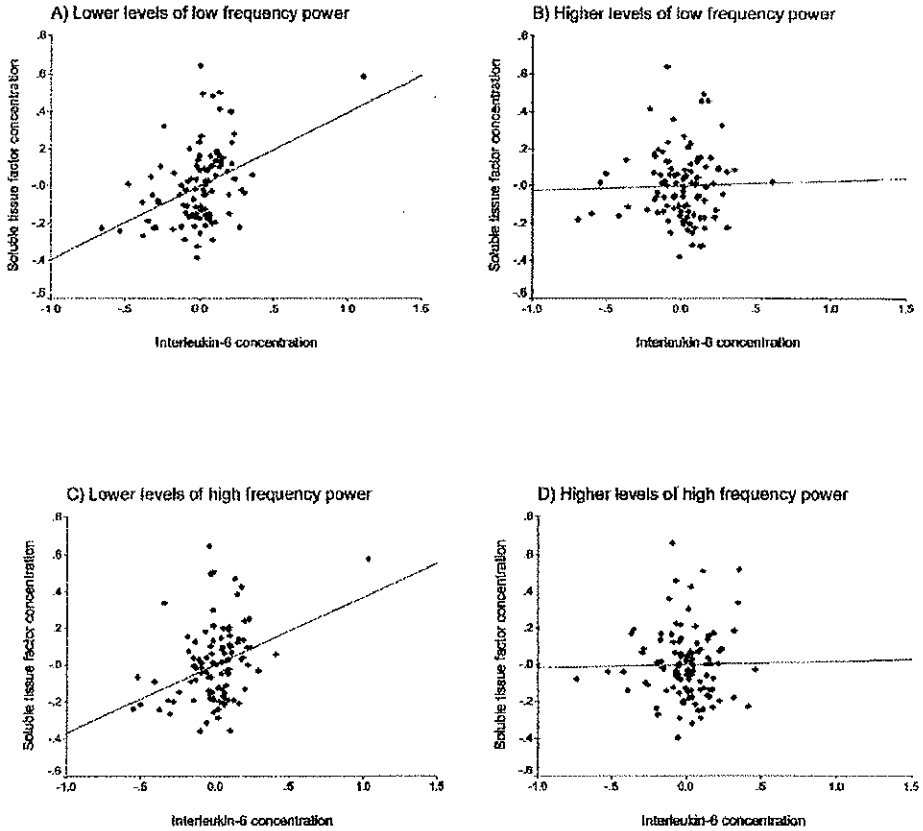

Figure 1. Relationship between IL-6 and sTF at different levels of heart rate variability
The partial regression plots depict the independent relationship with fit line between plasma levels of interleukin (IL-6) and soluble tissue factor (sTF) in the four groups of subjects as categorized by different levels of heart rate variability. There were significant relationships between IL-6 and sTF in the group with lower levels of low frequency power (Panel A) and in the group with lower levels of high frequency power (Panel C). IL-6 and sTF were not significantly related to each other in the group with higher levels of low frequency power (Panel B) and in the group with higher levels of high frequency power (Panel D). Adjustment was made for age, gender, ethnicity, smoking status, mean arterial blood pressure, and body mass index. Note the identical scaling of the x- and y-axes. All variables in the partial regression plots are residuals.

Table 1

Characteristics of the 102 subjects studied

Age [years]	36.1 ± 0.8 (23 – 52)
Gender [men / women]	50% / 50%
Ethnicity [black / white]	43% / 57 %
Current smoker [yes / no]	14% / 86 %
Mean arterial blood pressure [mmHg]	91.5 ± 1.1 (69.4 – 119.6)
Systolic blood pressure [mmHg]	125.2 ± 1.5 (96.0 – 168.7)
Diastolic blood pressure [mmHg]	74.7 ± 1.0 (53.7 – 100.0)
Body mass index [kg/m^2]	26.3 ± 0.5 (18.0 – 39.8)
Interleukin-6 [pg/mL]	1.4 ± 0.1 (0.1 – 9.0)
Soluble tissue factor antigen [pg/mL]	192.0 ± 11.2 (49.3 – 648.0)
Low frequency power [msec^2]	6.1 ± 0.5 (0.4 – 35.4)
High frequency power [msec^2]	4.5 ± 0.4 (0.3 – 16.1)
Low / high frequency power ratio	1.8 ± 0.1 (0.1 – 6.5)

Values are given as means ± SE with range or percentages

Table 2

Correlation matrix for heart rate variability measures

	LF power	HF power	LF / HF power ratio
Age	-0.32[c]	-0.46[c]	0.16
Gender	-0.10	0.15	-0.27[b]
Ethnicity	-0.32[c]	-0.13	-0.20[a]
Smoking	-0.09	-0.20[a]	0.12
Mean arterial blood pressure	-0.25[a]	-0.31[b]	0.09
Body mass index	-0.40[c]	-0.44[c]	0.05
Interleukin-6	-0.35[c]	-0.39[c]	0.05
Soluble tissue factor antigen	-0.03	-0.22[a]	0.21[a]

The columns show the Pearson correlation coefficients (*r*) between subjects' characteristics and heart rate variability measures.

[a]Significance levels of correlations: $p \leq 0.05$

[b]Significance levels of correlations: $p \leq 0.01$

[c]Significance levels of correlations: $p \leq 0.001$

Coding was −0.5 (men) or +0.5 (women) for gender; −0.5 (white) or +0.5 (black) for ethnicity; and −0.5 (not smoking) or +0.5 (smoking) for current smoking status

LF, low frequency; HF, high frequency

Table 3

Table 3a. Regression model for low frequency power predicting interleukin-6
Table 3b. Regression model for high frequency power predicting interleukin-6

Variables entered	Standardized β-coefficient	P-value	Delta R^2
Age	0.03	0.798	0.022
Gender	-0.15	0.160	0.001
Ethnicity	0.09	0.369	0.030
Smoking status	0.20	0.049	0.026
Mean arterial blood pressure	-0.20	0.087	0.012
Body mass index	0.12	0.253	0.038
Low frequency power	-0.31	0.007	0.066

Variables entered	Standardized β-coefficient	P-value	Delta R^2
Age	-0.03	0.783	0.022
Gender	-0.08	0.450	0.001
Ethnicity	0.15	0.135	0.030
Smoking status	0.17	0.087	0.026
Mean arterial blood pressure	-0.18	0.119	0.012
Body mass index	0.08	0.469	0.038
High frequency power	-0.36	0.002	0.085

The entire model accounted for 19.5% of the IL-6 variance (F=3.26, df=1,94, P=0.004)

The entire model accounted for 21.4% of the IL-6 variance (F=3.66, df=7,94, P=0.002)

Table 4

Table 4a. Regression model for LF power and IL-6 predicting soluble tissue factor
Table 4b. Regression model for HF power and IL-6 predicting soluble tissue factor

Variables entered	Standardized β-coefficient	P-value	Delta R^2
Age	0.14	0.187	0.055
Gender	-0.15	0.142	0.055
Ethnicity	-0.22	0.030	0.020
Smoking status	-0.08	0.399	<0.001
Mean arterial blood pressure	0.22	0.058	0.014
Body mass index	0.07	0.509	0.011
Interleukin-6	0.27	0.007	0.061
Low frequency power	0.13	0.233	0.008
LF power X IL-6 interaction	-0.28	0.002	0.075

Variables entered	Standardized β-coefficient	P-value	Delta R^2
Age	0.11	0.336	0.055
Gender	-0.15	0.145	0.055
Ethnicity	-0.23	0.021	0.020
Smoking status	-0.12	0.218	<0.001
Mean arterial blood pressure	0.25	0.039	0.014
Body mass index	0.03	0.803	0.011
Interleukin-6	0.25	0.014	0.061
High frequency power	0.03	0.834	<.001
HF power X IL-6 interaction	-0.25	0.032	0.053

The entire model accounted for 29.7% of the sTF variance (F=4.32, df=9,92, P<0.001)

The entire model accounted for 26.8% of the sTF variance (F=3.74, df=9,92, P<0.001)