Hormones and Biomarkers in Student Pilots Before and After Rehabilitation from Airsickness

Anita Greco; Paola Verde; Chiara De Nuccio; Camilla Spanu; Luisa Minghetti; Marco Lucertini

INTRODUCTION: Airsickness (AS) affects many aviators and has been associated with hormonal and other biomarker variations. An analysis of hormones and biomarkers potentially predicting an individual's adaptation to AS was performed.

METHODS: Plasma levels of vasopressin, cortisol, ghrelin, C-reactive protein, substance P, antioxidant capacity, and 15-F2t-isoprostane were analyzed in seven student pilots (five men and two women) affected with incapacitating AS and undergoing a rehabilitation program. Peripheral blood was sampled before and after a nauseogenic Coriolis Stress Test (CST) at the beginning and end of rehabilitation.

RESULTS: All individuals were sensitive and vomited upon initial CST, while no symptoms were provoked by the final one. No significant differences between men and women were observed. After return to real flight activity, one man was still affected with AS (fail case). Higher levels of vasopressin and ghrelin were detected in this individual before the initial CST, with respect to the rest of the sample. A cortisol peak was observed in all subjects after the initial CST (average from 6288–29,861 pg · mL^{-1}), but only in the fail case at the final CST (from 10,040–63,050 pg · mL^{-1}). No relevant changes were observed for C-reactive protein, substance P, and antioxidant capacity, but 15-F2t-isoprostane was significantly reduced after rehabilitation in all subjects with respect to the first recording.

DISCUSSION: Although various hormonal/biomarker changes can be observed during rehabilitation from AS, cortisol plasma levels were noted as a potentially promising parameter for predicting the success of desensitization.

KEYWORDS: vestibular system, motion sickness, endocrine system, nausea and vomiting.

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Motion sickness (MS) represents a common issue in aviation medicine, with the absolute incidence ranging from about 10 to almost 40%, as reported in several studies conducted on student pilots[1–3] and civil aviation passengers.[4]

Under different provocative stimuli, MS has also been associated with derangements of hormonal plasma levels[5–7] and of other biomarkers,[8,9] which have been pointed out as further indicators of the human multisystem response to a provocative motion environment. These complex changes might also be an interesting tool in the analysis of MS time course, potentially representing a reliable marker of the individual's adaptation process.

In the case of airsickness (AS), such an aspect might play a particularly significant role in those few individuals who do not rapidly adapt to the flight environment (so called slow adaptors), who represent the potential target for specific countermeasures against the incapacitating effects of AS on aircrew performance and readiness.[1,10,11]

Although the link between vestibular system and MS has been fully demonstrated (for a review, see Golding[12]), currently no easy, inexpensive, and rapid laboratory tests can adequately identify slow adaptors and their response to a rehabilitative approach.[13] Thus, in this study some hormonal and biochemical parameters potentially influenced by the adaptation process to MS were investigated in a group of student pilots severely affected with AS undergoing a dedicated rehabilitation program.

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From a practical point of view, most of the rehabilitation programs aim at accelerating the adaptation process with the use of a nauseogenic stimulus within a laboratory setting, as induced by specific exercises like the head tilt while rotating in the yaw axis on a rotatory chair, with consequent onset of a strongly nauseogenic Coriolis effect.[14] Since hormonal and metabolic parameters variations are among the possible sources of biomarkers for neuro-otology and the link between hormonal derangements and vestibular related disorders (MS included) has been adequately reported (for a review, see El Khaiti et al.[15]), research of parameters potentially predicting the rehabilitation outcome might provide useful tools in approaching this specific category of patients. Moreover, findings in this area of research could help in gaining a better insight into the complex human response to a nauseogenic environment.

METHODS

Subjects

Seven student pilots (five men and two women, with an age range of 20–27 yr) from the Italian Air Force (ItAF) Flight Training School at Latina AFB exhibiting recurrent episodes of incapacitating AS during their flight certification course were enrolled for the present research. They were all physically fit for flight duties according to ItAF regulations, and none was affected with audio-vestibular disorders during a standard clinical investigation (including patient's history, pure tone audiometry, impedance test, and caloric stimulation). Moreover, they were not using any medication and over-the-counter pharmaceuticals (i.e., without prescription).

The experimental protocol adhered to the principles of the Declaration of Helsinki and was approved by the local Institutional Review Board (Rome University: no. 2055/2020). All individuals participating in the study signed an informed consent and their participation in this experiment could be interrupted at any moment without any consequences for the rehabilitation program. The participants were asked to consent to undergo four venipunctures for the acquisition of blood samples; the first two were performed before, and the second ones at the end of the rehabilitation program. This aspect was the sole difference from the standard ItAF treatment for AS. All subjects were regularly referred by the local flight surgeon to the ItAF Aerospace Medicine Department at Pratica di Mare AFB to undergo an AS desensitization, according to the standard procedure described elsewhere[11].

Equipment and Materials

The analysis of each plasma sample was conducted by immunoenzymatic or spectrophotometric assay, according to the characteristics of the single hormone/biomarker, and following manufacturer instructions. All samples were analyzed in duplicate for data confirmation.

In more detail, arginine vasopressine (AVP) and ghrelin (GHRE) were measured by enzyme immunoassay kits (Phoenix Pharmaceuticals, Burlingame, CA, United States). For AVP. the detection limit was <70 pg · mL^{-1}; range of linearity: 70–830 pg · mL^{-1}; and intra- and interassay coefficients of variance (CVs) <15%. For GHRE, the detection limit was 130 pg · mL^{-1}; range of linearity: 130–1610 pg · mL^{-1}; and intra- and interassay CVs <10%.

Cortisol (CORT) was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Arbor, MI, United States); the detection limit was 35 pg · mL^{-1}; range of linearity: 100–4000 pg · mL^{-1}; and intra- and interassay CVs <10%. C-reactive protein (CRP) was measured by ELISA (Elabscience, Houston, TX, United States); the detection limit was 9 pg · mL^{-1}; range of linearity: 15.6–1000 pg · mL^{-1}; and intra- and interassay CVs <10%. Substance P (SP) was measured by an ELISA kit (ElabScience); the detection limit was 47 pg · mL^{-1}; range of linearity: 58–5000 pg · mL^{-1}; and intra- and interassay CVs <10%.

The antioxidant capacity (AOC) quantification was determined with the use of an assay kit (P.A.O., MED.DIA, San Germano Vercellese, Italy) which evaluated the reduction of copper (Cu) from Cu++ to Cu+ by the activity of all antioxidants present in the sample. The assay was found to be linear from 1–1000 nmol · L^{-1} of uric acid (r = 0.99, P < 0.001). Sensitivity was 22 nmol · L^{-1} of reductive capacity. Both intra- and interassay variability showed a CV lower than 4%.

The 15-F2t-Isoprostane (IsoP) level was measured with an enzyme immunoassay kit (Cayman Chemical) with a detection limit of 2 pg · mL^{-1}, range of linearity of 2–500 pg · mL^{-1}, an intra- and interassay CV <10%, and an anti-15-F2t-IsoP antibody cross-reactivity with other IsoPs < 2%. The method for AOC and IsoP analysis in the control group was exactly the same.

Procedure

In agreement with the ItAF protocol, each individual was preliminarily evaluated with a Coriolis Stress Test (CST), during which the subject is seated on a rotatory chair with his/her eyes closed, and performs active movements in pitch (one forth and back head tilt every 4 s) while rotating in the yaw axis. The Coriolis' effect evoked by this procedure is highly nauseogenic and disorienting,[14] and triggers an adaptation process that in most cases also results in a desensitization from AS[3,10,16].

The CST includes four consecutive steps separated by three 5-min intervals, with clockwise (CW) and counter-clockwise (CCW) directions of chair rotation, at two different speeds (90° and 150° · s^{-1}). The standard CST sequence is: 2 min of CW rotation at 90° · s^{-1} (step 1); 2 min of CCW rotation at 90° · s^{-1} (step 2); 3 min of CW rotation at 150° · s^{-1} (step 3); and 3 min of CCW rotation at 150° · s^{-1} (step 4).[11]

The patient's status is continuously monitored via a Misery Scale (MiSc), which is a specifically validated questionnaire indicating the level of discomfort, ranging from 0 (well-being) to 10 (vomiting).[17] The completion or not of the CST (due to a vomiting episode or to the patient's severe discomfort) and the score reached at the end of each session is a helpful baseline to calibrate the level of the nauseogenic stimulus to be adopted at the beginning of the desensitization program.

Despite the lack of predictive value for the actual success of rehabilitation, as documented in a previous report,[16] in this

study the CST was repeated in all subjects at the end of rehabilitation (CST2) to generate a vestibular stimulus identical to the preliminary one (CST1) under the same laboratory conditions. Blood samples were also collected before and after this second CST (pre- and post-CST2). Globally, a complete CST (i.e., when the sequence is not interrupted by the patient's discomfort and/or vomiting) lasts about 30 min.

According to the standard ItAF rehabilitation approach, after the CST1, a personalized ground-based desensitization program involving increasingly more intense nauseogenic exercises on a rotatory chair is administered. This program implies a progressive increase of the speed rotation, with the initial speed setting and head movement rate based upon the subject's behavior throughout CST1.

During the rehabilitation program (i.e., from day 2 to day 9 in **Fig. 1**), active head movements, both in pitch and roll in a randomized way according to the operator's indication, are performed by the subject during rotation in the yaw axis to provoke a nauseogenic stimulus, which is continuously repeated at a rate of one complete cycle (i.e., head tilt and back to upright) every 4–5 s for at least 10 min, unless interrupted by a vomiting episode. The speed of rotation is progressively increased according to a subject's capability of adapting to the nauseogenic stimulus. Once the final target chair velocity of $120° \cdot s^{-1}$ is reached (both CW and CCW) without the onset of significant symptoms (i.e., MiSc level ≤3) for at least 10 min of exposure, other exercises are also administered, with the use of different tools, as an off-axis rotation (i.e., adding a linear component), or a sustained 360° rotation in roll at different speeds. Although the main part of the rehabilitation program is the standard exercises on the rotatory chair, in our experience, the adding of different types of stimuli is initially strongly nauseogenic in most cases, and followed by a further very rapid process of adaptation. The aim of this part of the rehabilitation process is the increase in the number of stimuli participating in the desensitization process, facilitating the subject's capability of coping with new and unexpected environmental situations. The duration of this program is 2 wk (i.e., 10 working days), and no anti-MS medications or over-the-counter pharmaceuticals are administered.

The goal of this protocol is the resolution of incapacitating AS episodes, which could potentially impair the student pilot's performance during real flight missions conducted under the instructor pilots' supervision. Thus, the resolution of incapacitating AS is the only parameter actually considered for a final evaluation of the individual outcome,[16] separating the successful cases from failures. This type of information is obtained by the local flight surgeon after a pilot returns to flight activity (i.e., post-rehabilitation) and is the only criterion adopted to evaluate the treatment outcome.

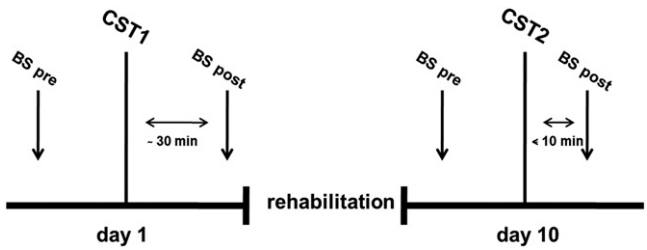

Fig. 1. Representation of the different experimental steps across the 2-wk rehabilitation period (corresponding to 10 working days). The location of the four blood samplings (BS) is also indicated (i.e., before and after each CST), along with the different time interval separating each CST.

In this study, at the beginning and at the end of rehabilitation, peripheral blood was sampled before and after each CST. In both CST1 and CST2, the first venipuncture (basal recording) was performed between 08:30 and 09:00, while the second one was performed between 09:15 and 10:00 according to the duration of the test and the physical status of each individual. Due to this last aspect, the post-CST blood sampling could be performed only about 30 min after CST1 because of the severe sense of malaise and discomfort affecting all subjects after the acute vomiting episode which characterized the CST1 of all patients. On the contrary, after CST2, the venipuncture could be performed within a few minutes due to the complete lack of symptoms in all individuals.

In our subjects, the main goal was undergoing a standard rehabilitation program for AS, and so a sufficiently long recovery-time after CST1 was allowed due to the presence of severe MS symptoms, despite the potential negative effects on the analysis of some parameters, as in the case of hormones having an extremely short half-life. The different steps of the experimental protocol are summarized Fig. 1.

The four samples (i.e., pre- and post-CST1 and CST2) were immediately heparinized and separated by centrifugation at 2500 relative centrifugal force for 15 min at 4°C, and the plasma was stored at −80°C until testing. The following three hormones, potentially related to MS, were analyzed:

1. AVP, or antidiuretic hormone, whose increase in plasma samples has been described during nauseogenic and stressful stimulation by several studies (e.g., among others, Eversmann et al.,[5] Farmer et al.,[6] and Grigoriev et al.[7]).
2. CORT, a steroid hormone released by the adrenal glands in response to stress. In a stressful motion environment, it may be a marker of the high intersubject variability of susceptibility to MS.[5,7,9]
3. GHRE, an orexogenic gastric hormone, stimulating appetite and food intake in humans[18]. Nausea has also been associated with a reduction in the levels of plasma GHRE when a visual nauseogenic stimulus was administered in normal individuals[6].

Due to previous literature reports, and to their potential relationship with MS symptoms and signs, these further four biomarkers were also investigated:

1. CRP, which is released into the bloodstream in response to the inflammation induced by a nauseogenic stimulus.[9]
2. SP, a neuropeptide acting as pain modulator in the nervous system and as a paracrine messenger in the gastrointestinal tract. In both functions, SP mediates the nausea and vomiting cascade, while its plasma levels have been related to MS in an animal model.[8]

3. Total AOC, an index of the potential response to the oxidative stress, as determined by the cumulative action of all the antioxidant molecules and of their synergic interactions, and whose plasma level increases after exposure to a stressful activity such as a hypobaric chamber training in student pilots.[19]
4. IsoP, a sensitive index of the effects of oxidative stress, documenting the amount of AOC activation; also, in this case, its plasma level increased after exposure to various stressors.[19,20]

Unfortunately, only in the case of AOC and IsoP, a control population could be obtained from a previous study from our laboratory,[19] where 34 student pilots (32 men and 2 women) with a mean age of 23 yr were investigated before and after exposure to a hypobaric chamber training so their recordings could be compared to the pre-CST1 data of this present sample.

Statistical Analysis

Statistical analysis was carried out by using statistical software STATA 17 and was conducted on the variation of each hormone/biomarker throughout the rehabilitation program. A two-way ANOVA for repeated measures was conducted to analyze the interaction between the results obtained before and after rehabilitation (pre vs. post), and before and after each CST (CST1 vs. CST2). Post hoc analysis was conducted with the Tukey's HSD (honestly significant difference) test. The criterion of significance was set at $P < 0.05$. For the analysis of possible derangements in single individuals with respect to the rest of the population, the so called "empirical rule" (or "3 standard deviation limit") was adopted, so that only variations greater than 3 SD were taken into account.

RESULTS

All student pilots were highly sensitive to CST1, which was in three cases interrupted during the second step (CCW rotation at 90° · s^{-1}), and at the beginning of the third (CW rotation at 150° · s^{-1}) in the other four due to a vomiting episode (level 10 of the MiSc). In 2 wk the rehabilitation program was completed (Fig. 1), and CST2 did not evoke any relevant symptoms in anyone, with a MiSc never exceeding the level of 1 (i.e., a mild stuffy sensation).

Nevertheless, at the return to real flight activity, only six individuals became resilient to AS (positive outcomes: POs), since in one man the local flight surgeon reported no significant changes in AS sensitivity (fail case: FC). Interestingly, this individual did not report any MS symptoms when exposed to the final CST2 following the rehabilitation period (MiSc level 0).

Fig. 2 summarizes the average score for the MiSc throughout the rehabilitation period from day 2 to day 9 in the POs. In the same table, the mean velocity of chair rotation in each specific day is also indicated. **Table I** summarizes the levels of the seven hormones/biomarkers obtained from the four blood specimens collected before and after CST1 and CST2 (data in the POs: mean and SD). The values measured from the FC are separately shown in the third row for each parameter. Within the PO group, no significant differences were detected between the four men and the two women.

The interaction between pre- and postrehabilitation, and the pre- and post-CST conditions exhibited significant changes for CORT [$F(5) = 10.2$, $P = 0.0242$]. Moreover, CORT was the only hormone whose blood levels exhibited a significant variation (+23,573 pg · mL^{-1} increase) in the POs after CST1 (Tukey's HSD test: $P < 0.045$; $t = 3.79$), with a similar pattern in the FC (+29,050 pg · mL^{-1}). On the contrary, after CST2, a CORT increase could be detected for the sole FC (+53,010 pg · mL^{-1}), while a mean decrease of 4511 pg · mL^{-1} in the POs was recorded.

Due to the particular results of CORT plasma levels, its values in each subject are specifically indicated for every blood sampling in **Fig. 3**, separating the FC from the other POs.

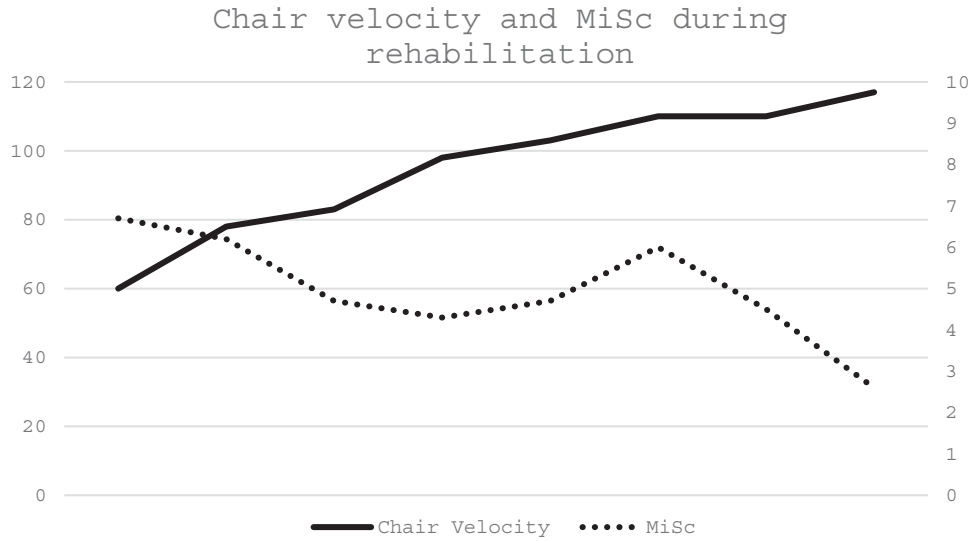

Fig. 2. Representation of the average rotatory chair velocity (full line—left ordinate in ° · s^{-1}) and MiSc score (dotted line—right ordinate) as observed in the PO sample and recorded from day 2 to day 9 of rehabilitation. The MiSc peak at day 7 is due to the introduction of different types of nauseogenic exercises, as reported in the Procedure paragraph.

Table I. Levels of Hormones/Biomarkers Collected from Subjects Undergoing Rehabilitation for Airsickness.

HORMONE/ BIOMARKER	OUTCOME	PRE-CST1 (mean ± SD)	POST-CST1 (mean ± SD)	PRE-CST2 (mean ± SD)	POST-CST2 (mean ± SD)	STAT
AVP, pg · mL^{-1}	POs	1.38 (0.24)	1.65 (0.65)	1.50 (0.45)	1.30 (0.22)	NS
	FC	3.06^	3.28	1.86	1.73	^ 7 SD
GHRE, pg · mL^{-1}	POs	1.34 (0.20)	1.63 (0.66)	1.50 (0.46)	1.28 (0.21)	NS
	FC	2.91^	3.14	1.88	1.74	^ 7 SD
CORT, pg · mL^{-1}	POs	6288° (5491)	29,861° (23,689)	11,208 (7077)	6697 (5396)	°$P < 0.045$
	FC	9100	38,150	10,040	63,050^	^ 10 SD
CRP, pg · mL^{-1}	POs	1.30 (0.24)	1.36 (0.09)	1.41 (0.03)	1.40 (0.04)	NS
	FC	1.42	1.44	1.42	1.42	
SP, pg · mL^{-1}	POs	588 (41)	635 (75)	586 (43)	589 (43)	NS
	FC	611	483	496	455^	^ 3 SD
AOC, µM	POs	1233 (315)	1239 (341)	1250 (346)	1228 (284)	NS
	FC	922	671	633	700	
Iso-P, pg · mL^{-1}	POs	27° (0.9)	21 (4.0)	15° (6.9)	16 (5.1)	°$P < 0.027$
	FC	29	17	11	18	

Hormones/biomarkers with related measurement units (first column), obtained from subjects undergoing rehabilitation and recovered (POs) or not (FC) from airsickness (second column). Blood samples were collected before and after CST1 and CST2 (third-sixth column). For POs, mean ± SD (in brackets) are shown, while the FC data are separately indicated. The last column shows the statistical comparison for the PO group. For the FC, only differences of at least 3 SD from the PO average are reported.
AVP: arginine vasopressin; CORT: cortisol; GHRE: ghrelin; CRP: C reactive protein; SP: substance P; AOC: antioxidant capacity; IsoP: 15-F2t-isoprostane; POs: positive outcomes; FC: fail case; CST: Coriolis stress test; Stat: statistical significance; NS: not significant; SD: standard deviation.

This figure also shows a wide interindividual variation in the response in the POs following CST1, ranging from 9337–76,180 pg · mL^{-1} (vs. a basal 2790–17,130 pg · mL^{-1} range), while findings in the FC did not differ from the rest of the sample. Moreover, no relationships were observed between the basal CORT levels and the clinical outcome, or the subject's behavior during the rehabilitation process. A slightly wider data distribution before the CST2 was also observed with respect to the pre-CST1 findings (compare the different SD values in Table I), although even in this case no relationships could be detected with the clinical outcome or with the global behavior during the rehabilitation process.

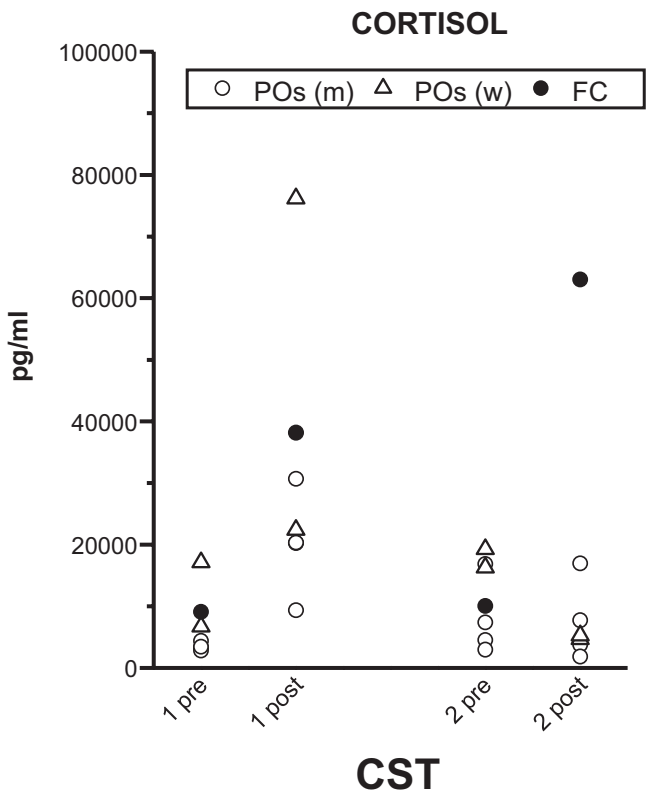

Fig. 3. CORT plasma levels (ordinates in pg · mL^{-1}) as recorded in each individual (i.e., both POs and FC) in the four analysis sessions (abscissas: pre- and post-CST1, and pre- and post-CST2). FC: black circles; POs (men): white circles; POs (women): white triangles.

As documented in Table I, AVP and GHRE did not show significant changes throughout the different test sessions in the POs. However, the basal recording from the FC (i.e., before CST1) showed much higher plasma levels with respect to those recorded in the POs (+7 SD in the case of both AVP and GHRE), while values similar to those of POs were observed in the other three blood samplings (i.e., post-CST1, pre- and post-CST2).

As to the other biomarkers, no significant changes of CRP and AOC could be detected in any of the four different blood samples in the POs, while a derangement of the SP at the post-CST2 venipuncture in the FC barely reached the adopted criterion of 3 SD. In the POs, IsoP showed a statistically significant reduction between the pre-CST1 and the pre-CST2 recordings (Tukey HSD: $P < 0.027$; $t = -4.34$). However, a similar pattern was also observed in the FC.

Finally, the comparison between the basal (i.e., pre-CST1) AOC and IsoP levels observed in the POs and those observed in the control population showed very small differences (i.e., 1233 ± 315 vs. 1192 ± 193 µM for AOC, and 27 ± 9 vs. 27 ± 10 pg · mL^{-1} for IsoP), that was not statistically significant.

DISCUSSION

MS is a common disorder affecting many individuals exposed to actually or virtually moving environments. In the case of AS, symptoms are usually limited to the early exposures to the flight environment, while in just a few cases a particularly low

capability of adaptation is exhibited.[1,2,10] Among aircrew members, the slow adapting category represents the potential target for a physical rehabilitation program due to the negative effects of anti-MS drugs on flight safety requirements (mainly related to drowsiness and visual impairments).[21,22] Therefore, various rehabilitative approaches have been developed in different laboratories to treat these particular patients (e.g., among others, Dobie and May,[1] Bagshaw and Stott,[10] and El Khiati et al.[15]).

In this study, the success rate of desensitization was 86%, in agreement with previous literature reports.[10,16] Physical evaluation tests, such as the CST2, performed at the end of a rehabilitation program did not efficiently predict the treatment outcome,[16] and this was confirmed by the present investigation, where the only FC had a negative outcome, as well as the other POs.

Therefore, a reliable set of biochemical parameters indicating the success of rehabilitation could represent a more useful tool in the management of these patients, potentially adding further information on the individual's actual capability of adaptation, as well as on the retention of its benefits. Previous reports on hormonal and other biomarkers' variations induced by MS represent an interesting area of investigation for this purpose. Moreover, the time course of their variations throughout a desensitization program might be per se an interesting parameter, which may add information on the complex relationship existing between the vestibular system and the endocrine response.[15] To our knowledge, none of the seven biomarkers analyzed in this study was ever monitored under such rehabilitative conditions.

However, a major "weak point" of the present study is represented by the small sample that could be recruited, which was due to the relatively low number of airsick individuals undergoing rehabilitation. In previous studies, CORT, GHRE, and AVP have already been pointed out as sensitive indicators of MS (compare, among others, Eversmann et al.,[5] Farmer et al,[6] and Girgoriev et al.[7]).

In the case of AVP, other authors reported its increase during and immediately after MS episodes,[5–7] although its plasma levels rapidly decayed within a few minutes after stimulus cessation due to the prompt activation of liver and kidney vasopressinases.[5,9,23] In our study, the time lapse separating the end of CST1 from the following venipuncture (about 30 min) was probably sufficient to "normalize" AVP values so that its small but still detectable increase (+0.27 pg · mL^{-1}) did not reach the criterion set for statistical significance.

Due to its sensitivity to MS, AVP has been extensively investigated in different contexts where a high sensitivity to nauseogenic stimuli was reported,[5-7] along with a crucial role of its V_1 receptors in the genesis of nausea and vomiting.[24] Kohl et al. also reported higher basal AVP levels in subjects who were less susceptible to the nauseogenic effects of ground-based motion stimuli.[25] On the contrary, in our data, the only individual who exhibited a relatively high basal AVP level was the FC (see Table I), whose performance during rehabilitation was very similar to the other subjects. Due to our criterion of considering only individuals who do not develop posttreatment incapacitating AS episodes in real flight environments as actually rehabilitated, such a finding further emphasizes the lack of a completely clear relationship between ground-based exercises and real in-flight situations.

After rehabilitation, the blood sampling could be performed just a few minutes after CST2 due to the substantial absence of symptoms in all individuals. Therefore, in contrast with the previous post-CST1 findings, in this case the low AVP values were much more reliable, and the lack of significant variations was most probably secondary to the positive effects of rehabilitation. However, according to our data, AVP was not predictive of the success of our treatment.

Interestingly, in this study, the data from GHRE was extremely similar to those of AVP, with the FC showing higher basal levels (+7 SD), and no changes throughout all the other experimental steps in each individual (i.e., both in POs as in the FC). A previous report from Farmer et al.[6] documented an opposite behavior between AVP and GHRE during a nauseogenic visual stimulation, with a decrease of plasma GHRE and a concurrent increase of AVP.

As in the case of AVP, the very rapid metabolism of GHRE, with a pulsatile secretion and a catabolism of a few minutes,[18,26] along with the long delay between the end of CST1 and the blood sampling procedure, can explain our findings. Moreover, a different provoking stimulus was used in the two studies (i.e., visual in their case vs. vestibular in ours). Therefore, both GHRE and AVP might be considered two potentially interesting parameters for separating subjects positively responding to rehabilitation from nonresponders, although such a finding certainly needs further investigation due to the low number of individuals that could be analyzed in this study.

The behavior of CORT plasma levels in this investigation is of particular interest due to its clear relationship with the actual level of in-flight adaptation reached by each subject. After CST1, CORT plasma levels showed a major increase in all subjects, which resulted in statistically significant oucomes in the POs (compare Table I and Fig. 3); such a finding was not observed during CST2, where a mean decrease of 4511 pg · mL^{-1} was detected, but in the FC, where a behavior similar to the prerehabilitation findings was recorded (+53,010 pg · mL^{-1}). This particular response might be considered an interesting marker for the lack of in-flight adaptation, despite a negative CST2.

Although with some differences between their studies, other authors have outlined different CORT findings between men and women exposed to nauseogenic stimuli.[9,27] In the present research, the data from the only two women could not produce significant gender-related differences, although they exhibited relatively high pre-CST1 and pre-CST2 CORT levels (see Fig. 3). From the practical point of view, their clinical outcome was positive as well as in four out of the five men. Therefore, our data are insufficient to support any hypothesis on a differential behavior between men and women in these experimental situations. However, our single FC findings may suggest the use of CORT as an index of a successful adaptation process.

In this study, other biomarkers related to inflammation, such as CRP and SP, were analyzed. In the PO sample, they did not exhibit any significant variation, while in the FC SP level was 3 SD lower with respect to that observed in the POs. Due to our small sample size, to the difficult interpretation of this result, and to the barely reached statistical significance, such a finding certainly deserves further investigation.

The antioxidant capability was analyzed using AOC and IsoP. Globally, the total AOC was substantially insensitive to the effects of the nauseogenic stimulus in all subjects, both before and after rehabilitation. Only a minor and not significant reduction of the AOC was observed in the FC after the CST1, which remained stable at a relatively low level after rehabilitation. However, such a finding was severely influenced by the low number of volunteers that could be recruited for this study. Notably, in a wider control population undergoing hypobaric chamber training, no significant AOC changes were detected before and after the exposure to that stressor, and AOC levels were substantially similar to those recorded in this study[19].

Although global AOC did not exhibit relevant changes, IsoP levels significantly decreased from their basal values after rehabilitation (i.e., pre-CST1 vs pre-CST2 data). Such a result was detected both in the POs as in the FC, and might highlight the activation of a response to an oxidative stress induced by the MS rehabilitation process. This could potentially be pointed out as a sensitive marker to monitor the amount of such a stressor.

In this case, the stimulus was much stronger than in the control group, since in that case no significant differences were detected before and after the exposure to hypoxia.[19] Nevertheless, also in this case, the low number of individuals tested in this study must be considered, along with the absence of a different response between the POs and the FC.

In conclusion, for practical aeromedical purposes, the main result of this study was the predictive CORT behavior in separating POs from the FC. To our knowledge, this is the first time that this analysis has been performed on aircrew members undergoing this type of treatment, with the possibility of in-flight control of laboratory findings. The possible easier usage of salivary samples to analyze free CORT levels, as performed by other researchers,[9,27] could further facilitate future studies.

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