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Modulatory effects of L-carnitine plus L-acetyl-carnitine on neuroendocrine control of hypothalamic functions in functional hypothalamic amenorrhea (FHA)

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ABSTRACT

Functional hypothalamic amenorrhea (FHA) is a relatively frequent disease due to the combination of metabolic, physical, or psychological stressors. It is characterized by the low endogenous GnRH-induced gonadotropin secretion, thus triggering the ovarian blockade and a hypoestrogenic condition. Up to now various therapeutical strategies have been proposed, both using hormonal treatment as well as neuroactive compounds. Since carnitine, namely L-acetyl-carnitine (LAC), has been demonstrated to be effective in the modulation of the central hypothalamic control of GnRH secretion, we aimed to evaluate whether a combined integrative treatment for 12 weeks of LAC (250 mg/die) and L-carnitine (500 mg/die) was effective in improving the endocrine and metabolic pathways in a group of patients ($n = 27$) with FHA. After the treatment, interval mean LH plasma levels increased while those of cortisol and amylase decreased significantly. When patients were subdivided according to baseline LH levels, only hypo-LH patients showed the significant increase of LH plasma levels and the significant decrease of both cortisol and amylase plasma levels. The increased 17OHP/cortisol ratio, as index of the adrenal activity, demonstrated the reduced stress-induced adrenal activity. In conclusion, our data sustain the hypothesis that the integrative administration of LAC plus L-carnitine reduced both the metabolic and the neuroendocrine impairment of patients with FHA.

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Introduction

Secondary amenorrhea is a not so rare event and when no endocrine or systemic factor is recognized, a hypothalamic blockade has to be suspected. Typically, such hypothalamic dysfunction can occur in all women during fertile life, with no difference between adolescence as well as during adult life [1–3]. It is known as functional hypothalamic amenorrhea (FHA), and it is associated with metabolic, physical, or psychological stress (after severe dieting, heavy training, intense emotional events, or a combination of them) [4,5] with or without body weight loss [6].

A specific correlation exists between loss of weight and amenorrhea [1,6] and when the loss of weight is below a critical point and the ratio between fat and muscular masses is reduced, the loss of menstrual cyclicity occurs. Amenorrhea is a frequent symptom after dieting as well as during intense training of dancers or runners [7]. High energy consumption and reduced food intake with psychological stressors such as emotional, familial, or working problems may have a relevant negative impact on food intake and this can amplify the stress response to fasting [8]. Often many patients show affective disorders (neuroticism, somatization, anxiety) associated to a psychological stressor recorded as heavy negative event, and these mix of situations lead to the disruption of the hypothalamus–pituitary activity, and as a consequence, there is the abnormal and later the stoppage of the ovarian function [9].

Various hormones, neurotransmitters, and neuromodulators are involved in the control of GnRH (PRL, cortisol, opioids, noradrenaline, dopamine, etc.) [4] and various putative treatments have been proposed to restore the activity of the reproductive axis from estroprogestins, estriol [10] to neuroactive compounds such as pivagabine, naltrexone [11,12].

Since, recently integrative neuroactive compound, i.e. acetyl-L-carnitine (ALC) [13] has been reported to modulate hypothalamic function, we aimed to evaluate whether the combination of ALC with a metabolically active compounds, i.e. L-carnitine, was able to improve the neuroendocrine blockade in a group of patients with weight loss FHA.

Materials and methods

Subjects

Twenty-seven amenorrheic patients ($n = 27$), mean age of 26.5 ± 2 (mean \pm SEM) were selected for this study, after giving their informed consent, among those referring to the Center for Gynecological Endocrinology, University of Modena and Reggio Emilia, Italy, between 2011 and 2015. All patients underwent to our clinical protocols for assessments of their disease for FHA.

Patients were selected on the basis of the following criteria: (a) presence of amenorrhea in the last 6 months, (b) no metabolic diseases, (c) body weight stable in the last 6 months and

within the normal ranges for age and height, i.e. a body mass index (BMI) not below 19 kg/m², (d) history of emotionally stressful events preceding the onset of amenorrhea, such as problems within the family, at school, at work, or of psycho-social stress; psychiatric diseases were excluded using DSM-IV criteria [14], (e) no intense training for agonistic purposes, (f) exclusion of adrenal, thyroid, or PRL diseases.

Patients were subdivided according to their LH plasma levels (mean of three samples collected over a span of 30 min: 0, +15, +30): Group A (LH ≤ 3 mIU/ml, *n* = 15), Group B (LH > 3 mIU/ml, *n* = 12).

All patients were invited not to change their life-style and to undergo the following endocrine evaluations before and after 12 weeks of integrative treatment with a combination of L-carnitine (500 mg die per os) and ALC (250 mg die per os) plus an integration of L-arginine, N-acetyl-cisteyn, and Vitamins E and C as antioxidant (Proxeed Women, Alfasigma, The Netherland): (a) GnRH test (10 µg in bolus) performed from time -20, -10, 0 to +100 (sampling every 10 min) and (b) baseline hormonal parameters [LH, FSH, prolactin (PRL), estradiol (E2), androstenedione (A), 17-hydroxy-progesterone (17OHP), TSH, fT3, fT4, insulin, cortisol, amylase, and testosterone (T)]. Integrative treatment was continued at least up to the end of the second round of endocrine evaluations.

Vaginal US was performed before and after the treatment to evaluate the changes of the thickness of the endometrium.

Assay

All samples from each subject were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using a previously described immunofluorometric assay (IFMA) [15,16]. The sensitivity of the assay expressed as the minimal detectable dose was 0.1 IU/ml. The cross-reactivities with free α- and β-subunits of LH, FSH, and TSH were less than 2% [16]. Intra-assay and inter-assay coefficients of variation were 4.5% and 7.1%, respectively.

Plasma E2, 17OHP, A, cortisol, and T were determined by radioimmunoassay (Radim, Pomezia, Rome, Italy) as previously described [17]. Based on two quality control samples the average within- and between-assay coefficients of variation were 3.7% and 8.8%.

Plasma insulin was determined using an immunoradiometric assay (Biosource Europa S.A., Nivelles, Belgium). Based on two quality control samples the average within- and between-assay coefficients of variation were 4.1% and 11.2%.

Statistical analysis

LH response to the GnRH bolus was computed as the difference (Δ_{max}) between the maximum height of the LH response and the LH plasma levels observed at the sampling interval immediately before the stimulation.

Data were tested for statistically significant differences between the groups (before and after treatment interval) after analysis of variance (one-way ANOVA) by the use of Student's *t*-test for paired and unpaired data, as appropriate.

Data are expressed as mean ± SEM.

Results

Table 1 summarizes the hormonal parameters of all patients before and after 12 weeks of integrative treatment. The treatment significantly improved LH plasma levels while significantly

Table 1. Hormonal parameters of all patients with FHA under study (*n* = 27), mean ± SEM.

FHA (<i>n</i> = 27)	LH (mIU/ml)	FSH (mIU/ml)	E2 (pg/ml)	A (pg/ml)	T (ng/ml)	T (ng/dl)	Insulin (µIU/ml)	Cortisol (µg/dl)	17OHP (ng/ml)	17OHP/Cortisol	Amylase (mIU/ml)	TSH (µIU/ml)	fT3 (pg/ml)	fT4 (pg/ml)	BMI
Baseline	4.4 ± 1.1	5.5 ± 0.4	21.4 ± 3.7	261.6 ± 29.4	0.27 ± 0.04	3.6 ± 0.4	16.4 ± 0.6	96.9 ± 7.4	1.0 ± 0.07	0.06 ± 0.004	92 ± 7.7	2.1 ± 0.2	2.5 ± 0.1	10.9 ± 0.5	19.8 ± 0.3
Under treatment	5.1 ± 0.9**	5.7 ± 0.5	21.6 ± 4.5	217.0 ± 24.7	0.21 ± 0.02	5.4 ± 1.1	14.9 ± 0.7*	92 ± 7.7*	1.04 ± 0.1	0.07 ± 0.005	92 ± 7.7*	2.2 ± 0.2	2.7 ± 0.1	10.6 ± 0.3	20.2 ± 0.3***

* *p* < 0.05.

** *p* < 0.01 versus baseline.

*** *p* < 0.03.

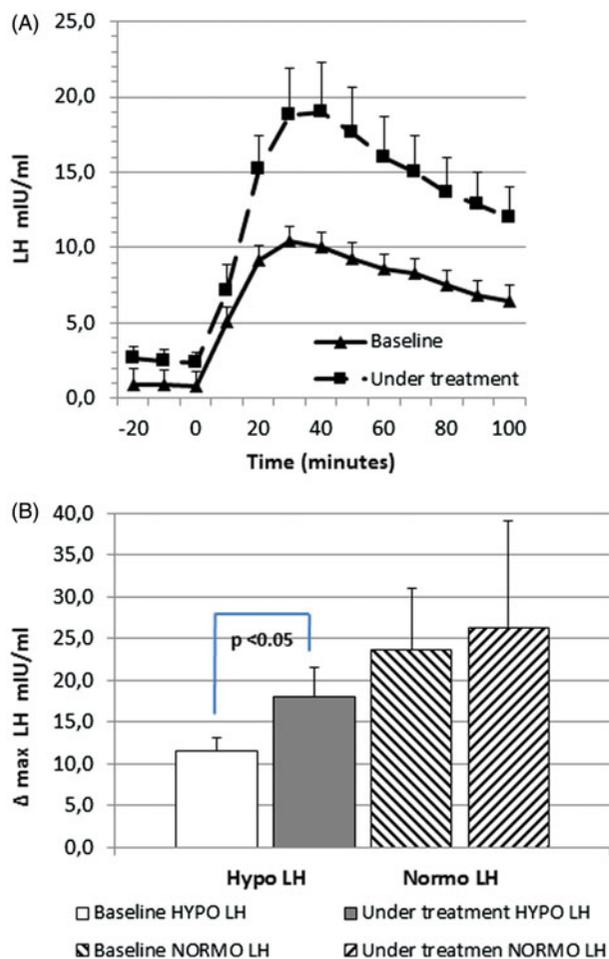


Figure 1. Panel A: Improved GnRH-induced LH response in hypo-LH FHA patients. Panel B: Maximal response (Δ_{\max}) of LH to GnRH bolus in the two groups of FHA patients. Hypo-LH subjects showed the significant increase of the LH response under integrative treatment. No changes in normo-LH subjects.

combination modulates not only the reproductive axis but also metabolism adrenal axis since our patients had the significant decrease of both cortisol and amylase plasma levels. Such effect has not been described before in patients with FHA under ALC only administration.

Indeed ALC has been demonstrated to modulate specific neuroendocrine areas both in experimental animals [21,22] as well as in humans [13,19] protecting the hypothalamic functions from chronic stress. Indeed in rats ALC counteracts the decrease of beta-endorphin contents at the medio-basal hypothalamus (MBH) level and the increase at the anterior pituitary (AP) level and such treatment did not increase circulating beta-endorphin [21,22] and avoided testosterone plasma levels decrease in chronically stressed male rats [22].

Previous studies on FHA showed that ALC improved LH secretion and that such effect was probably modulated by a specific activity exerted and the hypothalamic level, mainly on GnRH-secreting neurons [13,19]. In addition, the positive modulation of ALC on stress-induced blockade of the reproductive axis has been ascribed to ALC ability to acetylate -OH groups such as those on many aminoacids (i.e. serine, threonine, tyrosine) thus modulating protein structure adding another level of control over hormone and neuropeptides (i.e. opioids) conformation and function, decreasing their efficiency and role in determining a dysfunctional state such as FHA [13,23].

Our data are in agreement with these reports [13,19] since mean LH plasma levels increased but were greatly improved in hypo-LH patients. Interestingly, carnitine administration also greatly improved LH response to GnRH stimulation test, thus suggesting that the pituitary have increased not only the ability to respond to the GnRH stimulation but also to synthesize and store LH, as previously suggested [19].

In the present study, we used higher amount of L-carnitine than ALC and this let us infer that probably the quicker metabolic destiny of L-carnitine inside the cells than ALC was at the basis of the effects we observed since it is the esterification of carnitine to acyl-carnitine that permits the transfer of acyl-CoA and acetyl-CoA across mitochondrial membranes activating the metabolism [24].

This combination of carnitines (ALC plus L-carnitine) also disclosed a modulation on the adrenal axis since cortisol plasma levels decreased in the hypo-LH subjects. Such observation is sustained by the recent hypothesis that carnitine metabolism in the hypothalamus and more specifically at the level of NPY (Neuropeptide-Y) and POMC (pro-opio-melanocortin) neurons constitute a 'sensing mechanism that simultaneously integrates nutrient and hormone information to control the output of neurons' [24,25]. According to this hypothesis, most of the actions of the 'metabolically active' hormones such as ghrelin, leptin, or insulin at the POMC or NPY neurons level may depend and vary according to the nutrient status and carnitine metabolism is critical in the integrative processing [24] and the integrative carnitine administration might be at the basis of the reduced cortisol plasma levels of the hypo-LH patients. In addition, this could also explain most of the positive effects observed in patients with FHA under carnitine administration since they often show eating disorders and are classically underfed and/or retain minimal amount of the energy derived by their feeding due to the excessive training. It is well known that, classically, the elevation of amylase levels in plasma is due to a pancreatitis but when dealing with patients with FHA and/or anorexia nervosa/bulimia, the hyperamylasemia is due to excessive secretion of the salivary-type amylase [26]. Indeed severe body weight reduction increases amylase plasma levels [27]. Moreover, fT3 plasma levels were significantly increased by carnitine administration in hypo-LH patients thus supporting a consistent change in the dynamics of the metabolic control of energy production/dispersion. Probably, the positive changes of amylase and fT3 plasma levels are a direct/indirect effect induced by the carnitine-triggered metabolic improvements which are positively modulating glucose, aminoacids as well as fatty acid metabolisms [24].

In conclusion, our data show the specific positive effect and role for the integrative administration of carnitines as a combination of ALC and L-carnitine in patients with FHA and low LH levels. Our data confirm the hypothesis that carnitine integration in patients with FHA improves neuronal function and facilitate both nutrient/metabolic and hormonal modulations at the hypothalamic level and sustain that hormonal signaling in the brain varies according to the nutrient/metabolic status as recently reported [24].

Disclosure statement

The authors have nothing to declare.

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