Autonomic cardiac control during the cold pressor test in normal subjects

Running title: Cardiac autonomic modulation during cold pressor test.

Key words :

Heart rate variability; spectral analysis; detrended fluctuation analysis; fractal; cold pressor test

Authors.

Laurent MOUROT[1], Malika BOUHADDI[1, 2], Alain BOUSSUGES[3], Jacques REGNARD[1, 2]

1 - Université de Franche Comté, EA 3920 and IFR133, Physiologie, Faculté de Médecine, Besançon, France

2 - Centre Hospitalier Universitaire, Explorations fonctionnelles - Physiologie, Besançon, France

3 - Université de la Méditerranée, EA 3280, Physiopathologie et action thérapeutique des gaz sous pression, Marseille, France and Institut de Médecine Navale du Service de Santé des Armées, Toulon, France

Address of correspondence:
MOUROT Laurent
Telephone: +33 3.81.66.56.54
Telefax: +33 3.81.66.56.14
e-mail: mourotlaurent@hotmail.com
Laboratoire de Physiologie
Faculté de Médecine et de Pharmacie
F-25030 Besançon Cedex FRANCE

Abstract (186 words)

The cold pressor test (CPT) triggers in healthy subjects a vascular sympathetic activation and an increase in blood pressure. The heart rate (HR) response to this test is less well defined, with a high inter-individual variability. We used traditional spectral analysis together with the non-linear detrended fluctuation analysis to study the autonomic control of HR during a 3-min CPT. 39 healthy young subjects (23.7 ± 3.2 years, height 180.4 ± 4.7 cm and weight 73.3 ± 6.4 kg) were divided into two groups according to their HR responses to CPT. Twenty subjects have a sustained increase in HR throughout the test with reciprocal autonomic interaction, i.e. increase in sympathetic activity and decrease vagal outflow. In the 19 remainders, HR decreased after an initial increase, with indication of involvement of both sympathetic and vagal outflow. Baseline evaluation of the subjects revealed no difference between the two groups. A higher sympathetic activity at the skin level during CPT was nevertheless present in the group with decreased HR. Further studies are needed to explain why healthy subjects react differently to the CPT and if this has potential clinical implications.

Introduction

The cold pressor test (CPT) is typically performed by immersing a subject's hand into ice water (1-5 C°) for a short period of time (1-6 minutes) while measuring blood pressure (BP) and heart rate (HR). In normal subjects, a vascular sympathetic response increased peripheral resistances and a sustained increased BP is observed (Victor *et al.* 1987, Fagius *et al.* 1989, Weise *et al.* 1993, Stancak *et al.* 1996, Sendowski *et al.* 2000, Cui *et al.* 2002).

The HR response is less well defined, more variable on an individual basis (Jauregui-Renaud *et al.* 2001, Glenn & Ditto 2004) and not homogeneous for the entire CPT period. Two major patterns could be distinguished with either an increased (LeBlanc *et al.* 1975, Shibahara *et al.* 1996, Jauregui-Renaud *et al.* 2001, Dishman *et al.* 2003) or an unchanged HR (Weise *et al.* 1993, Sendowski *et al.* 1997, Cui *et al.* 2002, Fu *et al.* 2002). The latter response appeared less to be a "true" unchanged HR than a biphasic alteration, with an initial increase followed by a slow decrease that could return toward the control values (Victor *et al.* 1987, Stancak *et al.* 1996, Sendowski *et al.* 1997, Cui *et al.* 2002). Contrary to the vascular control, the autonomic HR control needs to be precise during CPT. Initially, a decrease in cardiac vagal outflow was accepted together with the sympathetic involvement (Frey *et al.* 1980a, Dishman *et al.* 2003, Tulppo *et al.* 2005, Wirch *et al.* 2006). However, these changes were not always found. An increase in vagal activity (Frey *et al.* 1980b, Shibahara *et al.* 1996, Sendowski *et al.* 1997, Glenn & Ditto 2004) induced by baroreceptor activity stimulation has also been hypothesized. This vagal stimulation should occur concomitantly to the persistent sympathetic involvement leading to cardiac autonomic co-activation (Weise *et al.* 1993). However, to our knowledge, this was not verified, mainly because in these previous reports the cardiac autonomic control was studied by spectral analysis of heart rate variability (HRV). However, this method may not offer a proper assessment of the HR dynamics during CPT because of limitations inherent in its stationary hypothesis (Task Force 1996).

New analysis techniques, such as analysis of fractal scaling exponents by detrended fluctuation analysis (DFA), have been developed to probe features in HRV that are not detectable by traditional analysis method. With DFA, the short-term (from 4 to 11 beats) fractal organization in human HRV is expressed as a scaling exponent named α_1. Changes in α_1 allowed highlighting cardiac autonomic co-activation or reciprocal changes in vagal / sympathetic activity (Tulppo *et al.* 2001a, Tulppo *et al.* 2005). Therefore, DFA may help to describe the cardiac autonomic status during CPT.

The aim of the present research was to study the autonomic control of HR during CPT. For that purpose, subjects were divided into two groups according to their HR response, and their autonomic control profiles were studied by means of linear and non-linear HRV analysis. We hypothesized that in the group with sustained increased HR, α_1 would increased, suggesting a decrease in cardiac vagal outflow together with sympathetic activation, while in the group with an increase followed by a decrease in HR, α_1 would decrease suggesting cardiac autonomic co-activation. Differences in baseline autonomic characteristics were suspected to explain these two HR responses.

Methods

Subjects

Forty male students (age 23.6 ± 3.2 years mean ± SD; height 180.4 ± 4.6 cm; and weight 73.4 ± 6.4 kg) voluntarily participated in the study. That was the first time that they have to perform a cold pressor test. Their medical history and a medical examination were used to discard subjects with cardiovascular, pulmonary, or metabolic diseases. The subjects were normotensive and none was taking any medication. The study protocol complied with the Helsinki declaration for human experimentation. The subjects were informed of the

organization and details of the study and signed an informed consent form, which was approved by our local ethics committee.

Testing protocol

The subjects were instructed to fast for at least 3 h before testing, and were asked to refrain from ingesting beverages containing caffeine and alcohol and not to exercise during the 24 h preceding each test. They were studied in the supine position in a quiet, dimly lighted room (ambient temperature, 26°C to 27°C). Before each test, the subjects, wearing short pants and T-shirts, rested 20 min to ensure hemodynamic stabilization. Then, baseline measurements started for 10 min. Subsequently, the subject immersed its left hand to the wrist into a 0-1°C water bath for a period of 3 minutes, followed by removal of the hand from the bath and continuation of recording for another 5 minutes.

The HR changes during the first part of the CPT test were roughly the same whatever the subjects. During the first minute, HR continuously rose until an initial peak. According to the HR changes after this peak, the subjects were divided into two groups. One group (CPTi) was constituted with subjects that further increased or maintained their HR during the second and third minutes of the test. The second group (CPTd) was constituted with subjects that decreased their HR by more than 5 beats per minute (mean over 10 sec). To properly describe these HR alterations, times of maximal HR, time of minimal values after this maximal, and the corresponding HR values (mean over 10 sec) were considered. If HR decreased less than 5 beats, the HR was considered to be maintained (Figure 1).

Hemodynamic measurements

Beat-to-beat BP was measured continuously using the Finometer® (Finapres Medical System, Amsterdam, The Netherlands). This device measures arterial pressure through a cuff wrapped around the middle phalanx of the middle finger. It has been demonstrated that finger BP recordings can accurately reproduce the beat-to-beat changes in intra-arterial blood pressure induced by a cold pressor stimulus (Parati *et al.* 1989). Arterial pulse pressure (PP, mmHg) was calculated from systolic (SBP) minus diastolic (DBP) blood pressure. The arterial pressure signal was analyzed using the Beatscope Software (TNO-TPD, Biomedical Instrumentation). Heart rate (HR) was derived from the beat to beat arterial pressure wave. Stroke volume (SV) was analysed by the Modelflow (Bogert & van Lieshout 2005). Cardiac output (CO) was calculated as the product of HR and SV and total peripheral resistance (TPR) by dividing mean arterial pressure (MAP) by CO.

To study heart rate variability (HRV), R-R intervals were obtained from a standard ECG.

Cardiovascular autonomic nervous activity evaluation

Firstly, baseline autonomic nervous profile of the subjects were evaluated thanks to heart rate (HRV) and blood pressure (BPV) variability analyses performed on a time series of 256 cycles selected during the baseline period, according to standard recommendations (Task Force 1996). The corresponding hemodynamic data are presented in Table 1. Secondly, HRV analysis was performed on a one minute-length basis to evaluate the cardiac autonomic nervous response to the CPT. Three different minutes were chosen for this evaluation: during the baseline period (corresponding hemodynamic data presented in Table 2), and during the second and third minute of CPT. Since maximal discomfort occur in the first minute of cold stimulus, the first minute was not addressed in the present study (Hilz *et al.* 2002).

All the R-R intervals, SBP and DBP values used for analyses were edited initially by visual inspection to exclude all the undesirable beats (i.e., to ensure that each analysis for the segment was free of movement artifact and/or sharp transient in the signal due to premature beats) which accounted for less than 1% in every subjects.

Spectrum analysis was performed with the coarse graining spectral analysis (CGSA) method (Yamamoto & Hughson 1991) that separates and permits simultaneous quantification of the contribution of the harmonic and fractal components of the total spectral power (TP), even when the data length is short (Yamamoto & Hughson 1991). From the harmonic component (HP) the integrated powers (ms^2) in the low- (LF: 0.04-0.15 Hz) and high- frequencies (HF: 0.15-0.50 Hz) were computed (Yamamoto & Hughson 1991). The very low frequencies (0-0.04 Hz) were not addressed in the present study. With HRV, the HF power normalized to the total spectral power (HF nu) was used as an indicator of parasympathetic activity. Despite controversial results, the LF/HF ratio is often used as an indicator of sympathetic activity (Yamamoto & Hughson 1991) and was used with this meaning.

The heart beat times series exhibits pattern of non linear process and is non-stationary during CPT. To better delineate the cardiac autonomic control, we used the DFA method to quantify the fractal-like scaling properties of the R-R interval data (Peng *et al.* 1995). The algorithm computes R-R intervals fluctuations in several windows of different sizes and finally creates a log–log curve, the slope of which defines the scaling exponent α_1. This short-term scaling exponent was computed for small (four to 11 beats) time scales. Details of DFA have been described previously (Peng *et al.* 1995, Tulppo *et al.* 2001a, Tulppo *et al.* 2001b, Tulppo *et al.* 2005).

Spontaneous Baroreflex sensitivity

Sequences of three or more beats in which the SBP and the following R-R interval changed in the same direction (either increasing or decreasing), which reflect the HR response to spontaneous variations in BP, were considered as spontaneous baroreflex (SBR) sequences. A linear regression was calculated for each of these sequences, and an average regression slope was calculated for all such sequences detected during each chosen recording epoch. This slope is considered as depicting the sensitivity of the cardiac SBR ($ms.mmHg^{-1}$) (Bahjaoui-Bouhaddi *et al.* 2000).

Temperature

Subjects' skin temperatures of the two hands (middle of the third metacarpus of the palmar side) were measured continuously by means of thermistor surface contact probes [series 400, type 409B, Yellow Springs Instrument (YSI); accuracy ± 0.1°C] fixed on the skin with thin, air-permeable, adhesive surgical tape. The probes were applied on the centre of the palmar surface.

Statistical methods

Standard statistical methods were used for the calculation of mean ± SD. Two comparisons were performed. The baseline characteristics, the time of maximal and minimal HR (and the corresponding values) of the subjects of the two groups were compared thanks to an unpaired t-test. Finally, the responses to the cold pressor test within each group were evaluated with a one-way ANOVA with repeated measures. When appropriate, post-hoc t-tests for paired data with Bonferroni correction were performed. Statistical significance was

accepted at the $p<0.05$ level. Statistical analyses were performed using SigmaStat® software (SPSS Inc, Chicago, USA).

Results

Cold pressor test and measurements were successfully performed in all but one subject, which was removed from the results presented here. In this subject, beat-by beat blood pressure could not reliably be evaluated during the entire test's duration. Without this subject, the characteristics of the subjects were age 23.7 ± 3.2 years, height 180.4 ± 4.7 cm and weight 73.3 ± 6.4 kg. Based on the HR response to the CPT, 20 subjects formed the CPTi group and 19 were assigned to the CPTd group.

Baseline characteristics of the two groups.

Anthropometric and hemodynamic data and indexes derived from analyses of HRV and BPV are given in Table 1. At baseline, no significant differences were found between CPTi and CPTd.

Hemodynamic responses during the CPT (Table 2)

For the two groups, significant increases in BP and TPR were observed during the second and third minutes of CPT. For the CPTi group, the increases between baseline and the third minute were 14.7 ± 10.4 %, 19.1 ± 14.6 %, and 16.7 ± 23.9 %, for SBP, DBP and TPR, respectively. For the CPTd group, the increases were 12.5 ± 7.7 %, 15.0 ± 10.2 %, and 17.8 ± 17.0 %, for SBP, DBP and TPR, respectively (Table 2). PP also increased, but the increases reached the significant level only for the third minute (9.6 ± 10.4 % for CPTi and 9.5 ± 10.7 % for CPTd).

In the CPTi group, times of maximal and minimal HR were 1.0 ± 0.2 and 2.2 ± 0.8 min, respectively. The corresponding HR values were 62.5 ± 8.3 and 59.9 ± 6.7 bpm, respectively. The HR during min 2 (61.2 ± 8.5 bpm) and min 3 (60.1 ± 7.4 bpm) were not different from the peak HR. HR was significantly higher at minute 2 and 3 compared to baseline (Figure 2). In the CPTd group, time of maximal HR (1.0 ± 0.1 min) and maximal HR (65.0 ± 4.3 bpm) were not significantly different from the CPTi group. The time for minimal HR was also not significantly different from the CPTi group (2.5 ± 0.4 min). However, the minimal HR (50.9 ± 7.1 bpm) was significantly lower. The HR during min 2 (59.3 ± 7.2 bpm) and min 3 (56.0 ± 6.9 bpm) were significantly lower than the peak HR. HR was higher during the second minute of the CPT compared to baseline and the third minute ($p<0.05$).

The palmar surface temperature of the left (immersed) hand decreased significantly for the two groups (Table 2) at the second (47.6 ± 22.7 % for CPTi and 55.9 ± 13.6 % for CPTd) and third minutes (50.1 ± 24.6 % for CPTi and 58.8 ± 15.3 % for CPTd). The palmar surface temperature of the right (non-immersed) hand also decreased at the second and third minutes. However, the decreases were not statistically significant for the CPTi group (1.6 ± 4.8 % for the second minute and 1.5 ± 6.5 % for the third minute) while the significant level was achieved for the CPTd group (1.5 ± 2.7 % for the second minute and 1.7 ± 2.3 % for the third minute).

Cardiovascular autonomic responses during the CPT

In the CPTi group, in absolute values, LF increased from 686 ± 654 ms^2 during baseline to 1617 ± 1551 ms^2 and 1623 ± 1454 ms^2 during the second and third minutes, without reaching the significant level. HF changed (without statistical differences) from 1045 ± 945 to 2040 ± 2548 and 1598 ± 2049 ms^2 (second and third minutes, respectively). This was accompanied

by a significant decrease in HFnu and by a significant increase in the LF/HF ratio and the scaling exponent α_1 (Figure 2). The slope of SBR changed from 25.1 ± 9.3 to 24.0 ± 13.5 and 28.9 ± 19.7 ms.mmHg^{-1} at the second and third minutes (no significant differences).

In the CPTd group, LF increased (no significant difference) from 701 ± 654 ms² during baseline to 1155 ± 915 ms² and 1033 ± 792 ms² during the second and third minutes. HF significantly increased from 720 ± 732 to 1530 ± 1441 and 1630 ± 2657 ms² at the second and third minutes. This was accompanied by a trend for an increase in HFnu and for decrease in the LF/HF ratio, but without reaching the significant level (Figure 2). Contrary to the changes observer in the CPTi group, the scaling exponent α_1 decreased significantly during minute 2 and further during minute 3 (Figure 2). The slope of SBR changed from 25.2 ± 5.8 to 23.1 ± 8.8 and 21.2 ± 11.9 ms.mmHg^{-1} at the second and third minutes (no significant differences).

Discussion

In healthy human subjects, CPT triggers an increase in BP (Victor *et al.* 1987, Fagius *et al.* 1989, Stancak *et al.* 1996, Jauregui-Renaud *et al.* 2001, Cui *et al.* 2002). This may be due to an increased CO during the initial period of the test with little increase in muscle sympathetic nerve activity, while an increase in this activity heightens peripheral resistances in the later period (Victor *et al.* 1987, Yamamoto *et al.* 1992). PP also increases, mainly at the end of the test (Stancak *et al.* 1996). The results of the present study are in accordance with these observations, whatever the group concerned (Table 2).

The increased CO is mainly due to changes in HR since SV appears unaltered (Dishman *et al.* 2003). A maintained HR elevation was found throughout CPT compared to baseline (LeBlanc *et al.* 1975, Shibahara *et al.* 1996, Jauregui-Renaud *et al.* 2001). However, a lot of studies also reported a marked increase in HR followed by a slow decrease (Victor *et al.* 1987, Stancak *et*

al. 1996, Sendowski *et al.* 1997, Cui *et al.* 2002). This bi-phasic pattern was observed in about half of the tested subjects in the present study. The decrease in HR is difficult to explain since the CPT was initially thought to induce a general sympathetic activation with no change or a decrease in vagal outflow (Frey *et al.* 1980a, Dishman *et al.* 2003, Tulppo *et al.* 2005, Wirch *et al.* 2006). That was the aim of this study to precise the autonomic control of HR during CPT.

Non-invasive evaluation of the autonomic control of heart rate in real-life conditions is possible by means of HRV analysis (Task Force 1996). However, the results of studies using this technique during CPT are inconsistent (Weise *et al.* 1993, Jauregui-Renaud *et al.* 2001, Dishman *et al.* 2003, Glenn & Ditto 2004, Tulppo *et al.* 2005, Wirch *et al.* 2006). These results, obtained at a group level, may be explained by the fact that both HR and the changes in HRV indexes appeared highly variable on an individual basis (Jauregui-Renaud *et al.* 2001, Glenn & Ditto 2004). The subjects of the present study were divided in two groups, according to their HR responses to CPT. For both groups, maximal HR was observed at the beginning the CPT test (Victor *et al.* 1987), while minimal HR was observed later, during the latest minute. Thus, in the two groups the temporal HR changes during the test were roughly identical. However, the magnitude of HR changes was different since after this initial increase only minimal HR changes were observed in the CPTi group while HR progressively decreased in the CPTd. In the CPTi group, the spectral analysis results indicated a persistent decreased cardiac vagal outflow (HFnu) and increased sympathetic activity (LF/HF ratio). In the CPTd group, the spectral analysis revealed opposite changes in cardiac autonomic regulation. The changes in spectral analysis indexes likely correctly depicted the cardiac autonomic control, because they were in accordance with the HR alteration. They also were in accordance with previous studies with similar changes in HR.

A number of studies dealing with HRV have shown that R-R intervals exhibit patterns suggestive of non-linear processes. Parameters arising from non-linear methods have therefore been identified. The short-term scaling exponent (α_1) of the detrended fluctuation analysis (DFA), computed for small (four to 11 beats) time scales, is one such parameter (Peng *et al.* 1995).

Tulppo and colleagues observed that α_1 increased during CPT. In their study, HR, muscle sympathetic nerve activity and LF/HF ratio increased while the HF index of HRV spectral analysis decreased, suggesting a generalized cardiovascular sympathetic involvement (Tulppo et al. 2005). Tulppo and colleagues suggested that when physiological changes in autonomic regulation occurred with reciprocal interplay, the fractal correlation of HR dynamics increased (Tulppo *et al.* 2001a, Tulppo *et al.* 2005). The results concerning the CTPi group were in accordance with these results. On the other hand, α_1 decreased when both the sympathetic and vagal activity increased (Tulppo *et al.* 2005). Such a co-activation was suspected but not verify during CPT (Weise *et al.* 1993). We observed that α_1 decreased in the CPTd group. In healthy subjects, the result of cardiac autonomic co-activation is a decreased HR (Levy 1971, Tulppo *et al.* 2005), which is consistent with our results. The enhancement of vagal outflow during CPT is likely a baroreflex correction to the sustained blood pressure increase in the latter part of the CPT. Indeed, the CPT shifts the baroreflex curve expressing the relationship between heart rate and systolic blood pressure to high blood pressures but does not alter its sensitivity (Cui *et al.* 2002). The baroreflex is thus capable of appropriately modulating HR during CPT.

It is unclear why subjects react to CPT with reciprocal changes in cardiac autonomic control while other increased the activity of the two branch of the cardiac autonomic nervous system. A distinct baseline autonomic nervous activity (e.g., enhanced sensitivity of the baroreflex, higher vagal outflow) was suspected in the CPTd group. However, no statistical differences

were found in the baseline hemodynamic and autonomic characteristics (Table 1) and thus we could not confirm our hypothesis. A significant decrease in the palmar surface temperature of the non-immersed hand during the test was found only in the CPTd group. This suggests a higher sympathetic tone to the skin in this group (Kistler *et al.* 1998). A different involvement of the pain receptors could be argued since the sensation of pain has been suggested to play a major role in HR regulation during CPT (Victor *et al.* 1987). However, this was not evaluated in the present study.

Limitations

We did not impose the breathing pattern. The subjects spontaneously adapted their tidal volume and breathing frequency but they were encouraged not changing their breathing pattern. Despite reported controversial results, it has been shown that the amplitude of respiratory-related heart rate oscillations increases at a given respiratory rate as the tidal volume increases (Saul *et al.* 1989). During a cold pressor test, tidal volume and minute ventilation usually increases (Wirch *et al.* 2006). In the present study, the respiratory-related heart rate oscillations did not change (CPTd) or decreased during CPT, i.e. were opposite to what would be expected from the changes observed in the ventilatory pattern (Saul *et al.* 1989), so the effect of ventilation was considered minimal. Nevertheless, we can not rule out a potential flaw due to ventilation in our results.

Implications

CPT has been used for the diagnosis of cardiovascular reactivity in normotensive and hypertensive subjects and the responses to CPT may help to identify normotensive candidates at future risk of suffering from hypertensive disease. Most of the time, the pressor response is based on the BP changes, with little or no attention on the HR alteration. In the present study,

the mean changes in BP were similar in the two groups. However, half of the subjects reacted with sign of cardiac autonomic co-activation that decrease α_1. Fractal organization is flexible, and breakdown of this scale-invariance (self-similarity) may lead to a more rigid and less adaptable system. A decrease in α_1 has indeed been observed in various disease states or with advancing age, and appeared as the most potent HRV indicator of a facilitated spontaneous onset of fibrillation (Vikman *et al.* 1999). A breakdown in the short-term fractal organization in human HR dynamics during CPT could have potential clinical implication, but this had to be verified with further studies.

Conclusion

In the present study, we used both linear and non-linear method of heart rate variability analysis to study the autonomic control of heart rate during the cold pressor test (CPT). It was found that in half of the tested subjects, reciprocal changes in cardiac autonomic regulation induced a sustained increased in HR. In the other subjects, CPT induced a decrease in HR after an initial increase, likely due to the co-activation of vagal and sympathetic outflow at the heart level which was highlighted by a change in HR dynamics from fractal toward more random HR organization.

References

BAHJAOUI-BOUHADDI M, CAPPELLE S, HENRIET MT, DUMOULIN G, WOLF JP ,REGNARD J: Graded vascular autonomic control versus discontinuous cardiac control during gradual upright tilt. *J Auton Nerv Syst* **79**: 149-155, 2000.

BOGERT LW ,VAN LIESHOUT JJ: Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol* **90**: 437-446, 2005.

CUI J, WILSON TE ,CRANDALL CG: Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans. *Am J Physiol Heart Circ Physiol* **282**: H1717-1723, 2002.

DISHMAN RK, NAKAMURA Y, JACKSON EM ,RAY CA: Blood pressure and muscle sympathetic nerve activity during cold pressor stress: fitness and gender. *Psychophysiology* **40**: 370-380, 2003.

FAGIUS J, KARHUVAARA S ,SUNDLOF G: The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles. *Acta Physiol Scand* **137**: 325-334, 1989.

FREY MA, SELM EA ,WALTHER JW, JR.: Reflex cardiovascular responses to cold exposure of the face or foot. *Jpn Heart J* **21**: 665-679, 1980a.

FREY MA, SIERVOGEL RM, SELM EA ,KEZDI P: Cardiovascular response to cooling of limbs determined by noninvasive methods. *Eur J Appl Physiol Occup Physiol* **44**: 67-75, 1980b.

FU Q, LEVINE BD, PAWELCZYK JA, ERTL AC, DIEDRICH A, COX JF, ZUCKERMAN JH, RAY CA, SMITH ML, IWASE S, SAITO M, SUGIYAMA Y, MANO T, ZHANG R, IWASAKI K, LANE LD, BUCKEY JC, JR., COOKE WH, ROBERTSON RM, BAISCH FJ, BLOMQVIST CG, ECKBERG DL, ROBERTSON D ,BIAGGIONI I: Cardiovascular and sympathetic neural responses to handgrip and cold pressor stimuli in humans before, during and after spaceflight. *J Physiol* **544**: 653-664, 2002.

GLENN D ,DITTO B: Vagal tone biofeedback: respiratory and non-respiratory mediated modulations of vagal tone challenged by cold pressor test. *MacGill J Medicine* **7**, 2004.

HILZ MJ, AXELROD FB, BRAESKE K ,STEMPER B: Cold pressor test demonstrates residual sympathetic cardiovascular activation in familial dysautonomia. *Journal of the Neurological Sciences* **196**: 81-89, 2002.

JAUREGUI-RENAUD K, HERMOSILLO AG, MARQUEZ MF, RAMOS-AGUILAR F, HERNANDEZ-GORIBAR M ,CARDENAS M: Repeatability of heart rate variability during simple cardiovascular reflex tests on healthy subjects. *Arch Med Res* **32**: 21-26, 2001.

KISTLER A, MARIAUZOULS C ,VON BERLEPSCH K: Fingertip temperature as an indicator for sympathetic responses. *Int J Psychophysiol* **29**: 35-41, 1998.

LEBLANC J, DULAC S, COTE J ,GIRARD B: Autonomic nervous system and adaptation to cold in man. *J Appl Physiol* **39**: 181-186, 1975.

LEVY MN: Sympathetic-parasympathetic interactions in the heart. *Circ Res* **29**: 437-445, 1971.

PARATI G, CASADEI R, GROPPELLI A, DI RIENZO M ,MANCIA G: Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension* **13**: 647-655, 1989.

PENG CK, HAVLIN S, STANLEY HE ,GOLDBERGER AL: Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos* **5**: 82-87, 1995.

SAUL JP, BERGER RD, CHEN MH ,COHEN RJ: Transfer function analysis of autonomic regulation. II. Respiratory sinus arrhythmia. *Am J Physiol* **256**: H153-161, 1989.

SENDOWSKI I, SAVOUREY G, BESNARD Y ,BITTEL J: Cold induced vasodilatation and cardiovascular responses in humans during cold water immersion of various upper limb areas. *Eur J Appl Physiol Occup Physiol* **75**: 471-477, 1997.

SENDOWSKI I, SAVOUREY G, LAUNAY JC, BESNARD Y, COTTET-EMARD JM, PEQUIGNOT JM ,BITTEL J: Sympathetic stimulation induced by hand cooling alters cold-induced vasodilatation in humans. *Eur J Appl Physiol* **81**: 303-309, 2000.

SHIBAHARA N, MATSUDA H, UMENO K, SHIMADA Y, ITOH T ,TERASAWA K: The responses of skin blood flow, mean arterial pressure and R-R interval induced by cold stimulation with cold wind and ice water. *J Auton Nerv Syst* **61**: 109-115, 1996.

STANCAK A, JR., YAMAMOTOVA A, KULLS IP ,SEKYRA IV: Cardiovascular adjustments and pain during repeated cold pressor test. *Clin Auton Res* **6**: 83-89, 1996.

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* **93**: 1043-1065, 1996.

TULPPO MP, HUGHSON RL, MAKIKALLIO TH, AIRAKSINEN KE, SEPPANEN T ,HUIKURI HV: Effects of exercise and passive head-up tilt on fractal and complexity properties of heart rate dynamics. *Am J Physiol Heart Circ Physiol* **280**: H1081-1087, 2001a.

TULPPO MP, KIVINIEMI AM, HAUTALA AJ, KALLIO M, SEPPANEN T, MAKIKALLIO TH ,HUIKURI HV: Physiological background of the loss of fractal heart rate dynamics. *Circulation* **112**: 314-319, 2005.

TULPPO MP, MAKIKALLIO TH, SEPPANEN T, SHOEMAKER K, TUTUNGI E, HUGHSON RL ,HUIKURI HV: Effects of pharmacological adrenergic and vagal modulation on fractal heart rate dynamics. *Clin Physiol* **21**: 515-523, 2001b.

VICTOR RG, LEIMBACH WN, JR., SEALS DR, WALLIN BG ,MARK AL: Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* **9**: 429-436, 1987.

VIKMAN S, MAKIKALLIO TH, YLI-MAYRY S, PIKKUJAMSA S, KOIVISTO AM, REINIKAINEN P, AIRAKSINEN KE ,HUIKURI HV: Altered complexity and correlation properties of R-R interval dynamics before the spontaneous onset of paroxysmal atrial fibrillation. *Circulation* **100**: 2079-2084, 1999.

WEISE F, LAUDE D, GIRARD A, ZITOUN P, SICHE JP ,ELGHOZI JL: Effects of the cold pressor test on short-term fluctuations of finger arterial blood pressure and heart rate in normal subjects. *Clin Auton Res* **3**: 303-310, 1993.

WIRCH JL, WOLFE LA, WEISSGERBER TL ,DAVIES GA: Cold pressor test protocol to evaluate cardiac autonomic function. *Appl Physiol Nutr Metab* **31**: 235-243, 2006.

YAMAMOTO K, IWASE S ,MANO T: Responses of muscle sympathetic nerve activity and cardiac output to the cold pressor test. *Jpn J Physiol* **42**: 239-252, 1992.

YAMAMOTO Y ,HUGHSON RL: Coarse-graining spectral analysis: new method for studying heart rate variability. *J Appl Physiol* **71**: 1143-1150, 1991.

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Figures and Legends

Figure 1. Changes in blood pressure and heart rate in a representative subject of the CPTi (A) and CPTd (B) groups.

Figure 2. Changes in heart rate (HR), in normalized high frequency (HFnu) and low- to high frequency ratio (LF/HF) of heart rate variability and in short-term fractal scaling exponent ($\alpha 1$), measured at baseline and during the second and third minute of cold hand immersion in the group with increased (CPTi) or decreased (CPTd) HR response to the cold pressor test (CPT).

Figure 1.

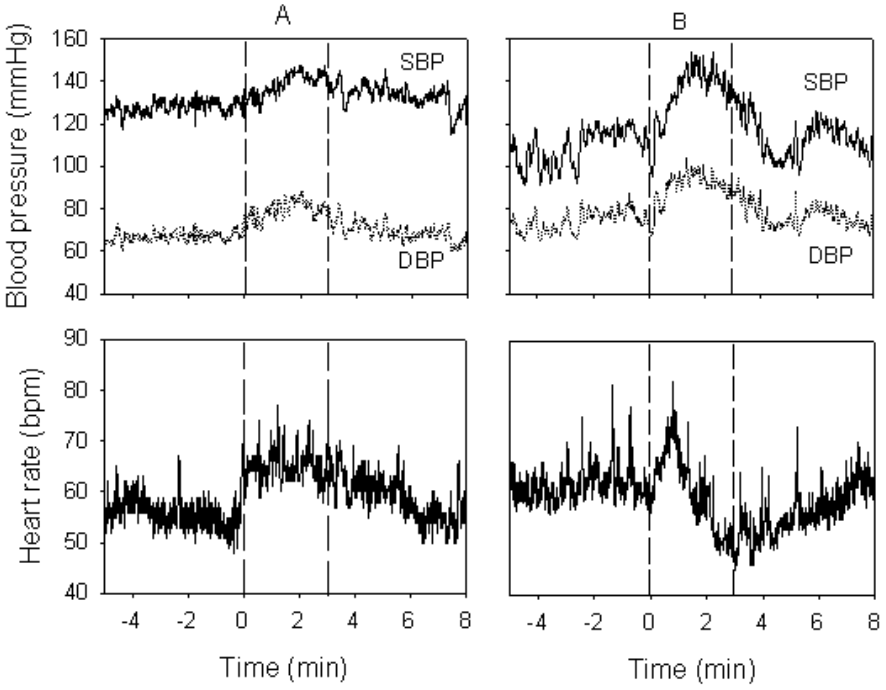
A
B
Blood pressure (mmHg)
160
140
120
100
80
60
40
SBP
DBP
SBP
DBP
Heart rate (bpm)
90
80
70
60
50
40
-4
-2
0
2
4
6
8
Time (min)
Time (min)

Figure 2.

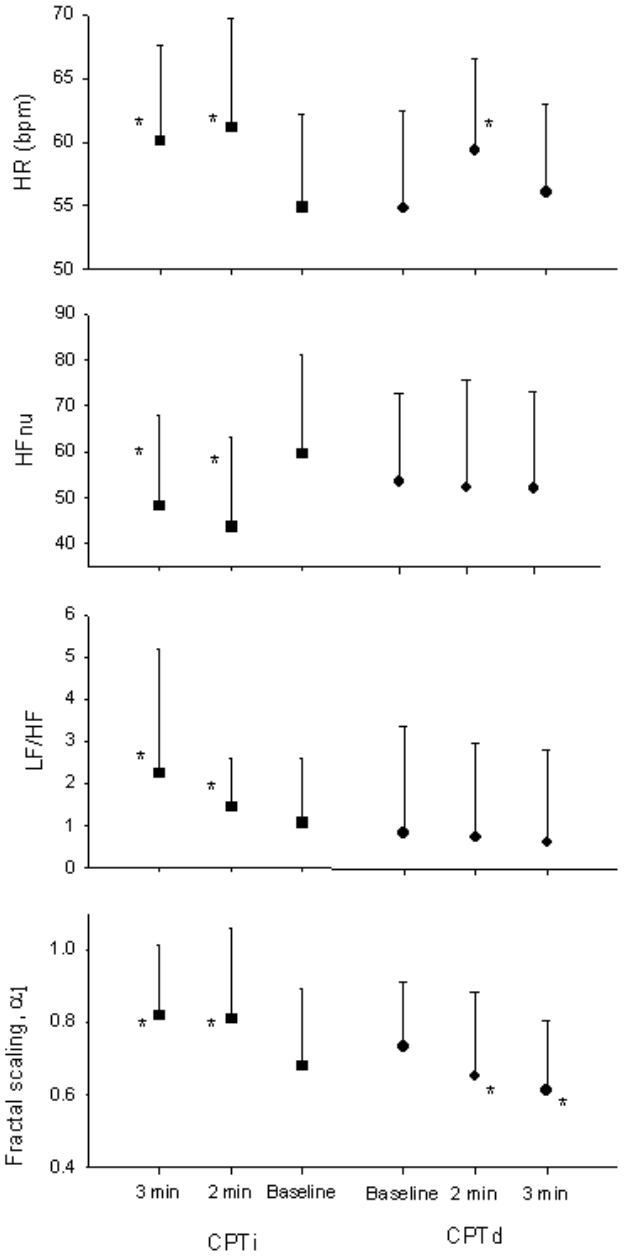
HR (bpm)
70
65
60
55
50
HF nu
90
80
70
60
50
40
LF/HF
6
5
4
3
2
1
0
Fractal scaling, α1
1.0
0.8
0.6
0.4
3 min
2 min
Baseline
Baseline
2 min
3 min
CPTi
CPTd

Table 1. Anthropometric and baseline hemodynamic and autonomic status of the subjects.

			CPTi	CPTd
	n =		20	19
Anthropometric data	Age	years	23.0 ± 3.4	24.4 ± 3.0
	Height	cm	179.9 ± 4.6	180.9 ± 4.9
	Weight	kg	73.3 ± 7.7	73.3 ± 4.9
Hemodynamic data	HR	bpm	53.3 ± 6.9	54.2 ± 7.2
	SBP	mmHg	126.1 ± 10.2	126.5 ± 7.2
	DBP	mmHg	70.3 ± 5.5	70.2 ± 5.3
	PP	mmHg	55.8 ± 8.9	56.3 ± 6.8
	SV	mL	101.1 ± 13.9	99.6 ± 11.9
	CO	$L.min^{-1}$	5.4 ± 0.9	5.4 ± 1.1
	TPR	(PRU)	1.02 ± 0.23	1.03 ± 0.18
Linear HRV analysis	Total Power	ms^2	5027 ± 3902	4450 ± 3968
	LF	ms^2	479 ± 547	407 ± 459
	HF	ms^2	515 ± 552	677 ± 1302
	HFnu	n.u.	0.11 ± 0.10	0.13 ± 0.12
	LF/HF		3.28 ± 5.93	2.48 ± 4.30
Linear BPV analysis	Total Power	ms^2	24.4 ± 19.6	20.0 ± 18.3
	LF	ms^2	2.9 ± 1.8	2.6 ± 2.5
	LFnu	ms^2	15.8 ± 18.9	19.0 ± 42.0
Baroreflex sensitivity		$ms.mmHg^{-1}$	24.6 ± 10.3	29.0 ± 9.8
Non linear HRV analysis	$\alpha 1$		0.68 ± 0.21	0.73 ± 0.18

CPTi and CPTd = group of subjects with secondary increased or decreased heart rate after the initial increased during the cold pressor test. HR = heart rate; SBP and DBP = systolic and diastolic blood pressure; PP pulse pressure; SV = stoke volume; CO = cardiac output; TPR =

total peripheral resistances. LF = low frequency; HF = high frequency; nu = normalized units; $\alpha 1$ = short-term fractal exponent.

Table 2. Hemodynamic changes during the cold pressor test.

			One minute Baseline	Second minute	Third minute
	SBP	mmHg	125.8 ± 10.3	143.9 ± 20.6 *	145.0 ± 22.8 *
	DBP	mmHg	70.1 ± 5.5	84.0 ± 11.3 *	83.5 ± 11.9 *
CPTi	PP	mmHg	55.7 ± 9.3	59.9 ± 14.2	61.4 ± 14.7 *
	SV	mL	101.4 ± 13.8	94.4 ± 19.3	97.6 ± 15.6 *§
n = 20	CO	$L.min^{-1}$	5.4 ± 0.9	5.7 ± 1.4	5.6 ± 1.0
	TPR	(PRU)	1.02 ± 0.21	1.18 ± 0.34 *	1.18 ± 0.29 *
	T° left hand	°C	33.0 ± 1.4	17.0 ± 7.2 *	16.2 ± 7.9 *
	T° right hand	°C	32.0 ± 1.1	31.5 ± 2.1	31.3 ± 2.4
			Baseline	Second minute	Third minute
	SBP	mmHg	126.5 ± 7.5	140.9 ± 15.0 *	141.9 ± 13.7 *
	DBP	mmHg	71.6 ± 5.3	84.4 ± 6.3 *	82.0 ± 7.3 *
CPTd	PP	mmHg	54.9 ± 6.7	56.5 ± 11.4	59.9 ± 9.8 *§
	SV	mL	99.7 ± 10.9	93.7 ± 16.8	99.4 ± 14.7
n = 19	CO	$L.min^{-1}$	5.5 ± 1.1	5.5 ± 1.0	5.5 ± 0.9
	TPR	(PRU)	1.03 ± 0.20	1.20 ± 0.20 *	1.19 ± 0.27 *
	T° left hand	°C	32.3 ± 1.2	14.3 ± 4.6 *	13.4 ± 5.1 *
	T° right hand	°C	31.7 ± 1.1	31.2 ± 1.3 *	31.1 ± 1.3 *

CPTi and CPTd = group of subjects with secondary increased or decreased heart rate after the initial increased during the cold pressor test. SBP and DBP = systolic and diastolic blood pressure; PP pulse pressure; SV = stoke volume; CO = cardiac output; TPR = total peripheral resistances

* significantly different from baseline; § significantly from the second minute of the cold pressor test at $p < 0.05$.