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Muscle Metaboreflex Contribution to Sinus Node Regulation During Static Exercise
Insights From Spectral Analysis of Heart Rate Variability

Ferdinando Iellamo, MD; Paolo Pizzinelli, MD; Michele Massaro, MD; Gianfranco Raimondi, MD; Giuseppe Peruzzi, MD; Jacopo Maria Legramante, MD

Background—it is currently assumed that during static exercise, central command increases heart rate (HR) through a decrease in parasympathetic activity, whereas the muscle metaboreflex raises blood pressure (BP) only through an increase in sympathetic outflow to blood vessels, because when the metaboreflex activation is maintained during postexercise muscle ischemia, BP remains elevated while HR recovers. We tested the hypotheses that the muscle metaboreflex contributes to HR regulation during static exercise via sympathetic activation and that the arterial baroreflex is involved in the HR recovery of postexercise muscle ischemia.

Methods and Results—Eleven healthy male volunteers performed 4-minute static leg extension (SLE) at 30% of maximal voluntary contraction, followed by 4-minute arrested leg circulation (ALC). Autonomic regulation of HR was investigated by spectral analysis of HR variability (HRV), and baroreflex control of heart period was assessed by the spontaneous baroreflex method. SLE resulted in a significant increase in the low-frequency component of HRV that remained elevated during ALC. The normalized high-frequency component of HRV was reduced during SLE and returned to control levels during ALC when BP was kept elevated above the resting level while HR recovered.

Conclusions—the muscle metaboreflex contributes to HR regulation during static exercise via a sympathetic activation. The bradycardia that occurs during postexercise muscle ischemia despite the maintained sympathetic stimulus may be explained by a baroreflex-mediated increase in parasympathetic outflow to the sinoatrial node that overpowers the metaboreflex-induced cardiac sympathetic activation. (Circulation. 1999;100:27-32.)

Key Words: exercise ■ muscles ■ heart rate ■ nervous system, autonomic ■ baroreceptors

Static exercise is characterized by increases in arterial pressure (AP) and heart rate (HR). Two neural mechanisms have been implicated in these responses. One mechanism activates central neuronal circuits that control somatomotor and cardiovascular systems, establishing changes in parasympathetic and sympathetic efferent activity that determine the cardiovascular responses (“central command”). In the other mechanism, the changes in autonomic efferent activity are caused reflexly by stimulation of somatic afferents sensitive to metabolites produced within the contracting muscles (“exercise pressor reflex” or “muscle metaboreflex”).

The current thinking is that during static exercise, the rise in AP occurs mainly via an increase in sympathetic activity to blood vessels due to muscle metaboreflex activation, whereas the increase in HR occurs mainly through a decrease in parasympathetic activity to the sinus node due to central command. This general view came from the observation that during postexercise circulatory occlusion, a maneuver that maintains muscle metaboreflex activation while removing the central command, the increases in AP, vascular resistance, and sympathetic nerve activity to resting muscles are kept elevated above resting levels, whereas HR fully recovers.

However, Maciel et al and Martin et al reported a reduced HR response to static exercise after administration of ß-adrenergic blocking drugs, suggesting an involvement of the sympathetic nervous system in HR regulation, although the mechanism underlying the sympathetic contribution (ie, central versus reflex) has not been determined. More recently, O’Leary provided evidence that sympathetic activation originating from the muscle metaboreflex contributes substantially to the HR increase during exercise in the conscious dog, inasmuch as parasympathetic blockade with atropine did not affect the increase in AP and HR that occurred during exercise but did prevent the fall in HR during postexercise circulatory occlusion. The hypothesis advanced was that the fall in HR during postexercise muscle ischemia despite a
maintained increase in sympathetic outflow was caused by a
sudden rise of parasympathetic activity at the cessation of
exercise, due to the loss of central command or to arterial
baroreflex mechanisms, which overpowered the sympathetic
activation. Whether these data can be extrapolated to humans
is unknown, because neural control of circulation at rest and
during exercise may differ between dogs and humans owing
to differences in baseline autonomic tone, pumping capacity,
and oxidative capacity of muscles.2

The present study was undertaken to test the hypotheses
that in humans, the muscle metaboreflex contributes to HR
regulation during static exercise via sympathetic activation
and that the arterial baroreflex could be involved in HR
recovery during postexercise circulatory occlusion. Auto-
nomic regulation of HR has been investigated by means of
power spectral analysis of HR variability, a technique cur-
cently used to derive noninvasive indexes of the different
neural components regulating the sinoatrial node.9 Arterial
baroreflex control of HR has been assessed by analysis of the
relationship between beat-by-beat spontaneous fluctuations in
arterial blood pressure and RR interval.10

Methods

Subjects

Eleven healthy male volunteers, aged 26.0 ± 2.4 years, participated in
this study. All participants gave written informed consent. None
were taking medications, and all were nonsmokers and not involved
in regular physical activity. The protocol was approved by the Ethics
Committee of the Department of Internal Medicine of our university.

General Procedure

Static exercise consisted of 1 leg-knee extension (SLE) performed in
the seated position with the trunk supported by the chair back of a
computer-based dynamometer apparatus (REV 9000, Technogym).
The inferior third of the leg was attached to the distal end of the
moveable lever arm of the dynamometer. A pneumatic cuff was
placed as high as possible on the thigh to allow experiments with
arrested circulation to the leg.

SLE was performed at 30% of maximal voluntary contraction,
previously determined as the highest force developed by the subjects
in 3 trials. A visual feedback2 allowed subjects to hold constant the
muscle tension during static contractions. Subjects were instructed to
avoid Valsalva maneuver and to relax all of the muscle not involved
in contraction.

Experimental Protocol

The experiments were performed in the morning in a laboratory at
ambient temperature (22°C to 24°C). Subjects were required not to
eat or to drink coffee for ≥2 hours. Each subject performed in
random order the exercise or no-exercise protocol. The exercise
protocol consisted of 5 minutes’ rest followed by 4 minutes of SLE. Eight
seconds before cessation of SLE, the pneumatic cuff on the
exercising leg was rapidly inflated to suprasystolic levels
(250 mm Hg), and arrested leg circulation (ALC) was maintained for
4 minutes in the postexercise period. The no-exercise protocol
consisted of 5 minutes’ rest followed by 4 minutes of thigh
circulatory occlusion. A 20- to 30-minute rest period separated the
exercise and no-exercise protocols.

Recorded Variables

Subjects were connected to a defibrillator supplied with an oscillo-
graphic screen (Hewlett-Packard [HP] 43120A); a chest lead pro-
vided the ECG signal. Arterial pressure was continuously and
noninvasively measured from the third finger of the nondominant
hand by Finapres (Ohmeda, model 2300). This device provided
accurate estimates of changes of intra-arterial pressure during labor-
atory tests, including exercise tests.11 The arm with the instrumented
finger was held extended at the heart level by means of a pulley
arrangement.5,12 Respiration was assessed by a pneumotachograph-
(Fleisch No. 3) pressure transducer set (HP 47304A) connected to a
face mask worn by the subjects. The 3 analog signals were sampled
at 300 Hz per channel by an analog/digital board (Data Translation
2831) inserted into a personal computer and stored for subsequent
analyses.

Power Spectral Analysis

A derivative-threshold algorithm provided continuous series of RR
interval (tachogram) from the ECG signal. Stationary sections of
tachograms of appropriate length were selected according to guide-
lines on standards of measurements for HR variability.9 The variance
of RR interval was evaluated. The harmonic components of RR-
interval variability were evaluated by the autoregressive method, in
which the autoregressive coefficients are estimated by the Yule-
Walker method.13 We checked the validity of the model by testing the
whiteness of the prediction error, and we chose the optimal
model order by applying the Akaike information criterion.14 The
center frequency and associated power of each relevant oscillatory
component were automatically calculated by the residue method.
Two main components were considered: that in the frequency band
from 0.04 to 0.15 Hz (low frequency; LF) and that in the range 0.15
to 0.4 Hz (high frequency; HF), which is synchronous with respira-
tion. The very-low-frequency component (< 0.03 Hz) was not
addressed and was considered as a DC component.9 We calculated
the power density of each spectral component in absolute values
(ms²) and normalized units, which we obtained by dividing the
absolute power of each spectral component by total power after
having subtracted from it the power of the DC component, if present,
and multiplying this value by 100. The normalization procedure is
particularly helpful in allowing comparisons between subjects or
experimental conditions characterized by large differences in total
power or DC noise.9,15 Spectral analysis of the respiratory signal was
performed on the signal sampled once for every cardiac cycle by a
procedure similar to that described for RR interval. These spectra
were used to assess the main respiratory frequency and to locate the
respiratory component of the power spectral analysis of RR-interval
variability.

Spontaneous Baroreflex Analysis

Details of this analysis have been described previously.3,12 Briefly,
the beat-by-beat time series of systolic AP (SAP) and RR interval are
scanned by a computer to identify sequences of ≥3 consecutive beats
in which SAP and RR interval of the following beat change in the
same direction (either increasing or decreasing). A linear regression
is applied to each individual sequence, and the mean slope of the
SAP/RR interval relationship, obtained by averaging all slopes
computed within a given test period, is calculated and taken as a
measure of the spontaneous baroreflex sensitivity (BRS) for that
period. This technique provided reproducible results during many
laboratory tests, including static exercise.16

Statistical Analysis

The significance of differences in the reported variables among the
different experimental periods was evaluated by ANOVA for re-
peated measures with Bonferroni adjustment for multiple compar-
isons. Values are presented as mean ± SE. Differences were consid-
ered statistically significant when P was <0.05.

Results

SLE produced a significant increase in AP and a significant
decrease in RR interval. During ALC, AP remained signifi-
cantly increased above resting levels, whereas RR interval
returned to control. On release of occlusion, AP also returned
to control (Table 1 and Figure 1).
The RR-interval variance was not significantly different from that at seated rest during both SLE and ALC. Spectral analysis of RR-interval variability demonstrated the presence of 2 clearly separated major oscillatory components in an LF and HF band in all experimental periods (Figure 2).

The LF component significantly increased above rest during SLE and remained elevated during ALC, both in absolute values and normalized units. It returned to baseline during recovery (Table 1). The power of the HF component was significant (Table 1) during SLE and remained elevated during ALC, both in absolute values and normalized units. It returned to baseline during ALC (14.8 ± 3.1, 9.1 ± 1.9, and 13.4 ± 3.1 ms/mm Hg during rest, SLE, and ALC, respectively; F = 10.43; P < 0.001). Relating SAP to HR instead of RR interval did not substantially modify the above results (−1.30 ± 0.21, −1.10 ± 0.18, and −1.24 ± 0.20 beats·min⁻¹·mm Hg⁻¹ during rest, SLE, and ALC, respectively; F = 3.84; P < 0.05). An example of spontaneous baroreflex from 1 subject during rest, SLE, and ALC is shown in Figure 3. No significant differences were found between +RR/+SAP and −RR/−SAP sequences either at rest (14.9 ± 2.7 versus 13.8 ± 3.2 ms/mm Hg) or during SLE (9.0 ± 2.1 versus 8.8 ± 1.7 ms/mm Hg). None of the considered variables were significantly affected by circulatory occlusion of the thigh without exercise (Table 2).

**Discussion**

The main findings of this study are 2-fold. The first is that in humans, the muscle metaboreflex contributes to HR regulation during static exercise through a sympathetic stimulation. The second new finding is that the arterial baroreflex may be involved in the recovery of HR during postexercise muscle ischemia despite the maintenance of sympathetic activation.

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**Figure 1.** Recording from 1 subject showing example of simultaneous time series of RR interval (RRI; top), mean AP (MAP; middle), and respiration (RESP; bottom) during SLE followed by arrested circulation of the exercising leg. Arrows from left to right indicate start of exercise and start and end of ALC, respectively. au indicates arbitrary units.

**TABLE 1.** Cardiovascular Responses and Spectrum Analysis of RR-Interval Variability During Exercise and Postexercise ALC

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>ALC</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP, mm Hg</td>
<td>118.2±2.9</td>
<td>144.7±5.7*</td>
<td>140.6±6.3*</td>
<td>121.7±3.8</td>
</tr>
<tr>
<td>DAP, mm Hg</td>
<td>74.8±2.7</td>
<td>90.3±3.6*</td>
<td>87.8±4.0*</td>
<td>75.0±2.9</td>
</tr>
<tr>
<td>RR interval, ms</td>
<td>817.9±45.3</td>
<td>687.8±33.2*</td>
<td>770.2±45.3</td>
<td>804.6±49.6</td>
</tr>
<tr>
<td>Variance, ms²</td>
<td>2952.4±1076.2</td>
<td>2804.3±765.7</td>
<td>3706.4±959.6</td>
<td>3658.1±1422.3</td>
</tr>
<tr>
<td>LF</td>
<td>1158.6±361.7</td>
<td>1991.9±492.2*</td>
<td>2409.3±684.6*</td>
<td>1289.5±479.8</td>
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<tr>
<td></td>
<td>63.8±6.1</td>
<td>82.8±4.5*</td>
<td>78.9±6.4*</td>
<td>63.3±5.5</td>
</tr>
<tr>
<td></td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
<td>0.08±0.01</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>HF</td>
<td>793.5±467.5</td>
<td>304.0±96.9</td>
<td>895.2±453.8</td>
<td>836.6±434.4</td>
</tr>
<tr>
<td></td>
<td>28.9±6.2</td>
<td>14.0±3.9*</td>
<td>19.0±6.2</td>
<td>28.7±5.0</td>
</tr>
<tr>
<td></td>
<td>0.28±0.01</td>
<td>0.31±0.02</td>
<td>0.27±0.02</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>Respiration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.28±0.01</td>
<td>0.31±0.02</td>
<td>0.29±0.02</td>
<td>0.28±0.02</td>
</tr>
</tbody>
</table>

DAP indicates diastolic AP; NU, normalized units; Hz, center frequency of oscillatory components of RR interval and respiratory variability signals. Values are mean±SE. *P<0.05 vs rest.

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The center frequency of both the LF and HF components did not change significantly during either SLE or ALC. The HF center frequency in the RR-interval variability spectrum was close to that in the respiratory variability signal (Table 1). During exercise, respiratory rate did not change significantly from the resting value of 0.28±0.02 Hz.

BRS was significantly decreased from rest during exercise and returned to control values during ALC (14.8±3.1, 9.1±1.9, and 13.4±3.1 ms/mm Hg during rest, SLE, and ALC, respectively; F = 10.43; P < 0.001). Related SAP to HR instead of RR interval did not substantially modify the above results (−1.30±0.21, −1.10±0.18, and −1.24±0.20 beats·min⁻¹·mm Hg⁻¹ during rest, SLE, and ALC, respectively; F = 3.84; P < 0.05). An example of spontaneous baroreflex from 1 subject during rest, SLE, and ALC is shown in Figure 3. No significant differences were found between +RR/+SAP and −RR/−SAP sequences either at rest (14.9±2.7 versus 13.8±3.2 ms/mm Hg) or during SLE (9.0±2.1 versus 8.8±1.7 ms/mm Hg). None of the considered variables were significantly affected by circulatory occlusion of the thigh without exercise (Table 2).

**TABLE 2.** Cardiac Response to Circulatory Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>ALC</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>145.2±3.9</td>
<td>147.2±3.6*</td>
<td>147.6±3.8*</td>
<td>148.1±3.6*</td>
</tr>
<tr>
<td>RR interval, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>817.9±45.3</td>
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<td>770.2±45.3</td>
<td>804.6±49.6</td>
</tr>
<tr>
<td>BRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hz</td>
<td>0.28±0.01</td>
<td>0.31±0.02</td>
<td>0.29±0.02</td>
<td>0.28±0.02</td>
</tr>
</tbody>
</table>

*P<0.05 vs rest.
A spectral analysis of short-term fluctuations of RR interval, 2 main components can be identified, in LF and HF bands, which are predominantly linked to a relative increase in sympathetic and parasympathetic activity, respectively.9,15,17–21

By applying this methodology, we observed a decrease in the HF component of the RR-interval spectra during SLE, in agreement with the results of Taylor et al22 during static handgrip. This would reflect the well-known decrease in parasympathetic outflow to sinus node occurring during exercise. Concomitantly with the decrease in HF oscillations, there was an increase in the lower-frequency oscillations of HR that, by reflecting an increased sympathetic modulation to the sinoatrial node, would indicate a significant contribution of this autonomic division to HR regulation during static exercise. The relevance of the sympathetic nervous system in regulating HR is further stressed by the fact that the increase in the LF component did occur despite the HF component that was present in sympathetic discharge variability,23 which could have restrained LF enhancement, because vagal outflow decreases during exercise. Interestingly, the increase in the LF component was significant both in absolute values and normalized units. In this context, it is of note that total RR-interval variance was not significantly changed from rest during exercise, probably because HR values attained during SLE were not so dramatic. Thus, in our study, extremes of stimulation of the sinus node did not occur, allowing us to emphasize the strengths of the spectral analysis technique24 rather than their limitations, at variance with other studies25,26 that used various protocols of dynamic exercise that drastically reduced HR variability. Obviously, large interindividual differences in total power or in DC noise present at rest may persist during exercise, making it necessary to use normalized units to better assess the distribution of power in defined spectral components.15,19,20 This aspect is exemplified by our results with the HF component. During postexercise circulatory occlusion, the RR interval returned to control, whereas AP remained significantly elevated above rest, indicating a maintained activation of the muscle metaboreflex. During ALC, the HF component returned to rest, whereas the LF component remained significantly elevated. After release of circulatory occlusion, the HF component did not feature significant changes from rest and ALC, whereas the LF component returned to control, indicating the muscle metaboreflex as the effective stimulus in maintaining elevation of the LF component during postexercise muscle ischemia. Overall, these results strongly suggest that the muscle metaboreflex contributes to HR regulation during static exercise via a sympathetic excitation, this latter being maintained during postexercise ischemia, similar to what occurs for the vasculature3,5 despite the return of HR to control levels.

The present study is the first to provide experimental indication that the muscle metaboreflex contributes to HR regulation during static exercise in humans and might contribute to the theory that muscle metaboreflex may even be able to increase muscle blood flow by increasing cardiac output in addition to increasing blood pressure by vasoconstriction.

**Baroreflex Control of HR**

We found that during exercise, BRS was significantly reduced, but it was restored during ALC. The restoration of BRS to the resting level during postexercise ischemia, at the same time as AP was kept elevated and close to the exercise level by the muscle metaboreflex, could indicate that a vagally mediated baroreflex mechanism was responsible for...
the return of HR toward resting levels despite the maintained sympathetic activation. In this setting, the increased parasympathetic outflow induced by the arterial baroreflex would overpower the metaboreflex-induced cardiac sympathetic excitation, because muscle metaboreflex exerts few direct influences on parasympathetic outflow.²

The possibility that the loss of central command at the cessation of exercise could contribute to HR recovery cannot be excluded. However, the results of Bull et al²⁷ concerning HR recovery, with a maintained pressor response, during muscle ischemia after electrically induced static contractions support the concept in the present study that removal of central command is not invariably the cause of HR recovery during postexercise circulatory occlusion.

During exercise, the decrease in arterial baroreflex gain was associated with an increase in the LF component of the RR-interval spectra. This observation is in line with a recent study by Cooley et al.,²¹ which showed that LF oscillations in RR interval can be generated in the absence of vagally mediated baroreflex input. These findings support the concept of a central origin of the LF component in RR-interval variability and favor the notion that the LF component is mainly linked to sympathetic activity.¹⁸–²¹

Our findings confirm and extend those obtained by O’Leary⁸ in dogs under the influence of autonomic blocking drugs during exercise. We did not use pharmacological manipulations of the autonomic nervous system for several reasons. For example, atropine would alter both baseline vagal tone²⁸ and HR response to static exercise.²⁹ Furthermore, atropine virtually eliminates spontaneous baroreflex slopes,³⁰ whereas β-blockade enhances baroreflex sensitivity.³¹,³² Finally, results obtained after selective autonomic blockade must be viewed with caution, because interference with the activity of one division of the autonomic nervous system might lead to compensatory changes in the other that could obscure the relative contribution of each of the 2 components. This could lead to ambiguous results, particularly when multiple (redundant) control mechanisms are integrated in producing the net responses, as during exercise.¹ Therefore, our experimental approach allowed a distinctive insight into the autonomic regulation of HR during static exercise by having used nonperturbational techniques, without artificially isolating the influence of the different neural pathways.

**Study Limitations**
We considered the possibility that our results could have been influenced by respiratory adjustments to exercise, because the HF RR-interval spectral power declines as breathing fre-
crease in respiratory rate was very small, amounting to ~2 breaths/min. This small change in breathing frequency should have minimally affected the decrease in the HF component observed during exercise.18 Moreover, the possibility that changes in parasympathetic activity at the cessation of exercise could have contributed to some extent to the maintained increase in the LF component during ALC cannot be absolutely excluded.18,23 However, the finding of no significant differences in HF spectral power (and in BRS as well) between ALC and recovery when, on the contrary, LF returned to control would argue against a substantial contribution of vagal mechanisms to LF spectra during ALC.

Finally, the spontaneous baroreflex method reflects responses to rapid, transient changes in AP that are vagally mediated, whereas it does not enable us to investigate the slower sympathetic component of the baroreflex. This could be important during exercise when, in contrast to during rest, a substantial baroreflex bradycardia has been reported to occur via sympathetic inhibition, whereas the remaining parasympathetic component in the baroreflex response appears to be reduced.34 However, this problem should have a greater effect on the baroreflex control of sinus node during exercise intensities greater than those used in the present investigation.34 In conclusion, the results of this study suggest that the muscle metaboreflex contributes to HR regulation during static exercise via a sympathetic activation that is maintained during postexercise muscle ischemia. The bradycardia that occurs during postexercise circulatory occlusion despite the maintained sympathetic stimulus may be explained by an arterial baroreflex-mediated increase in parasympathetic outflow to the sinus node that overpowers the metaboreflex-induced cardiac sympathetic activation.

References