

Influence of a high carbohydrate diet on the functional activity of 5-HT_{1B/1D} receptors on human peripheral blood lymphocytes during intense military training

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ABSTRACT. The present study was undertaken to examine the effect of a high carbohydrate diet on the functional activity of 5-HT_{1B/1D} receptors in human peripheral blood lymphocytes, and on serum cortisol and plasma cytokine responses during intense military training. Thirty two male soldiers (mean age: 21 ± 2 years) were randomly assigned to two groups and received either 3200 kcal/24 h [13440 kJ; habitual diet group (HD)] or 4200 kcal/24 h [17640 kJ, high carbohydrate diet group (HCD)] by adding 1000 kcal (4200 kJ) of fruit jelly to the HD. They took part in a three-week training program followed by a five-day combat course. Blood samples were collected from each group before entry into the commando training and after the five-day combat course. The results of [³⁵S] GTPγS binding assays showed that h5-HT_{1B/1D} receptors were desensitized after the training program in the HD group, whereas no change was observed between the beginning and the end of the military training in the HCD group [(HD : IC₅₀ = 100 ± 14 nM to 544 ± 178 nM; n = 16) and (HCD: IC₅₀ = 68 ± 14 nM to 101 ± 22 nM; n = 16)]. Serum cortisol was only significantly increased after the commando training in the HD group (from 532.2 ± 30 to 642 ± 45 nmol·L⁻¹, p < 0.05), whereas values were not significantly changed in the HCD group (441 ± 31 to 502 ± 40 nmol·L⁻¹). No changes were observed in IL-10, TNF-α and IFN-γ levels after the training program in either group. Carbohydrate ingestion or additional dietary energy during repeated bouts of high-intensity exercise could attenuate the alterations in immune function via 5-HT_{1B/1D} receptors and the action of 5-HT moduline, an endogenous tetrapeptide (Leu-Ser-Ala-Leu) that specifically modulates the sensitivity of 5-HT_{1B/1D} receptors.

Keywords: 5-HT_{1B/1D} receptors, T lymphocyte, multi-stressors, carbohydrate, training

Intense training in combination with inadequate recovery can result in a decrease in performance. Although training stress and a disturbance in homeostasis are thought to underlie overreaching and overtraining, the mechanisms remain unclear. Automatic imbalance [1], neuroendocrine changes [2], plasma cytokines [3], and glycogen depletion [4] have all been implicated. With respect to the latter, high-intensity training with limited recovery periods may result in reduced muscle glycogen levels, which may impair performance [4].

Few studies have documented the effect of high-carbohydrate (CHO) diets on performance during periods of normal and intensive training [5, 6]. Jacobs and Sherman [7] have suggested that a high-CHO diet may be required for optimal adaptation to training.

On the other hand, several studies have shown that mitogen-induced proliferation of T lymphocytes is decreased after acute exercise [8, 9]. It is recognized that intensive exercise is also associated with alterations in immunoregulatory hormones [10, 11]. Studies of endurance athletes suggest that ingestion of carbohydrates during sustained and prolonged exertion is associated with:

attenuated cortisol, growth hormone, epinephrine, pro- and anti-inflammatory cytokine responses, and fewer alterations in blood immune cell counts. It is also been described that decreases of granulocyte and monocyte phagocytosis and oxidative burst activity are limited by carbohydrate ingestion. Carbohydrate intake has also been found to blunt the rise in IL-6 and IL-1 receptor antagonist (IL-1ra) involved in the inflammatory cascade response to heavy exertion [12, 13].

The stress hormone cortisol may be involved in the attenuating effect of CHO supplementation on the exercise-induced impairment in T lymphocyte function. A reduction in T lymphocyte function [14, 15] and an elevation in cortisol [16] have been noted after intense prolonged exercise, and the immunosuppressive effects of cortisol are well established [17, 18]. The mechanism by which cortisol influences T lymphocyte function after exercise is unknown. Alison [19] has suggested a direct interaction of cortisol on the T lymphocyte surface via receptors. Other studies have indicated that cortisol impairs proliferative function indirectly by inducing changes in the composition of the cellular pool [20]. CHO ingestion has also been

shown to reduce cortisol release into the bloodstream and diminish changes to immune function [21]. In a recent study [22], we showed that h5-HT_{1B/1D} receptors in peripheral blood lymphocytes were desensitized after a military training program, suggesting the existence of a control on T cells, probably mediated by an endogenous tetrapeptide (Leu-Ser-Ala-Leu), 5-HT moduline. Indeed, the participation of 5-HT_{1B} and 5-HT-moduline in immunocompetent cell activity have been investigated. Grimaldi *et al.*, [23] in a series of experiments, have shown that 5-HT_{1B/1D} receptors are present in rodent thymocytes and splenocytes and in human lymphocytes (a human T lymphoblastoid cell line, CEM) [23]. They also found that the stimulation of 5-HT_{1B/1D} receptors significantly stimulated the proliferation of these cells, indicating that, under experimental conditions, the 5-HT_{1B/1D}-mediated serotonergic activity favoured the immune response via the stimulation of the proliferation of the immunocompetent cells. Interestingly, 5-HT-moduline affected this reaction, reversing the 5-HT_{1B/1D}-induced proliferation of the cell. This antagonistic effect of 5-HT-moduline is in good agreement with that observed on central 5-HT_{1B/1D} receptors, and confirms that the 5-HT_{1B/1D} peripheral receptor behaves like the central one as regards to its sensitivity to 5-HT-moduline. During an acute restraint stress, adrenal 5-HT moduline content has been found to be decreased, suggesting that the endogenous compound is involved in the modulation of systemic serotonergic activity under stress conditions [24]. This suggests that the circulating 5-HT moduline released from adrenals reaches peripheral targets.

In fact, a neurosecretory protein (NESP 55) has been described as a putative precursor for 5-HT moduline. NESP 55, a novel member of chromogranin is expressed in the adrenal medulla, pituitary, and brain [25].

In view of these observations, we hypothesize that repeated carbohydrate ingestion during military training including sleep deprivation, physiological and psychological stress, could reduce cortisol release into the bloodstream. This in turn, would attenuate the alterations in immune function via reduced desensitization of 5-HT_{1B/1D} human peripheral blood lymphocytes mediated by 5-HT moduline.

The present study was designed to examine the effect of repeated carbohydrate ingestion and/or an additional dietary energy on serum cortisol, functional activity of 5-HT_{1B/1D} receptors in human peripheral blood lymphocytes and on plasma cytokine responses during military training.

DONORS AND METHODS

A group of 32 male soldiers (mean age = 21 ± 2 years) from the French Military Officer School took part in a four-week military endurance training program. The subjects were trained and in good mental and physical condition. The mean maximal oxygen uptake (VO_{2max}) was 54.3 ± 1.0 ml.min⁻¹.kg⁻¹. The study was approved by a French medical ethics committee (Faculty of Medicine, Paris V, France), and the participants had all given voluntary written consent.

Description of the French Army Commando Training

The three-week training and the five-day combat course took place at the National Centre for commando training at Mont-Louis in the Pyrenees mountains in June. The three-week training included a sea phase (first week), followed by a mountain phase (altitude: 1600 m). During the training, subjects spent most of their time engaged in realistic combat training over rough terrain including heavy physical activity: swimming, walking and running, avoiding roads, lanes and trails while carrying back-packs of 11 ± 1.2 kg. Several parts of the training involved mountain climbing. For the entire training program, the total daily uphill and downhill walking distance was 11.2 km. The five-day course took place from 6 a.m. on day 1 until 6 a.m. on day 6; most of the physical activities were at night. Since the cadets slept outside at ambient temperatures during the three weeks of training, their sleep was disturbed, and since most of the physical activities were at night during the five-day course, subjects were also sleep-deprived.

During the five-day combat course, subjects were estimated to have continuous physical exercise activities corresponding to an average of 35 % of maximal oxygen uptake and a daily energy expenditure exceeding 5000 kcal.

Weight, height and percentage of body fat were measured before the training and after the five-day course. The percentage of body fat was determined using the measurements of four skinfolds (biceps, triceps, subscapular and suprailiac) [26].

Diet preparation

The subjects were randomly assigned to one of two groups (n = 16) differing with regard to the energy content of their diet. The basis for the two diets was the light-weight commando ration. One group (n = 16) identified as habitual energy diet (habitual diet: HD) received a complete ration with a mean energy content of 3200 kcal/24 h (13440 kJ/24 h) This ration of a mean mass of 900 g consisted of two freeze-dried meals (Lyofal, France) of 480 kcal (2016 kJ) each, kcal (5040 kJ) of bread in the form of biscuit and 1000 kcal (4200 kJ) of energy bars, chocolate, and candied fruit. The percentage of each class of nutrient in the total energy supplied was 55 % carbohydrates, 30 % lipids, 15 % proteins. The second group (n = 16) identified as high energy diet (high carbohydrate diet: HCD) received 4200 kcal/24 h (17640 kJ/24 h) by adding 1000 kcal (4200 kJ) of fruit jelly to the commando ration. The composition was 60 % carbohydrates, 25 % lipids, 15 % proteins. The rations were consumed during three main meals, only fruit jelly was eaten *ad libitum*. In spite of this high energy diet, the soldiers were in marked negative energy balance for the four weeks.

Study design

Subjects received the commando ration every day from the first day of the training to the last day of the combat course (one month). During the period of supplementation, they were asked to avoid other dietary products, which was readily checked as all the food was provided by the military quartermaster. In order to assess compliance, the

subjects were asked to report in a logbook the quantity of fruit jelly consumed each day.

Blood parameters

Blood samples were taken before the three weeks of the training program and at the end of the five-day course. The first samples were taken in the Officer School between 7 a.m. and 8 a.m. The second sample, at the end of the course, was taken between 6 a.m. and 7 a.m. during medical and scientific investigations in a military barracks. Thirty millilitres of venous blood were taken from an antecubital vein after 10-min rest supine. A portion of this was centrifuged for collection of serum and plasma, stored at -80°C and used for subsequent hormone determinations in our laboratory.

[^{35}S]GTP γ S binding to membrane preparations

LeucosepTM was purchased from Opopharma AG (Zurich, Switzerland). Ficoll-Paque was from Pharmacia (Uppsala, Sweden). [^{35}S]GTP γ S (1250 Ci/mmol) was obtained from NEN Life Sciences (Boston, MA, USA). L-694,247 was from Tocris Cookson (Ballwin, MO, USA). L-694,247 was initially dissolved in dimethyl sulfoxide (DMSO) and stored at -20°C in 10 μL aliquots (10^{-2} M). Membrane protein levels were estimated using the Bio-Rad kit (Hercules, CA, USA). Multiscreen filtration plate (FB glass fiber type) was from Millipore (Bedford, MA, USA). Hepes, GDP salt, ascorbic acid and phenantrolin were purchased from Sigma (St Louis, MA, USA). All buffered solutions were prepared fresh daily. The liquid scintillation cocktail was from Amersham (Catalog No. NBCS.104, Buckinghamshire, UK).

Lymphocytes were isolated from a whole blood sample, anticoagulated with EDTA according to the method of Boyum [27]. Seven millilitres of fresh blood were added per Leucosep tube containing 3 mL of Ficoll-Paque. After centrifugation at 1100 g for 10 min, the plasma layer was removed and lymphocytes were harvested by pipette and transferred to a Falcon tube. Lymphocytes were washed twice in 6 ml of phosphate buffered saline by centrifugation at 500 g for 15 min. The supernatant was discarded and the pellet was stored at -80°C .

The frozen pellet was resuspended in 2 ml of 20 mM Hepes containing 10 mM EDTA (pH = 7.4) and homogenized with an Ultra-Turrax apparatus. Five millilitres of 20 mM Hepes containing 10 mM EDTA were added and the sample was centrifuged for 12 min at 40,000 g at 4°C . The resulting pellet was resuspended in 20 mM Hepes containing 0.1 mM EDTA, homogenized and centrifuged as described above. The pellet was resuspended in 500 μL of 20 mM Hepes containing 0.1 mM EDTA. A Bradford protein assay was performed and 400 to 500 μg aliquots were then stored at -80°C .

Lymphocytes membranes (15 μg of protein in a total volume of 225 μL per well) were incubated in 20 mM Hepes solution, pH 7.4, containing 100 mM NaCl, 3 mM MgCl_2 , 0.2 mM ascorbic acid, 30 μM GDP and 100 μM phenantrolin for 30 min at 25°C on a MultiScreen filtration plate with increasing concentrations (10^{-10} to 10^{-4} M) of L-694,247. Twenty five μL of [^{35}S]GTP γ S (5 nM) were added for an additional incubation of 30 min. The 96-well

filtration plate was rapidly filtered and washed twice with 250 μL of cold 20 mM Hepes containing 3 mM MgCl_2 . The 96 filtrates were recovered and counted by liquid scintillation.

Quantification of cytokines

Plasma IL-10 and TNF- α concentrations were determined using quantitative high-sensitivity sandwich ELISA kits and concentrations of IFN- γ found using a sensitive sandwich ELISA kit, provided by R&D Systems (Minneapolis, MN, USA) and BioSource International, Inc (Camarillo, CA, USA). All samples and standards were analyzed in duplicate. Plasma IL-10, TNF- α and IFN- γ concentrations were determined from their respective standard curves by linear regression. The minimum detectable concentration were < 0.5 $\text{pg}\cdot\text{mL}^{-1}$ for IL-10, < 0.1 $\text{pg}\cdot\text{mL}^{-1}$ for TNF- α and < 8 $\text{pg}\cdot\text{mL}^{-1}$ for IFN- γ . The intra- and extra-assay coefficients of variation were: 8.5 % and 10.2 % for IL-10, 6.7 % and 8.2 % for TNF- α , 6 % and 8 % for IFN- γ . All samples were determined within the same assay.

Blood hormone and metabolite assays

Cortisol, testosterone, leptin and insulin concentrations were determined in duplicate by radioimmunoassay using commercial kits (DiaSorin, Beckman Coulter, Linco Research, France). The normal fasting ranges for lean men were: 193-690 $\text{nmol}\cdot\text{L}^{-1}$ for morning cortisol, 3.8 ± 1.8 $\text{ng}\cdot\text{mL}^{-1}$ for leptin. The limits of sensitivity were: 5.79 $\text{nmol}\cdot\text{L}^{-1}$ for cortisol, 0.18 $\text{nmol}\cdot\text{L}^{-1}$ for T, 0.05 $\text{ng}\cdot\text{mL}^{-1}$ for leptin and < 4 $\mu\text{U}\cdot\text{mL}^{-1}$ for insulin. The intra- and extra-assay coefficients of variation were: 7.7 % and 9.8 % for cortisol, 6.9 % and 10.3 % for T, 7.48 % and 4.02 % for leptin, 6.6 % and 6.2 % for insulin.

Epinephrine (EPI) and norepinephrine (NE) were assayed in plasma by HPLC with electrochemical detection. The chemical standard was 3,4-DHBA (dihydroxybenzylamine) (Sigma, Saint-Quentin Fallavier, France). The normal ranges were: < 100 $\text{ng}\cdot\text{L}^{-1}$ for EPI and 600 $\text{ng}\cdot\text{L}^{-1}$ for NE. The limits of sensitivity were: 9 $\text{ng}\cdot\text{L}^{-1}$ for EPI and 45 $\text{ng}\cdot\text{L}^{-1}$ for NE. The intra- and extra-assay coefficients of variation were 5 % and 9 %.

Plasma amino acids were determined by a modified gas chromatography (GC) method coupled with mass spectrometry [28]. Plasma glucose concentrations were analyzed using a colorimetric slide method (Glucose RTU, Biomérieux, Marcy-l'Etoile, France). FFA were measured in plasma samples using commercial colorimetric methods (NEFA, Half microtest, Roche Diagnostics, Meylan, France).

Statistical analysis

Binding experiments were analyzed under Prism 3.0 (Graphpad software, San Diego, CA, USA).

Statistica Version 6.1 (Statsoft, Tulsa, OK, USA) was used for all analysis. All data were expressed as mean \pm SEM. Habitual diet (HD) and high carbohydrate diet (HCD) groups were compared using Student's *t*-test. To analyze changes over time and between groups, a two-way repeated-measures ANOVA was used. If such an analysis revealed significant differences, a Newman-Keuls *post hoc* test was used to locate the specific differences.

Table 1

Characteristics of the subjects.

Age and body composition of the two groups: habitual diet (HD) and high carbohydrate diet group (HCD). Values are mean \pm SEM

Diet kcal/24 h	HD		HCD	
	3200		4200	
	Mean	SEM	Mean	SEM
Mass (kg)	76.4	2.1	78	2.0
Height (cm)	177.8	2.1	178.2	1.3
Body fat (%)	12.5	0.6	12.7	0.7
Age (yrs)	26.2	0.5	27	0.5

RESULTS

Subjects were randomly assigned to the habitual diet (HD; n=16) or the high carbohydrate diet (HCD; n = 16) groups. There were no significant differences between the two groups with respect to age (HD: 26.2 \pm 0.5 years and HCD: 27 \pm 0.5 years), height (HD: 177.8 \pm 2.1 cm and HCD: 178.2 \pm 1.3 cm), weight (HD: 76.4 \pm 2.1 kg and HCD: 78 \pm 2.0 kg), and body fat (HD: 12.5 \pm 0.6 % and HCD: 12.7 \pm 0.7 %) (table 1).

Binding of [³⁵S]GTP γ S measured in lymphocyte membranes of cadets in the two groups before the training program was stimulated by a specific agonist (L-694,247) in a dose-dependant manner (HD : IC50 = 100 \pm 14 nM; n = 16) (HCD: IC50 = 68 \pm 14 nM; n = 16). The corresponding binding curve was only shifted significantly to the right after the training in the HD group (IC50 = 544 \pm 178 nM; n = 16; p < 0.01), whereas the values did not differ in the HCD group (IC50 = 101 \pm 22 nM; n = 16) (table 2).

Blood hormones concentrations

Serum cortisol was only significantly increased after the commando training in the HD group (from 532 \pm 30 to 642 \pm 45 nmol·L⁻¹, p < 0.05), whereas values were not significantly changed in the HCD group (441 \pm 31 to 502 \pm 40 nmol·L⁻¹). This result showed a larger increase in mean cortisol serum concentrations in the HD group compared with the HCD group (p < 0.05) after the military training program.

After the training program in the two groups, there were decreases in leptin and testosterone (p < 0.001) and an increase in norepinephrine (NE) (p < 0.001), while epi-

nephrine (EPI) and insulin were not significantly changed. No significant differences between the two groups for these hormones were observed (table 3).

Blood metabolite concentrations

After the training program in the two groups, there were reductions in branched-chain amino acids (BCAA) (valine, leucine, isoleucine) (p < 0.01) and an increase in plasma free fatty acids (FFA) (p < 0.001), while blood glucose concentrations were not significantly changed. No significant differences were observed between the two groups for these metabolites (table 4).

Blood cytokines concentrations

The training program did not influence the measured resting serum cytokine concentrations. Plasma levels of cytokines are listed in table 5. There were no differences between the two groups.

DISCUSSION

The purpose of the present study was to examine the effect of repeated carbohydrate ingestion and/or an additional dietary energy on the functional activity of h5-HT_{1B/1D} receptors in human peripheral blood lymphocytes and on serum cortisol and plasma cytokine levels during intense military training.

The French commando training comprises three weeks of physical and psychological conditioning followed by a five-day combat course entailing energy restriction, sleep deprivation and psychological stress. This experimental pattern minimized variability in physical activity, amount of sleep and psychological pressure since it involved an homogeneous group of subjects who undertook the same physical activity, had an almost identical diet, similar amounts of sleep and faced the same psychological challenges. Moreover, in this study, the fact that all food was provided by the army limited the likelihood of subjects consuming other dietary products. In addition, the daily distribution of the commando ration enhanced dietary compliance. Furthermore, as part of the commando training, cadets were under medical surveillance throughout the study.

In the present study, we show that a high carbohydrate diet reduces serum cortisol levels and appears to block the

Table 2Binding of [³⁵S] GTP γ S.pIC50 (nM) and Emax (%) were measured before and after commando training in the habitual diet group (HD) and high carbohydrate diet group (HCD). Values are mean \pm SEM

[³⁵ S] GTP γ S Binding					
Diet		HD		HCD	
kcal/24 h		3200		4200	
		Mean	SEM	Mean	SEM
pIC50 (nM)	before	100	14	68	14
	after	544*	178	101	22
Emax (%)	before	21.7	5.5	23.7	8.4
	after	23.6	5.1	24.1	8.5

*p < 0.01 between before and after the commando training.

Table 3

Blood hormone concentrations measured before and after commando training. Cortisol, testosterone, insulin, leptin, norepinephrine and epinephrine concentration before and after commando training in the habitual diet group (HD) and high carbohydrate diet group (HCD)

Diet		HD		HCD	
kcal/24 h		3200		4200	
		Mean	SEM	Mean	SEM
Cortisol (nmol/L)	<i>before</i>	532.2	30	441	31
	<i>after</i>	642 ¹	35	502 ²	40
Testosterone (nmol/L)	<i>before</i>	12	0.59	12.32	0.55
	<i>after</i>	6.33 ³	0.4	6.91 ³	0.7
Insulin μU/L	<i>before</i>	17.78	2.93	13.54	1.01
	<i>after</i>	15.16	3.77	16.69	2.82
Leptin (ng/mL)	<i>before</i>	1.32	0.26	1.26	0.26
	<i>after</i>	0.38 ³	0.05	0.47 ³	0.06
Norepinephrine (ng.L-1)	<i>before</i>	519	50	534	53
	<i>after</i>	926 ³	67	1027 ³	100
Epinephrine (ng.L-1)	<i>before</i>	47	7	51	5
	<i>after</i>	56	11	52	4

Values are mean ± SEM.

¹ p < 0.05.

² p < 0.01.

³ p < 0.001 between before and after the commando training.

⁴ p < 0.05 between HD and HCD group.

Table 4

Blood metabolite concentrations before and after commando training. Blood glucose, plasma free fatty acids (FFA) and branched-chain amino acid (BCCA) concentration before and after commando training in the habitual diet group (HD) and high carbohydrate diet group (HCD)

Diet		HD		HCD	
kcal/24 h		3200		4200	
		Mean	SEM	Mean	SEM
Glucose (mmol/L)	<i>before</i>	4.81	0.18	4.98	0.14
	<i>after</i>	4.62	0.16	4.72	0.18
FFA (μmol/L)	<i>before</i>	156	23	200	34
	<i>after</i>	416 ¹	52	471 ¹	73
BCCA (μmol/L)	<i>before</i>	488	25	432	16
	<i>after</i>	408 ²	19	375 ³	12

Values are mean ± SEM.

¹ p < 0.001 between before and after the commando training.

² p < 0.01.

³ p < 0.05.

Table 5

Blood cytokine concentrations before and after commando training. Plasma IL-10, TNF-α and INF-γ concentration before and after commando training in the habitual diet (HD) and high carbohydrate diet group (HCD). No significant effects of the military training program on any of the measured serum cytokine levels were observed

Diet		HD		HCD	
kcal/24 h		3200		4200	
		Mean	SEM	Mean	SEM
IL-10 (pg/mL)	<i>before</i>	0.74	0.2	0.9	0.48
	<i>after</i>	0.29	0.13	0.66	0.41
TNF-α (pg/mL)	<i>before</i>	1.1	0.23	0.55	0.16
	<i>after</i>	0.78	0.15	0.83	0.18
INF-γ (pg/mL)	<i>before</i>	0.15	0.04	0.29	0.1
	<i>after</i>	0.19	0.04	0.33	0.11

Values are mean ± SEM.

desensitization of 5-HT_{1B/1D} receptors on T lymphocytes after intense military training.

Depletion of muscle glycogen results in fatigue and impaired performance [29]. The effects of repeated bouts of high-intensity exercise on muscle glycogen are well documented [30]. On the other hand, the effect of high carbohydrate (CHO) diets during periods of normal and intensive training on performance has been reported by few studies [5, 6]. Jacobs and Shermann [7] have suggested that a high-CHO diet may help optimize adaptation to training. CHO ingestion has been reported to attenuate the usual increase in cortisol concentration with intense prolonged exercise [14]. Consistent with these reports, we found that a high carbohydrate diet reduced cortisol levels at the end of the French commando training program. However, this high carbohydrate diet did not reduce the neuroendocrine disturbances to chemicals such as testosterone, insulin, leptin, norepinephrine and epinephrine that we had noted in a previous study [31].

Cortisol is a candidate agent for the attenuated reduction in T lymphocyte function after exercise, by dietary carbohydrate supplementation. We demonstrated that a high carbohydrate diet during periods of intense military training reduces the desensitization of h5-HT_{1B/1D} receptors on T lymphocytes that is observed in subjects with a habitual diet. The mechanisms remain unclear but may involve modification of the 5-HT moduline content. Indeed, in the central nervous system (CNS), the effects of physical training on 5-HT metabolism have been accounted for in terms of enhanced tryptophan availability [32] and/or a decrease in the sensitivity of 5-HT_{1B} receptors [33]. This latter study showed that 5-HT_{1B/1D} receptors were slightly desensitized in moderately trained animals and totally desensitized in intensely trained animals. The authors also noted that the desensitization was related to an increase in tissue 5-HT-moduline content. 5-HT moduline, an endogenous tetrapeptide (Leu-Ser-Ala-Leu) that specifically modulates the sensitivity of 5-HT_{1B/1D} receptors [34, 35] also regulates the peripheral serotonergic control of T cell proliferation. At the periphery, 5-HT affects immune function via an action on lymphocytes [36, 37], themselves regulated by 5-HT-moduline released from the adrenal medulla. This release may be stimulated by acute stress [24]. Sibella *et al.* [38] have found evidence for a direct serotonergic control of T cell proliferation mediated by h5-HT_{1B/1D} receptors. In a recent study [22], we demonstrated that intense military training reduced the sensitivity of T lymphocyte h5-HT_{1B/1D} receptors. A plausible hypothesis would be a specific action of 5-HT moduline on the sensitivity of 5-HT_{1B/1D} receptors. Interestingly, a neurosecretory protein (NESP55) has been described as a precursor of 5-HT moduline. The tetrapeptide 5-HT-moduline is present in the NESP55 sequence and is flanked by arginine residues enabling cleavage by prohormone convertases. NESP55 is stored in large, dense, secretory vesicles and is expressed in the adrenal medulla, the anterior and posterior pituitary and various regions of the brain [25]. In our subjects, 5-HT moduline levels may have been reduced by the high carbohydrate diet via cortisol, which would account for the absence of desensitization of h5-HT_{1B/1D} receptors on T lymphocytes. A rapid decrease in 5-HT moduline content in adrenal glands has been reported after stress, resulting from a rapid elimination of the peptide from adrenal glands into the blood [24].

After intense military training, combining stress and physical exercise, cortisol would be assumed to interact with adrenal glands via glucocorticoid receptors to induce 5-HT moduline production.

There is now abundant literature on the cytokine response to exercise. It is well documented that exercise affects local and systemic cytokine production, with similarities to the response to infection [39]. Cytokines play important roles in communication between cells and in cell signaling in response to infection and tissue injury. Indeed, strenuous physical exercise of limb muscles typically results in muscular soreness and injury, especially when the exercise is intense and prolonged as in long distance running [16].

Cytokines, such as TNF- α , IL-1 β , IL-6, IL-8 and cytokine inhibitors (interleukin-1 receptor antagonist IL-1ra, TNF-receptors) increase during exercise, akin to the acute inflammatory response [40]. During endurance exercise, proinflammatory cytokine production is down-regulated, and anti-inflammatory cytokines such as IL-1ra and IL-10 are up-regulated and there is an increase in IL-6 [41]. Strenuous prolonged exercise induces increases circulating levels of TNF- α , IL-1 β and IL-6. This is counterbalanced by cytokine inhibitors (IL-1ra, sTNF-r1 and sTNF-r2) and the anti-inflammatory cytokine IL-10 [42]. The magnitude of the changes differs markedly depending on the cytokine being examined, *i.e.* the plasma concentrations of IL-1 and TNF- α increase one- to two-fold, whereas IL-6 has been reported to increase over 100-fold after prolonged exercise [42].

The repetition of various exercises during a training program would be expected to induce adaptive responses; however, limited recovery, limited substrate availability and limited protein synthesis and the influence of other stressors may lead to fatigue and loss of function as observed in the overreaching or overtraining syndrome (OTS).

Cytokines have been implicated in the OTS [3]. It has also been suggested that the OTS is a response to excessive musculoskeletal stress, associated with insufficient rest and recovery. This, in turn, induces a local acute inflammatory response, which may evolve into chronic inflammation and produce systemic inflammation. Inflammation involves activation of circulating monocytes, which may synthesize large quantities of the proinflammatory cytokines, IL-1 β , IL-6 and TNF- α .

On the other hand, in immune cells, intracellular 5-HT is required for optimal synthesis of cytokines, such as interferon- γ (IFN- γ), a proinflammatory cytokine, and interleukin-10 (IL-10) a negative immunoregulatory cytokine. Extracellular 5-HT concentrations at or above serum values may suppress the production of IFN- γ [43]. In the light of these last observations, Maes *et al.* [44] examined the effect of 5-HT moduline on the stimulated production of IFN- γ , TNF- α and IL-10. They found that 5-HT moduline had negative immunoregulatory effects. Indeed 5-HT moduline (10^{-5} M and 10^{-6} M) significantly inhibits the production of IFN- γ and the IFN- γ /IL-10 ratio, moduline (10^{-5} M) significantly reduces the production of TNF- α . We failed to detect any significant differences in IL-10, TNF- α or INF- γ levels between the beginning and the end of the military training in either diet group. These results indicate that intense military training did not modify cytokine levels because in our situation, the intense military training did not induce overtraining syndrome, but

led rather to an alteration in the sensitivity of 5-HT_{1B/1D} receptors on T lymphocytes. Recent studies in our laboratory [31, 45] have shown that multi-stressors in the military training program induce the cellular responses of immunosuppression. These included a reduction in natural killers cell numbers (NK), the cells most responsive to physical and psychological restraints, and a suppression of mucosal immunity. There is scientific and anecdotal evidence that athletes have more risk of infection after a single intensive competitive event [46]. T lymphocytes play a central role in mounting and regulating the response to both intra- and extracellular pathogens.

Many studies have demonstrated that mitogen-induced proliferation of T lymphocytes is decreased after acute exercise [8, 9]. It is recognized that intensive exercise is also associated with alterations in several immunoregulatory hormones [10]. Although the mechanisms remain unclear, it has been reported that ingestion of carbohydrates during exercise attenuates the modifications in both immunoregulatory hormones and indices of immune function.

We propose an involvement of 5-HT_{1B/1D} receptors located on human peripheral blood lymphocytes via an action of 5-HT-moduline. In conclusion, we observed that the presence of multi-stressors in our military training program induced desensitization of h5-HT_{1B/1D} receptors on T lymphocytes. This desensitization was suppressed by ingestion of supplemental carbohydrates or additional dietary energy during the repeated bouts of high-intensity exercise. It would thus be of interest to evaluate immunosuppression by determining circulating levels of 5-HT-moduline in various stress situations.

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