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Associations of Metabolic Variables with Electrocardiographic Measures of Sympathovagal Balance in Healthy Young Adults

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1. Introduction

Although obesity affects African-Americans disproportionately to their representation in the U.S. population, few studies have been performed to elucidate the mechanisms of maintaining body fat stores in healthy adolescent or young adult African-Americans with low and high percentages of body fat, before they have developed obesity-related diseases. Research has suggested some explanations, such as distrust and hesitancy about invasive procedures, to account for the sparse representation of African-Americans as subjects in research (Hamilton et al., 2006; Farmer et al., 2007; Braunstein et al., 2008). Indeed, advances in our knowledge of the obese phenotype have been impeded by the lack of noninvasive technologies for measuring the impact of body fat on physiological regulatory mechanisms. However, this impediment has effectively been overcome by the advent of heart rate variability analyses for elucidating autonomic mechanisms (Lucini et al., 2002). Such analyses make it possible to differentiate a wide variety of conditions with common autonomic etiologies (Vanninen et al., 1996; Narkiewicz et al., 1998; Salo et al., 2000; Gutierrez et al., 2002; Pichon et al., 2004).

Previous studies have shown correlations between increments in vagal signaling and high frequency heart rate variability spectral power during controlled (paced) breathing (De Meersman et al., 1995; Sanderson et al., 1996; Badra, 2001). Although the percentage of body fat may be a determinant of heart rate variability spectral power measured at rest (Nagai et al., 2003; Chen et al., 2008), the influence of body fat on heart rate variability measurements was found to be nil when performed at rest and significant only during an autonomic challenge (Matsumoto et al., 1999). We have demonstrated positive correlations of the low frequency/high frequency heart rate variability spectral power ratio with the respiratory quotient before and after feeding (Millis et al., 2009) and negative correlations with the

percentage of body fat in healthy young adult/adolescent African-American males after overnight fasting; and the latter only during periods of uncontrolled, and not during periods of paced, breathing (Millis et al., 2010). Other researchers have reported that changes in the percentage of body fat may be correlated with changes in the very low frequency spectral power, a measure of sympathetic thermoregulatory and metabolic energy signaling (Fujibayashi et al., 2009). However, the significances of very low frequency spectral power to measures of body fat stores during trials of uncontrolled and paced breathing associated with different physiological states such as fasting or feeding remain unclear. Therefore, we designed this study to determine the role of sympathetic thermoregulatory and metabolic energy signaling in the same healthy adolescent/young adult African-American male population as previously reported (Millis et al., 2010) during trials of uncontrolled and paced breathing, associated with states of overnight fasting and 3 h post-feeding, the latter associated with the metabolism of foods. We tested the hypothesis that, in healthy resting subjects, the percentage of very low frequency power of heart rate variability, an indicator of sympathetic thermoregulatory and metabolic energy signaling, is significantly correlated with percent body fat, body temperature and energy expenditure.

2. Materials and methods

2.1 Study participants and design

This experimental protocol was approved by the Howard University Human Participants Institutional Review Board, and each subject provided informed consent. The study population of 10 healthy 18-20 year-old African-American male university students was recruited and 8 subjects were included in the experiment. Each subject was studied twice, on separate days at which time they ingested isocaloric (900 Cal) high-carbohydrate and high-fat beverages after overnight fasting. An unsupervised, self reported period of overnight fasting (mean ± SD 12 ± 2 h) was used to limit the potentially confounding effects of diet related differences in autonomic responsiveness that we have described in a previous report (Millis et al., 2009; Millis et al., 2010). Two subjects were excluded because of inadequate fasting as determined by respiratory quotient measurements >0.85. Other criteria for inclusion in the experiment were non-smoking status, absence of alcohol abuse (less than two standard alcohol drinks a day), absence of use of medication that could interfere with autonomic modulation, resting systolic/diastolic blood pressure < 140/90 mm Hg. Table 1 summarizes the relevant characteristics of this study group determined with the subjects at rest after overnight fasting. The respiratory quotient indicates utilization of fatty acids as the main energy substrate and the low frequency/high frequency ratio shows a predominance of vagal modulation of the heart rate.

2.2 Uncontrolled and paced breathing

The subjects were instrumented and instructed as to the experimental procedures. Subjects were instructed to breathe normally while lying recumbent at 45 degrees in a bed of the General Clinical Research Center (GCRC) at Howard University Hospital. Following the normal uncontrolled breathing protocol, subjects were instructed to perform 5 min of paced breathing by following a visual tracking image on a computer monitor for periodic durations of inspirations and expirations set to 12 breaths $\cdot$min^{-1} (0.2 Hz). Each subject practiced paced breathing for a period of 1-3 min and was then instructed to perform the paced breathing for the 5 min paced breathing trial during which time the

electrocardiogram signal was recorded using a Biopac MP100 data acquisition system (Biopac Systems, Santa Barbara, CA). The electrocardiogram electrodes were placed on the subject's chest in a standard three-lead position with recordings obtained from standard lead II.

Age (years)	19 ± 1
Weight (kg)	82 ± 25
Height (cm)	174 ± 20
Body mass index ($kg \cdot m^{-2}$)	27 ± 8
Systolic blood pressure (mm Hg)	130 ± 13
Diastolic blood pressure (mm Hg)	70 ± 10
Heart rate ($beats \cdot min^{-1}$)	65 ± 12
Respiratory quotient	0.75 ± 0.05
Energy expenditure ($Cal \cdot d^{-1}$)	1980 ± 369
Body temperature (°F)	97 ± 1

Values in mean ± standard deviation, n = 8

Table 1. Characteristics of study participants

2.3 Heart rate variability analyses

Heart rate was measured in $beats \cdot min^{-1}$. Fast Fourier transform analysis of the electrocardiogram RR intervals was used to spectrally decompose heart rate variability in the frequency domain. For the frequency domain analysis, vagal modulation was represented by the area under the high frequency power spectrum (HF: 0.15-0.4 Hz), sympathetic cardiovascular modulation by the area under the low frequency power spectrum (LF: 0.04-0.14) and cardiac sympathovagal balance by the ratio LF/HF has been previously reported in this population (Millis et al. 2010). In this study, we analyzed the area under the very low frequency power spectrum (VLF: 0.001-0.04 Hz) expressed as the power in raw ms^2 and in normalized units using specialized autonomic neural software (Nevrokard, Version 6.3, Ljubljana, Slovenia). All time and frequency domain analyses were carried out in accordance with the guidelines put forth by the *Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology* (1996). The very low frequency band (0.001-0.04 Hz) has been considered by the *Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology* (1996) guidelines to be too small to be a reliable indicator during short-term recordings. However, recent investigations have challenged this assertion (Nagai et al., 2005; Nagai et al., 2006; Fujibayashi et al., 2009) and we have found that 5-min recordings produced VLF measurements that represented a significant proportion (23%-52%) of the total heart rate spectral power expressed in normalized units. The present study was designed, partly, to test the physiological significance of the very low frequency band as an indicator of sympathetic thermoregulatory and metabolic energy signaling.

2.4 Anthropomorphic, cardiovascular and metabolic measurements

Axillary body temperature was measured after overnight fasting and 3 h after ingesting a 900 Cal beverage. Body weight and height were also measured (Detecto scale) and these values were used to compute body mass index as the quotient kg body weight/m^2 height.

Blood pressure was determined using an automated sphygmomanometer (Criticare Systems Model 506DXNT, Waukesha, WI). To validate the effectiveness of overnight fasting, respiratory quotient and resting energy expenditure were measured by indirect calorimetry using an isolated flow-directed breathing chamber (Deltatrac, SensorMedics, Yorba Linda, CA). The participants were taken to the Howard University Exercise Science Laboratory for an assessment of the body fat percentage measured by dual energy x-ray absorptiometric (DEXA) whole body scanning (LUNAR Model DPX-L DEXA, Madison, WI).

2.5 Statistical analyses

The study design consisted of a comparison and correlation analysis of measurements of body temperature and the area under the very low frequency power spectrum of heart rate variability measured at rest after overnight fasting and 3 h after ingesting a 900 Cal beverage, during trials of uncontrolled and paced breathing with the measurement of body fat percentage (n = 8, 18-20 year-olds). The significance of differences between the post-fasting and post-feeding states and between the uncontrolled and paced breathing trials was evaluated by analysis of variance using a multivariate general linear model with significance set at $P < 0.05$. A correlation analysis between the normalized and percentage units of the very low frequency band of heart rate variability, the low frequency/high frequency power ratio, body temperature, resting energy expenditure and body fat percentage measurements after overnight fasting and 3 h after ingesting a 900 Cal beverage (feeding state) was based on linear regression and Pearson's correlation coefficient during uncontrolled and paced breathing trials with significance at $P < 0.05$. A statistical software package was used for the computations and analyses (SPSS, Chicago, IL).

3. Results

Table 2 compares the body temperature, resting energy expenditure and heart rate variability very low frequency spectral power for the uncontrolled and paced breathing trials after fasting and feeding. Normalized and percentage units of very low frequency spectral power (VLFnu, VLF%) and body temperature for the uncontrolled breathing trials were increased 3 h after feeding; changes for the paced breathing trials were not significant.

Heart rate variability	Fasting spontaneous breathing condition	Fasting paced breathing condition	Feeding spontaneous breathing condition	Feeding paced breathing condition
Total power (nu)	122 ± 5	140 ± 19	202 ± 22*	141 ± 7**
Very low frequency (nu)	28 ± 5	43 ± 19	105 ± 22*	45 ± 7**
Very low frequency (%)	23 ± 3	31 ± 5	52 ± 4*	32 ± 3**
LF/HF	0.6 ± .09	0.8 ± .3	1.1 ± 0.2	1.2 ± 0.4

Values in mean ± standard error, nu = normalized units, n = 16
LF/HF = low frequency/high frequency
*Difference between fasting and feeding significant at P<0.01.
**Difference between spontaneous and paced breathing significant at P<0.01.

Table 2. Heart rate variability measurements after fasting and feeding

Table 3 shows that percent body fat was significantly correlated with body temperature during fasting (r=0.78, P<0.01) but not during feeding (r=0.13, P>0.1). Percent body fat was also significantly correlated with VLFnu and VLF% for the fasting uncontrolled breathing (r=-0.57 and -0.63, P<0.01) but not for the fasting or feeding paced breathing condition or for the feeding uncontrolled breathing condition (P>0.1). Body temperature was correlated with VLFnu and VLF% (r=-0.39, P<0.05 and r=-0.47, respectively, P<0.01) for the uncontrolled breathing trials after fasting but not for the uncontrolled or paced breathing trials after feeding (P>0.1).

Resting energy expenditure, before correction for inter-individual differences in body mass index and percent body fat, was not significantly correlated with VLF% (r=0.04, P>0.1). After correction, the resting energy expenditure/BMI and resting energy expenditure/percent body fat ratios were significantly correlated with VLF% (r=0.50, P<0.01 and r=0.82, P<0.001) for the uncontrolled breathing trials after fasting. The significant correlations between resting energy expenditure and VLFnu were the same as those for VLF%.

Correlate of very low frequency spectral power percentage	**Fasting, spontaneous breathing**	**Fasting, paced breathing**
Body fat (%)	-0.63**	-0.31
Body temperature (°F)	0.47**	0.19
Energy expenditure (Cal · d^{-1})	0.04	-0.13
Energy expenditure/body mass index	0.50**	0.18
Energy expenditure/percent body fat	0.82***	0.23

Values in Pearson's correlation coefficient, n = 16
*Significant at P<.05
**Significant at P<.01
***Significant at P<.001

Table 3. Correlations of very low frequency percentage of total spectral power with percentage of body fat, body temperature and resting energy expenditure after overnight fasting

Correlate of very low frequency spectral power of heart rate variability	**Feeding, spontaneous breathing**	**Feeding, paced breathing**
Body fat (%)	-0.41*	-0.15
Body temperature (°F)	0.41*	0.14
Energy expenditure (Cal · d^{-1})	0.53**	0.19
Energy expenditure/body mass index	0.54**	-0.35
Energy expenditure/percent body fat	0.35	-0.56**

Values in Pearson's correlation coefficient, n = 16
*Significant at P<.05
**Significant at P<.01
***Significant at P<.001

Table 4. Correlations of percentage of very low frequency spectral power with percentage of body fat, body temperature and resting energy expenditure after feeding

Table 4 shows that, after feeding, the direct correlation between VLF% and resting energy expenditure, uncorrected for body mass/fat, was significant for the paced breathing trials. The correlation between VLF% and energy expenditure/percent body fat was also significant for the paced breathing trials; however, the correlation was inverse (negative correlation) to that of the similarly corrected energy expenditure for the uncontrolled breathing trials. The significant correlations between resting energy expenditure and VLFnu were the same as those for VLF%.
Figure 1 depicts the results of linear regression analyses showing that physiological state was a determinant of the significant negative correlation between VLF% and percent body fat for the uncontrolled breathing trials after overnight fasting ($r=-0.63$, $P<0.01$) which was not significant for the uncontrolled breathing trials after feeding ($r=-0.31$, $P>0.1$).
Figure 2 shows the linear regression analyses demonstrating that respiration was a determinant of the significant correlation between VLF% and LF/HF for the uncontrolled breathing trials after fasting ($r=0.61$, $P<0.01$) which was not significant for the paced breathing trials after fasting ($r=0.34$, $P>0.1$). The correlation of VLF% and LF/HF was also significant for the uncontrolled breathing trials after feeding ($r=0.58$, $P<0.01$) and was not significant for the paced breathing trials after feeding ($r=0.14$, $P>0.1$).
Interference of paced breathing with the correlations between VLF% and body temperature after fasting and feeding are shown in Table 3.

4. Discussion

The main findings of this study are significant positive correlations between the very low frequency spectral power of heart rate variability and body temperature and significant negative correlations between percentage of body fat and both body temperature and the very low frequency power. These correlations were significant during trials of uncontrolled breathing after fasting but not during trials of paced breathing or after feeding. These findings suggest that, under steady-state conditions such as overnight fasting and uncontrolled breathing at rest, the very low frequency spectral power may be indicative of autonomic adaptations for maintaining metabolic energy stores which could contribute to the development of obesity-related diseases. The guidelines for standardizing heart rate variability measurements state that the very low frequency spectral power may represent too small a proportion of the total spectral power to be worthy of analysis during short intervals of electrocardiographic recordings (*Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology* 1996). More recent studies have suggested that the very low frequency power could, under appropriate conditions, be an indicator of sympathetic thermoregulatory and metabolic energy signaling (Nagai et al., 2005; Nagai et al., 2006; Fujibayashi et al., 2009). The significant positive correlation between body temperature and very low frequency power that we found during fasting, but not during paced breathing, corroborates the previous reports of a thermoregulatory and metabolic energy signaling function of the very low frequency band.
In a previous study, we reported the association of a low percentage of body fat with a shift in heart rate variability spectral power toward greater sympathetic modulation and the association of a high percentage of body fat with a shift in spectral power toward greater vagal modulation after overnight fasting (Millis et al., 2010). A shift in sympathovagal balance toward greater vagal modulation is reported during the ingestion of water (Routledge et al., 2002) and, thereby, implies that healthy individuals with high percentages of body fat may exhibit less sympathetic signaling activity during night-time hours than their leaner counterparts.

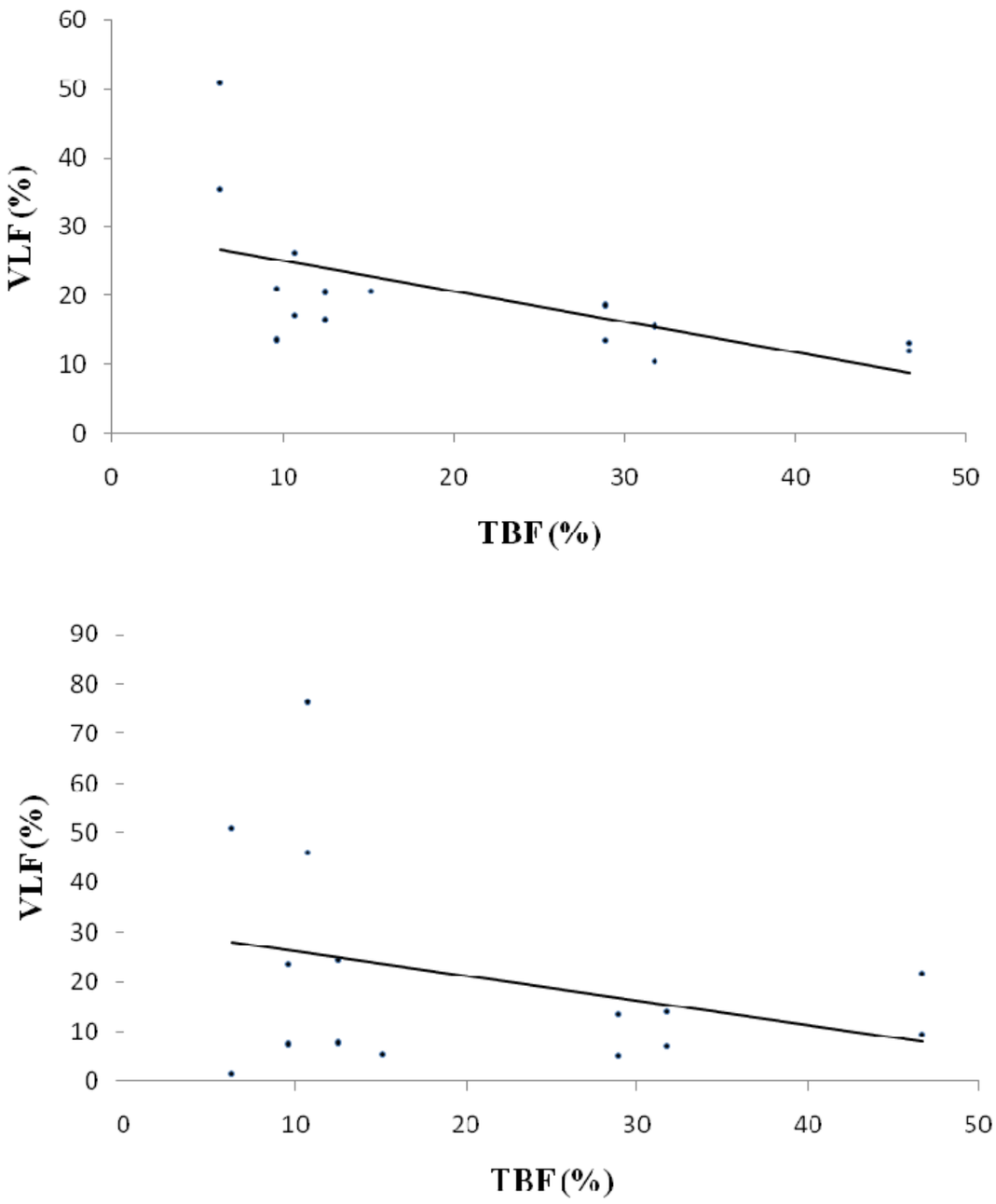

Fig. 1. **Correlation of very low frequency spectral power of heart rate variability with percentage of body fat.** Linear regression for heart rate variability percentage of very low frequency power (VLF) computed from fast Fourier transform analysis of the electrocardiogram RR intervals with percentage of body fat (TBF) measured by dual x-ray absorptiometric, DEXA whole body scanning for eight healthy 18-20 year-old African-American males during uncontrolled (spontaneous) breathing. Top: After overnight fasting, Pearson's correlation coefficient r=-0.63, P<0.01. Bottom: 3 h after feeding a 900 Cal beverage, Pearson's correlation coefficient r=-0.31, P>0.1.

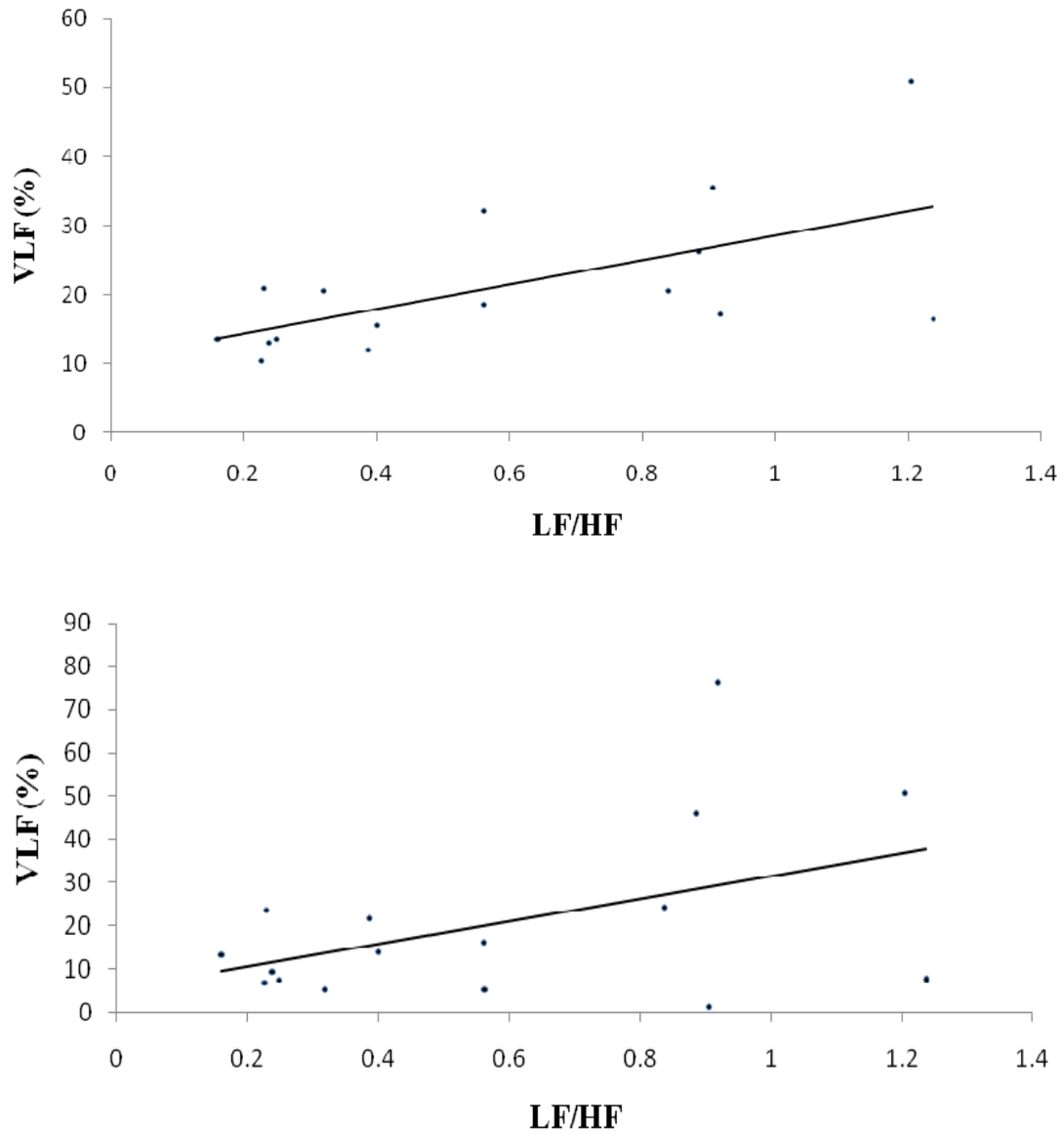

Fig. 2. **Correlation of very low frequency spectral power with low frequency/high frequency spectral power ratio of heart rate variability.** Linear regression for percentage of very low frequency power (VLF) with low frequency/high frequency (LF/HF) power ratio computed from fast Fourier transform analysis of the electrocardiogram RR intervals for eight healthy 18-20 year-old African-American males after overnight fasting. Top: During uncontrolled (spontaneous) breathing, Pearson's correlation coefficient $r=0.61$, $P<0.01$. Bottom: During controlled (paced) breathing at 0.2 Hz, Pearson's correlation coefficient $r=0.34$, $P>0.1$.

The metabolism of food produces a shift in sympathovagal balance toward greater sympathetic modulation, similar to that associated with postural changes (Piccirillo et al. 1998; Paolisso et al. 2000; Martini et al., 2001; Rabbia et al., 2003; Guizar et al., 2005; Kaufman et al., 2007a; Kaufman et al., 2007b; Nagai and Moritani, 2004). Higher body mass index is associated

with greater sympathetic responsiveness to postural changes, higher plasma leptin levels (Paolisso et al., 2000) and greater lipolytic activity of adipocytes (Berlan et al., 2002; Tentolouris et al., 2008). These findings suggest that inferences based on heart rate variability measurements of autonomic modulation vary with the physiological state. In the present study, we found significant correlations after overnight fasting but not after feeding. Moreover, we found that the aforementioned correlations were significant only during uncontrolled, and were obscured by paced, breathing. We previously reported that the correlations between percentage of body fat and the low frequency/high frequency spectral power ratio, a measure of cardiorespiratory sympathovagal balance, were masked by paced breathing and were observed only during trials of uncontrolled breathing (Millis et al., 2010).

The requirement for controlling respiratory frequency during measurements of heart rate variability is controversial. Paced breathing is thought, by some researchers, to be necessary for controlling the respiration-related variability (respiratory sinus arrhythmia) of the electrocardiogram inter-beat (RR) intervals on which heart rate variability measurements are based (De Meersman et al., 1995; Sanderson et al., 1996; Badra et al., 2001). Several mechanisms have been attributed to this requirement; e.g., respiratory sinus arrhythmia might be amplified by increased tidal volume (De Meersman et al., 1995). We previously reported no significant difference in low frequency/high frequency ratio during uncontrolled versus paced breathing at 0.2 Hz, (Millis et al., 2010). Respiratory frequency controlled at 0.17 Hz, 0.25 Hz and 0.33 Hz is reported to have no effect on low frequency power and to modulate high frequency power only (Sanderson et al., 1996). Increase in tidal volume is reported to increase high frequency power (Grossman et al., 2004; Pöyhönen et al., 2004) and paced breathing at 0.2 Hz, the respiratory frequency that we used, to increase tidal volume (Pinna et al., 2006). We also previously reported an increase in the low frequency spectral power, a measure of cardiovascular sympathetic modulation, associated with paced breathing at 0.2 Hz (Millis et al., 2010). The subjects were lying recumbent during both the paced and uncontrolled breathing conditions; thereby, ruling out changes in sympathetic modulation associated with changes in posture. However, increased low frequency power has been shown to occur in association with an increased respiratory rate during conditions of mental stress (Bernardi et al. 2000) and could have occurred in the present study because of experimental stress, differences in tidal volumes associated with paced breathing, or differences in respiratory frequency during the uncontrolled breathing trials. The mechanisms responsible for the interferences of paced breathing at 0.2 Hz with the correlation between percent body fat, body temperature and the very low frequency spectral power of heart rate variability are unknown. However, we found the correlations between very low frequency power and low frequency/high frequency power ratio were significant for the uncontrolled, but not for the paced, breathing trials. These findings suggest the possibility that the aforementioned interferences of paced breathing with the correlates of very low frequency power, are likely related to significant interactions between sympathetic thermoregulatory/metabolic signaling indicated by the very low frequency band and the cardiorespiratory sympathovagal signaling indicated by the low frequency/high frequency ratio, which need to be further elucidated. Similar interactions might also be responsible for our findings that, after feeding, the direct correlation between very low frequency power and resting energy expenditure, uncorrected for body mass/fat and the correlation between very low frequency power and the energy expenditure/percent body fat ratio were significant only for the paced breathing trials; however, the latter correlation was inverse (positive changed to negative correlation) to that of the similarly corrected energy expenditure for the uncontrolled breathing trials.

5. Conclusion

In this study, we showed that the very low frequency spectral power of heart rate variability was an autonomic signaling correlate of body temperature, resting energy expenditure and percentage of body fat in healthy adolescent/young adult African-American males. The correlations were significant after fasting and during uncontrolled breathing but not after feeding or paced breathing. We found associations of high body temperature, low resting energy expenditure and low percentage of very low frequency spectral power in individuals with high percentage of body fat during trials of uncontrolled breathing. These findings suggest that the very low frequency band of heart rate variability may be correlated with sympathetic thermoregulation and related to an autonomic adaptation for maintaining metabolic energy stores. A comparison between the healthy young adult/adolescent population that we studied and a young population affected by diabetes mellitus or the metabolic syndrome should further elucidate the role of the very low frequency band of heart rate variability for sympathetic metabolic signaling in populations at high risk for developing an obese phenotype.

6. Acknowledgments

Supported in part by grants from the Cooperative Extension Service of the University of the District of Columbia & United States Department of Agriculture, Center for Diet, Nutrition & Health, from the Mordecai Wyatt Johnson Fund and by the Howard University General Clinical Research Center grant [M01 RR10284]), funded through the National Institutes of Health National Center for Research Resources, and the VIDDA Foundation.

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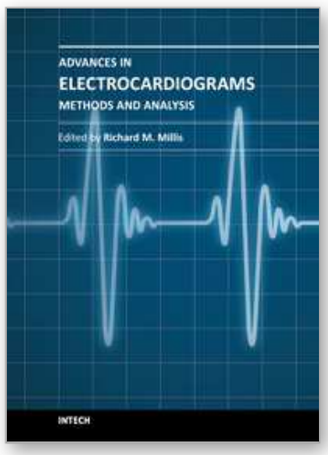

Advances in Electrocardiograms - Methods and Analysis
Edited by PhD. Richard Millis

ISBN 978-953-307-923-3
Hard cover, 390 pages
Publisher InTech
Published online 25, January, 2012
Published in print edition January, 2012

Electrocardiograms are one of the most widely used methods for evaluating the structure-function relationships of the heart in health and disease. This book is the first of two volumes which reviews recent advancements in electrocardiography. This volume lays the groundwork for understanding the technical aspects of these advancements. The five sections of this volume, Cardiac Anatomy, ECG Technique, ECG Features, Heart Rate Variability and ECG Data Management, provide comprehensive reviews of advancements in the technical and analytical methods for interpreting and evaluating electrocardiograms. This volume is complemented with anatomical diagrams, electrocardiogram recordings, flow diagrams and algorithms which demonstrate the most modern principles of electrocardiography. The chapters which form this volume describe how the technical impediments inherent to instrument-patient interfacing, recording and interpreting variations in electrocardiogram time intervals and morphologies, as well as electrocardiogram data sharing have been effectively overcome. The advent of novel detection, filtering and testing devices are described. Foremost, among these devices are innovative algorithms for automating the evaluation of electrocardiograms.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Richard M. Millis, Mark D. Hatcher, Rachel E. Austin, Vernon Bond and Kim L. Goring (2012). Associations of Metabolic Variables with Electrocardiographic Measures of Sympathovagal Balance in Healthy Young Adults, Advances in Electrocardiograms - Methods and Analysis, PhD. Richard Millis (Ed.), ISBN: 978-953-307-923-3, InTech, Available from: http://www.intechopen.com/books/advances-in-electrocardiograms-methods-and-analysis/associations-of-metabolic-variables-with-electrocardiographic-measures-of-sympatho-vagal-balance-in-

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