Muscle metaboreflex contribution to cardiovascular regulation during dynamic exercise in microgravity: insights from mission STS-107 of the space shuttle Columbia

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One of the most important features of prolonged weightlessness is a progressive impairment of muscular function with a consequent decrease in exercise capacity. We tested the hypothesis that the impairment in musculo-skeletal function that occurs in microgravity results in a potentiation of the muscle metaboreflex mechanism and also affects baroreflex modulation of heart rate (HR) during exercise. Four astronauts participating in the 16 day Columbia shuttle mission (STS-107) were studied 72–71 days before launch and on days 12–13 in-flight. The protocol consisted of 6 min bicycle exercise at 50% of individual $\dot{V}_{O_2,max}$ followed by 4 min of postexercise leg circulatory occlusion (PECO). At rest, systolic (S) and diastolic (D) blood pressure (BP), R-R interval and baroreflex sensitivity (BRS) did not differ significantly between pre- and in-flight measurements. Both pre- and in-flight, SBP increased and R-R interval and BRS decreased during exercise, whereas DBP did not change. During PECO preflight, SBP and DBP were higher than at rest, whereas R-R interval and BRS recovered to resting levels. During PECO in-flight, SBP and DBP were significantly higher whereas R-R interval and BRS remained significantly lower than at rest. The part of the SBP response (Δ) that was maintained by PECO was significantly greater during spaceflight than before (34.5 ± 8.8 *versus* 13.8 ± 11.9 mmHg, $P = 0.03$). The tachycardic response to PECO was also significantly greater during spaceflight than preflight (-141.5 ± 25.2 *versus* -90.5 ± 33.3 ms, $P = 0.02$). This study suggests that the muscle metaboreflex is enhanced during dynamic exercise in space and that the potentiation of the muscle metaboreflex affects the vagally mediated arterial baroreflex contribution to HR control.

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One of the most important features of prolonged weightlessness is a progressive impairment of muscular function (Ferretti *et al.* 1997; Zhang *et al.* 1997). Physiological modifications related to exposure to microgravity, e.g. altered blood volume distribution (Leach *et al.* 1996; Perhonen *et al.* 2001*a*), impaired myocardial function

All the investigators involved in this study want to pay tribute to the memory of the astronauts who lost their lives during this mission and to their families who still live and have to daily face the sorrow of losing their loved ones. Michael P. Anderson, David M. Brown, Laurel Blair Salton Clark, Kalpana Chawla, Rick Husband, William McCool and Ilan Ramon were not only invaluable research mates and science professionals, but also unique companions of the international research team for the years during which this mission was prepared. The scientific evidence we have been able to gather in spite of the abrupt interruption of the mission is the result of their superb professional performance, and represents the best we can do to contribute to their lasting memory.

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(Perhonen *et al.* 2001*b*), and a decrease in stroke volume (Shykoff *et al.* 1996), in addition to the alterations in muscular function (Convertino, 1996; Levine *et al.* 1996), might contribute to the reduced physical exercise capacity commonly reported after space flight (Convertino, 1996; Levine *et al.* 1996). The interplay between muscular (Ferretti *et al.* 1997; Zhang *et al.* 1997), haemodynamic and body fluid modifications occurring in microgravity (Watenpaugh & Hargens, 1996) might also alter the neural control mechanisms underlying the cardiovascular responses to exercise that are normally operating on the ground.

Whether and in which way microgravity-induced impairment of the muscular function affects the neural mechanisms of cardiovascular regulation is still poorly understood. Cardiovascular and autonomic responses to exercise have been reported to be either impaired (Pagani *et al.* 2001; Spaak *et al.* 2001) or preserved after simulated (Essfeld *et al.* 1993; Kamiya *et al.* 2000) or actual (Fu *et al.* 2002) microgravity conditions. However, the relative contribution provided by the different neural mechanisms involved in cardiovascular regulation during exercise, namely central command and the reflex drive from contracting muscles (Mitchell, 1990), has been directly addressed by two studies only, which suggested an unaltered muscle metaboreflex control of cardiovascular and sympathetic responses during simulated (Kamiya *et al.* 2000) or actual microgravity (Fu *et al.* 2002). No study has until now addressed the occurrence of possible changes in baroreflex control of HR during exercise in space.

All the studies performed so far dealing with cardiovascular responses to exercise during spaceflight have used static exercise of small muscle masses (i.e. handgrip) as a convenient model of muscular exercise. However, microgravity-induced muscular impairment involves mainly the antigravity muscles, i.e. leg muscles, which are those primarily involved in the reduced exercise capacity, whereas arm muscles are not ordinarily weight bearing and hence are less representative of the marked changes in the musculo-skeletal system induced by the microgravity environment (Ferretti *et al.* 1997; Zhang *et al.* 1997). In addition, cardiovascular responses to exercise and the underlying mechanisms are strongly dependant on the exercise mode, i.e. static *versus* dynamic, and the size of the muscle mass involved (Mitchell *et al.* 1980; Iellamo *et al.* 1999*a*).

Therefore our study was originally designed to investigate the cardiovascular responses to whole body dynamic exercise during and after spaceflight to test the hypothesis that the impairment in musculo-skeletal function that may occur in microgravity, e.g. decrease O_2 delivery and oxidative enzyme activity (Ferretti *et al.* 1997), results in a potentiation of the muscle metaboreflex mechanism during whole body dynamic exercise. In addition, since the arterial baroreflex plays a pivotal role in cardiovascular regulation during exercise (O'Leary, 1996; Iellamo, 2001*a*) and an impairment in arterial baroreflex control of heart rate has been reported in the resting state during spaceflight (Fritsch-Yelle *et al.* 1992), we also investigated whether and in which way the neural mechanisms underlying cardiovascular responses to whole body dynamic exercise affect the integrated baroreflex control of the sinoatrial node.

To accomplish this goal, moderate intensity, constant load bicycle exercise followed by postexercise occlusion of leg circulation to maintain the chemical stimulation of muscle afferents (i.e. metaboreflex activation) was planned to be performed before, during and after prolonged space flight, in the context of the ESA–NASA mission STS-107 of the space shuttle Columbia. The tragic end of the mission, involving the loss of the shuttle and its crew, prevented completion of the experimental protocol. The present paper therefore includes the results obtained during the ground experiments preceding launch and those from the experimental sessions performed during space flight in the context of this mission only.

Methods

Subjects

We studied four astronauts (three males and one female) of the 16 day mission STS-107 of the space shuttle Columbia. Their mean age was 45 years (range 42–49). All subjects were in excellent health, as determined by the National Aeronautics and Space Administration (NASA) medical evaluation board. The subjects gave their written consent to the experimental procedures after being informed of their aim and nature. The study was conducted under the guidelines issued by the NASA Johnson Space Center Human Research Policies and Procedure Committee and was approved by the Institutional Review Board at the institutions of the principal investigators. It conformed with the Declaration of Helsinki.

Instrumentation and experimental protocol

Experiments were performed on days 72–71 before launch and on days 12–13 in-flight. During the experiments the following signals were recorded: (1) continuous arterial pressure at the finger level by a modified Portapres device (Cardipres, Finapres Medical System, Aharnem, the Netherlands), (2) R-R interval from ECG (1 channel from Portapres), and (3) a respiratory signal from an inductance plethysmograph. All measuring devices were included in the Advanced Respiratory Monitoring System (ARMS, Damec, Odense, Denmark) module, a research facility provided by the European Space Agency which was available on the ground and on board the shuttle mission STS-107. This system is a multiuser facility

supporting human physiology research by measuring gas compositions during respiration of different gas mixtures, heart rate (HR), blood pressure (BP) and respiratory rate. Both pre- and in-flight, the study protocol consisted of 5 min rest followed by 6 min bicycle exercise at 50% of the workload producing the individual's maximal $\dot{V}_{O_2}$ at 1 *g* 15 s before cessation of exercise two pneumatic cuffs placed, as high as possible, on the thighs were automatically inflated to suprasystolic pressure and leg postexercise circulatory occlusion (PECO) was maintained for 4 min after the end of exercise. Exercise was performed in the seated posture both pre- and in-flight.

Data analysis

No special data pretreatment was required for our experiments. Data acquisition, digitalization (at 12 bit resolution and 100 Hz sampling rate) and storage were performed by the standard software which controlled ARMS during the experiments. In-flight signals were down-linked to Earth in real time to allow investigators to monitor the quality of the recordings and, in case of problems, to propose adequate solutions while the experiments were in progress, and this allowed the rescue of most of the in-flight data in spite of the loss of Columbia before landing.

Systolic (S) BP and diastolic (D) BP and the R-R interval were derived beat-by-beat from BP and ECG signals. All beat-by-beat values recorded during rest, exercise and PECO were averaged, separately for each experimental condition.

Analysis of baroreflex sensitivity

Baroreflex sensitivity (BRS) was dynamically assessed by the sequence technique as previously reported (Di Rienzo *et al.* 1985; Bertinieri *et al.* 1988; Parati *et al.* 1988; Iellamo *et al.* 1999*a*). Briefly, this technique is based on automatic scanning of the systolic (S) BP and R-R interval series, searching for spontaneous sequences of three or more consecutive heart beats characterized by a progressive increase in SBP, accompanied, with a one beat lag, by a progressive R-R interval lengthening or, *vice versa*, by a progressive SBP reduction followed by R-R interval shortening. For each sequence, the regression line is computed between SBP and R-R interval values, and the mean slope of the SBP–R-R interval relationship, obtained by averaging all slopes computed within a given test period, is calculated and taken as a measure of the average spontaneous BRS for that period.

Statistical analysis

Each variable was checked for normality of distribution by the Kolmogorov-Smirnof test. Since all variables were normally distributed, the significance of differences in the reported variables among rest, exercise and PECO (both pre- and in-flight) was evaluated by analysis of variance for repeated measures. Pairwise multiple comparison procedures were performed by the Student-Newman-Keuls test. Differences between pre- and in-flight data were compared by Student's paired *t* test. Values are presented as means ± S.E.M. Differences were considered statistically significant when P was < 0.05.

Results

Cardiovascular responses to exercise and postexercise leg circulatory occlusion

Pre-flight average maximal $\dot{V}_{O_2}$ of the four astronauts was 2684 ± 367.1 ml min^{-1}. Individual cardiovascular responses to exercise and PECO before and during spaceflight are reported in Table 1. Baseline cardiovascular variables did not differ significantly between pre- and in-flight measurements. Before spaceflight, during exercise R-R interval was significantly reduced, and SBP increased, although the increase did not reach statistical significance, whereas diastolic (D) BP did not change from resting values (Figs 1 and 2). During postexercise circulatory occlusion, R-R interval recovered toward resting values (Fig. 3), whereas SBP remained higher than the baseline level (albeit not significantly) and DBP significantly increased above exercise and resting values (Figs 1 and 2).

During spaceflight, SBP increased significantly during exercise, and remained significantly higher than at rest during PECO. DBP did not change from rest during exercise, but increased significantly above exercise and resting values during PECO (Figs 1 and 2). R-R interval decreased significantly during exercise, and remained significantly lower than the resting level during PECO (Fig. 3). The rise in SBP which occurred from rest to exercise and was maintained by PECO was significantly greater during spaceflight than before. A similar, albeit not statistically significant, trend was observed for DBP (Fig. 4). The tachycardic response to PECO was also significantly greater during spaceflight than preflight (-141.5 ± 25.2 *versus* -90.5 ± 33.3 ms, $P = 0.02$).

In baseline conditions, BRS decreased significantly from rest during exercise and recovered to resting level during PECO. During spaceflight, BRS decreased significantly during exercise and remained significantly lower than at rest also during PECO (Fig. 5).

No significant differences were detected in the number of spontaneous baroreflex sequences between preflight and in-flight in any experimental conditions (rest: 14.0 ± 4.5 *versus* 11 ± 5; exercise: 17.0 ± 2.1 *versus* 11.0 ± 2.2; PECO: 27.3 ± 2.7 *versus* 18.0 ± 3.7, $P > 0.05$ for all comparisons).

Table 1. Individual cardiovascular responses to exercise and postexercise muscle ischaemia

	Pre-flight			In-flight		
Astronaut	Baseline	Exercise	PECO	Baseline	Exercise	PECO
SBP (mmHg)						
1 (m)	126	149	166	168	185	217
2 (m)	107	136	130	106	143	143
3 (m)	122	162	130	114	165	157
4 (f)	125	135	109	102	111	111
Mean ± S.E.M.	120.0 ± 4.4	145.5 ± 6.4	134 ± 11.8	122.5 ± 15.4	151.0 ± 15.8*	157.0 ± 22.2*
DBP (mmHg)						
1	66	62	79	104	101	127
2	71	70	81	65	69	81
3	71	63	70	66	69	84
4	72	73	74	72	73	74
Mean ± S.E.M.	70.0 ± 1.4	67.0 ± 2.7	76.0 ± 2.5*	76.8 ± 9.2	78.0 ± 7.7	91.5 ± 12.0*
R-R interval (ms)						
1	639	494	482	611	451	425
2	694	575	561	730	508	560
3	829	618	817	770	497	698
4	578	481	518	538	497	400
Mean ± S.E.M.	685.0 ± 53.5	542.0 ± 32.8*	594.5 ± 75.9	662.3 ± 53.4	488.3 ± 12.7*	520.8 ± 68.7*
BRS (ms mmHg^{-1})						
1	5.0	1.8	3.0	4.0	2.0	1.5
2	3.8	3.2	4.4	8.2	4.7	5.0
3	3.8	1.2	4.5	4.7	4.5	3.8
4	4.9	2.4	4.0	6.4	3.4	3.4
Mean ± S.E.M.	4.4 ± 0.3	2.2* ± 0.4	4.0 ± 0.3	5.8 ± 0.9	3.6* ± 0.6	3.4 ± 0.7*

m, male; f, female; SBP, systolic blood pressure; DBP, diastolic blood pressure; BRS, baroreflex sensitivity; PECO, postexercise circulatory occlusion. *$P < 0.05$ *versus* baseline.

Discussion

This is the first study to investigate the neural mechanisms of cardiovascular regulation during whole-body dynamic exercise in space. The main findings of the present study are (1) that a microgravity environment results in an enhancement of the muscle metaboreflex mechanism in the regulation of the cardiovascular responses to dynamic exercise, and (2) that the microgravity environment affects the modulatory effect of the muscle metaboreflex on the integrated baroreflex control of the sinus node, with the latter mechanism being possibly involved in the cardiovascular response to dynamic exercise.

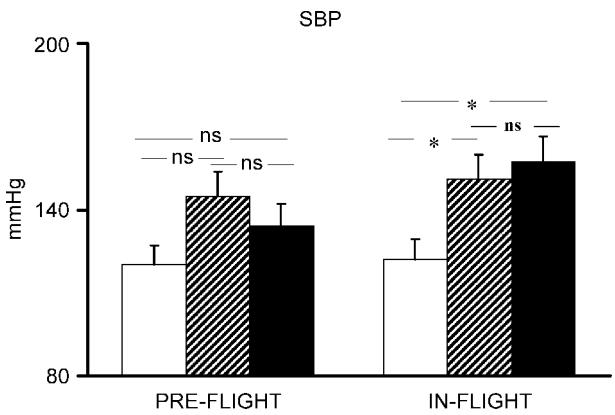

Figure 1. SBP response to exercise and postexercise circulatory occlusion preflight and in-flight
Open bars indicate rest; hatched bars, exercise; filled bars, postexercise circulatory occlusion. *$P < 0.05$.

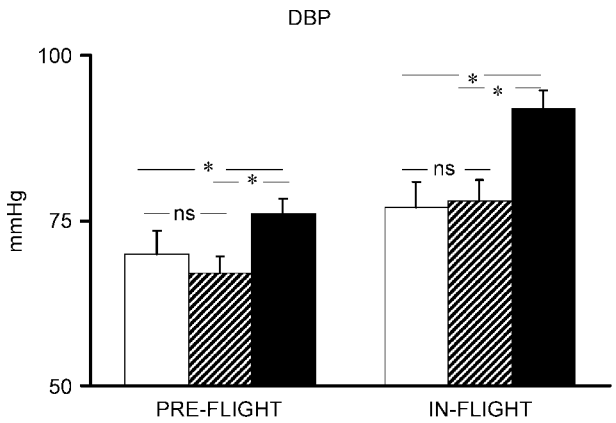

Figure 2. DBP response to exercise and postexercise circulatory occlusion preflight and in-flight
Open bars indicate rest; hatched bars, exercise; filled bars, postexercise circulatory occlusion. *$P < 0.05$.

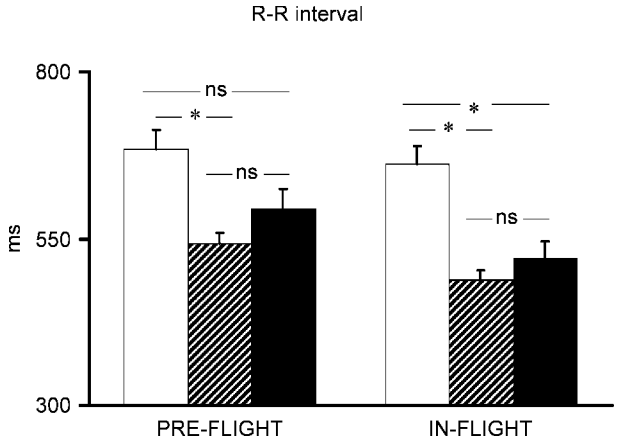

Figure 3. R-R interval response to exercise and postexercise circulatory occlusion preflight and in-flight
Open bars indicate rest; hatched bars, exercise; filled bars, postexercise circulatory occlusion. *$P < 0.05$.

Role of the muscle metaboreflex

When blood flow and oxygen delivery to contracting muscles are insufficient for the rate of metabolism, chemical products of muscle metabolism accumulate within the muscle and stimulate group III and IV afferents (Rotto & Kaufman, 1988). Activation of these afferents elicits a reflex increase in sympathetic activity to the heart and vasculature which increases HR and BP; this is termed the muscle metaboreflex (Kaufman *et al.* 1983; Mitchell, 1990). One of the most important consequences of a prolonged stay in a microgravity environment is a progressive impairment of the musculo-skeletal function, which becomes manifest as muscle atrophy and as functional changes in quadriceps muscle fibres, such as decreased oxidative enzyme activity, decreased mitochondrial volume density and decreased capillary length leading to decreased O_2 delivery to active muscles (Ferretti *et al.* 1997). These alterations are associated with a relative decrease in stroke volume at submaximal exercise work loads (Shykoff *et al.* 1996), resulting in a decreased O_2 transport capacity by the cardiovascular system which outweighs the decrease in $\dot{V}_{O_2}$ (Ferretti *et al.* 1997). The decrease in O_2 delivery and O_2 transport capacity to the active muscles might result in a greater engagement of the muscle metaboreflex during dynamic exercise (Sheriff *et al.* 1987). The volume reduction surrounding the interstitial muscle receptors, as a consequence of the relative dehydratation during spaceflight, which would affect mainly the lower body, might act in the same direction (Essfeld *et al.* 1993). Our results of a maintained and even increased SBP and DBP over and above resting and even exercise levels at the time of PECO during spaceflight would indicate that the muscle metaboreflex is enhanced during dynamic (bicycle) exercise in space. The part of the pressor and tachycardic responses (i.e. change from rest) that were maintained by PECO (i.e. by the muscle metaboreflex) was greater during spaceflight than on the ground, further supporting the conclusion of an augmented muscle metaboreflex activation during dynamic exercise in space. The possibility that some deconditioning might have resulted in a relatively greater involvement in central command signals to maintain the target level of effort, i.e. the work load at 50% of $\dot{V}_{O_2,max}$ determined on the ground, cannot be totally ruled out. However, we did not expect that actual workload during flight would be a relatively higher fraction of maximal O_2 consumption as determined preflight, since the decline in $\dot{V}_{O_2}$ at submaximal workload in space is small (Levine *et al.* 1996) and prolonged bed rest (42 days) led to a greater decrease in cardiovascular O_2 transport capacity, as a consequence of muscular alterations, than reduction in $\dot{V}_{O_2,max}$ (Ferretti *et al.* 1997).

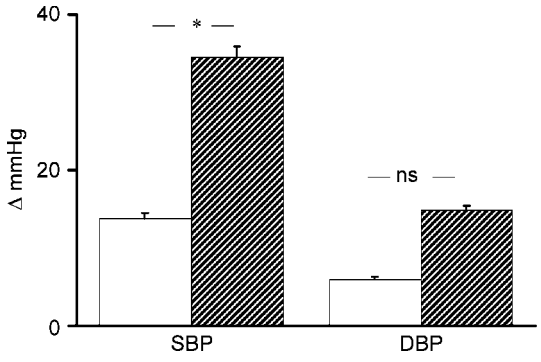

Figure 4. Changes (Δ) in exercise-induced SBP and DBP increase that were maintained by postexercise circulatory occlusion preflight (open bars) and in-flight (hatched bars)
*$P < 0.05$. *versus* preflight

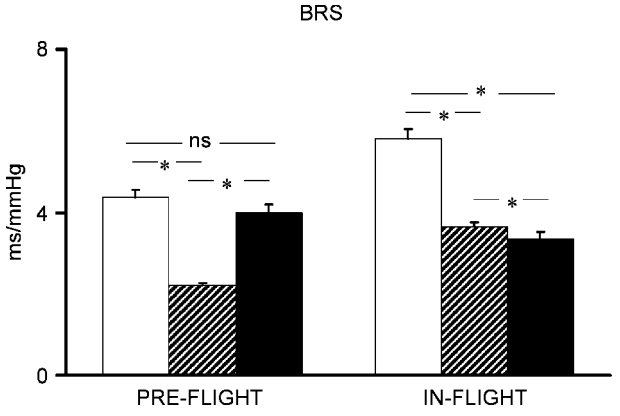

Figure 5. BRS response to exercise and postexercise circulatory occlusion preflight and in-flight
Open bars indicate rest; hatched bars, exercise; filled bars, postexercise circulatory occlusion. *$P < 0.05$.

Our finding of an enhanced muscle metaboreflex in space is partially at variance with previous reports of unaltered muscle metaboreflex contribution to cardiovascular regulation during exposure to simulated (Spaak *et al.* 2001) or actual (Fu *et al.* 2002) microgravity. The most likely explanation for these different findings relates both to the different mode of exercise and to the different muscle masses under investigation. Previous studies examined cardiovascular and neural responses to static exercise and postexercise circulatory occlusion of small (forearm) muscles (Kamiya *et al.* 2000; Fu *et al.* 2002) whereas we studied the responses to dynamic exercise of larger (leg) muscle masses. Both the type of exercise and the muscle masses engaged in muscular activity are strong determinants of both the cardiovascular responses and the relative contribution afforded by the different neural mechanisms involved in cardiovascular regulation (Mitchell *et al.* 1980; Galbo *et al.* 1987; Seals, 1993; Iellamo *et al.* 1997, 1999*a*). For example, HR regulating mechanisms are substantially different during dynamic as opposed to static exercise, with a predominant role of reflex drive from muscles in the former and of central command in the latter (Galbo *et al.* 1987; Victor *et al.* 1987; Victor & Seals, 1989; Rowell & O'Leary, 1990). In addition, the alteration in muscle function and structure induced by microgravity involves mainly the antigravity muscles, that is, leg rather than arm muscles, and this alteration would provide the substrate for the augmented muscle metaboreflex we observed. We speculate that the enhancement of the muscle metaboreflex might occur in the attempt to compensate, in part, for the reduced O_2 transport capacity occurring after exposure to a microgravity environment (Convertino, 1996; Shykoff *et al.* 1996; Ferretti *et al.* 1997). In fact, the metaboreflex has the capability to increase ventricular performance and O_2 delivery to active skeletal muscles during dynamic exercise and to induce peripheral vasoconstriction in non-active regions (O'Leary & Augustyniak 1998; O'Leary *et al.* 1999; Augustyniak *et al.* 2000), with the aim of eliminating any existing mismatch between O_2 delivery and O_2 demand in the exercising skeletal muscle during moderate intensity dynamic exercise. It is worth noting that a potentiation of the muscle metaboreflex has been reported in several (Piepoli *et al.* 1996; Greve *et al.* 1999; Hammond *et al.* 2000), although not all (Stern *et al.* 1991; Middlekauff *et al.* 1985, 2001; Sinoway & Li, 2005), studies in patients with heart failure, who feature some haemodynamic and muscles alterations (Lipkin *et al.* 1988; Sullivan *et al.* 1990; Drexler *et al.* 1992) like those ensuing in astronauts during spaceflight.

Unfortunately, missing data from the experiments that we could not perform after return to earth gravity prevents a full definition of the role of the muscle metaboreflex in regulating the cardiovascular responses to dynamic exercise on re-entry after microgravity exposure.

Role of the arterial baroreflex

In keeping with some (Bristow *et al.* 1971; Pagani *et al.* 1988; Lucini *et al.* 1995; Iellamo *et al.* 1998) but not all (Potts *et al.* 1993; Papelier *et al.* 1994; Norton *et al.* 1999; Ogoh *et al.* 2005) previous studies, before spaceflight we observed a decrease in BRS during dynamic exercise which recovered to resting levels during muscle metaboreflex activation by PECO. Differences in baroreflex testing methodology might account for these different results (see Iellamo, 2001*a*,*b* for review of this topic). Contrary to this, during exercise in microgravity the decrease in BRS was still maintained during the muscle metaboreflex activation, when central command (and muscle mechanoreceptor stimulation as well) was absent. These findings would indicate that a greater engagement of the muscle metaboreflex, as in microgravity conditions, with the attendant increase in sympathetic activity (Mark *et al.* 1985; Victor & Seals, 1989; O'Leary, 1993; O'Leary & Augustyniak 1998), is capable of blunting the integrated baroreflex mechanisms controlling heart period (Pagani *et al.* 1982; Gnecchi-Ruscone *et al.* 1987). The blunted reflex control of HR during exercise could be also due, at least in part, to central influences overcoming reflex HR modulation and aimed at increasing cardiac output, while preserving the reflex control of BP, thus excluding a full resetting of the arterial baroreflex (Mancia *et al.* 1982). However, central command was surely not operating during PECO. The maintained inhibition of the vagal–cardiac baroreflex modulation by the muscle metaboreflex could have contributed to the greater tachycardic response to PECO observed during spaceflight.

At variance with previous studies (Fritsch-Yelle *et al.* 1992) we did not observe significant changes in resting BRS during flight compared to preflight. Again, differences in baroreflex testing methodology might account for this different result. Previous studies (Fritsch-Yelle *et al.* 1992) employed the neck collar to assess the carotid–cardiac baroreceptor reflex whereas we evaluated the integrated arterial baroreceptor–HR reflex at the current, operating level of BP and HR. One advantage of the sequences technique is that it allows assessment of the baroreflex modulation of HR dynamically, relying on a natural stimulus of physiological magnitude, that is, spontaneous BP increases and decreases, without having to induce any pharmacological or mechanical disturbance from outside the cardiovascular system. As such, it appears particularly suitable for employment during space missions (Parati

et al. 2000). However, the spontaneous baroreflex method we used reflects the responses to rapid, transient changes in arterial pressure, which are vagally mediated, while it does not allow investigation of the slower sympathetic component of the baroreceptor–cardiac reflex (Ogoh *et al.* 2005).

How central command and the reflex drive from muscles affect the cardiac component of the arterial baroreflex during exercise is a surely complex and still undefined issue. Experimental evidence indicates that both these mechanisms are capable of modulating the arterial baroreflex during exercise, causing either a 'resetting' or a decrease in the gain of the baroreceptor–cardiac reflex (Iellamo, 2001*a*,*b*; Ogoh *et al.* 2002). It has been argued that such a modulating effect in the direction of 'resetting' or decreasing BRS is strongly dependent on the type as well as the intensity and the size of muscle masses engaged in exercise (Iellamo, 2001*a*). The maintained decrease in BRS during PECO during spaceflight, which was accompanied by greater tachycardic and pressor responses, would suggest that greater metaboreflex-induced sympathetic activation could be an adequate stimulus for decreasing the gain of the integrated baroreceptor–cardiac reflex. In this context, it may be pertinent to recall that sympathetic activation normally opposes the negative feedback mechanisms of baroreflex origin (Pagani *et al.* 1982; Gnecchi-Ruscone *et al.* 1987; Ferrari *et al.* 1991). On the other hand, the possibility of a direct action of the muscle metaboreflex on parasympathetic pathways seems unlikely because the metaboreflex operates principally via changes in sympathetic activity with little control over parasympathetic activity (O'Leary, 1993). The question of whether and to what extent muscle mechanoreceptor stimulation might contribute to the decrease in BRS observed during exercise cannot be answered by the present investigation. However, pure muscle mechano-reflexes were surely not engaged during postexercise circulatory occlusion.

The interest of the contribution provided by our paper relies on the fact that, to the best of our knowledge, it offers for the first time data on baroreflex modulation of the sinoatrial node during exercise and related mechanisms under actual microgravity conditions.

Limitations of the study

Some limitations of this study should be acknowledged. The first limitation is the small number of subjects, which is, however, unavoidable on space flight missions and is a feature of all previous papers published in this difficult field. Hence, the physiological interindividual variability of the results may have prevented the achievement of statistical significance in some instances, despite the appearance of clear trends. Our results on SBP response to exercise in baseline conditions is just an example of this. For this reason we have presented also individual data.

There are other possible confounding factors to be considered in the interpretation of our results, which are, however, a common feature of all manned space missions. These include, first, performing experiments in a shuttle environment, which is remarkably different from that of a standardized research laboratory; second, the psychological excitement which may be present both during baseline data collection and even more so during space flight, possibly leading to emotional arousal and interference with the subtle mechanisms involved in reflex cardiac regulation; third, the uninterrupted interaction with other crew members and the ground control staff, which might also have added noise to the physiological recordings; and, finally, the possible after-effects of previously performed experiments also planned during the mission on measurements that were supposed to be taken in 'control' conditions. All these factors, might have affected the results of our study, and might explain, for example, the low resting BRS values we observed both pre- and in-flight (Iellamo *et al.* 2002; Lucini *et al.* 2002). In an attempt to minimize as much as possible their effects, all the experiments, both pre- and in-flight, followed the same order, and were planned to be performed at approximately the same time during the day. Nevertheless, some of the above factors could have escaped control. Despite these limitations, we found consistent changes in the muscle metaboreflex and arterial baroreflex modulation of the sinus node during exercise in space as compared to baseline, which supports the physiological reliability of our data.

Conclusion

In conclusion, we found that the muscle metaboreflex contribution to cardiovascular regulation during moderate intensity dynamic leg exercise is enhanced during spaceflight and that such enhancement of the muscle metaboreflex affects the integrated baroreflex control of sinus node, suggesting that the latter mechanism may be involved in the cardiovascular response to dynamic exercise in actual microgravity conditions. We speculate that the enhancement of the muscle metaboreflex is a response to the reduced O_2 transport occurring after exposure to microgravity, with the aim of counteracting any existing mismatch between O_2 delivery and O_2 demand in the exercising skeletal muscle during moderate intensity dynamic exercise.

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