Treatment with carnitines, L-arginine and N-acetyl cysteine in patients affected by functional hypothalamic amenorrhea (FHA) induces hormonal and metabolic changes

Alessandro D Genazzani [1], Veronica Tomatis [1], Alba Manzo [1], Francesca Ressa [1], Martina Caroli [1], Martina Piccinini [1], Fedora Ambrosetti [1], Melania Arnesano [1], Giulia Despini [1], Blazej Meczekalski [2]

[1] Gynecological Endocrinology Center, Department of Obstetrics and Gynecology, University of Modena and Reggio Emilia, Modena, Italy; [2] Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland

ABSTRACT

Context: Functional hypothalamic amenorrhea (FHA) is a frequent stress-induced reproductive blockade, often reversible by removing stress factors. No specific therapeutic strategies have been defined for FHA, although a number of interventions have been proposed.

Aims: This study was conducted to evaluate the modulations induced by integrative administration of carnitines, L-arginine (LArg) and N-acetyl cysteine (NAC).

Design: Twenty-nine (n=29) patients with FHA were evaluated before and after an integrative treatment with L-carnitine (500 mg) and acetyl-L-carnitine (250 mg) combined with LArg (500 mg), NAC (50 mg), and vitamins E and C as antioxidants, administered daily. Hormonal plasma determinations of LH, FSH, prolactin, estradiol, cortisol, DHEAS, androstenedione, testosterone, progesterone, insulin, and amylase were performed before and after 12 weeks of integrative treatment.

Results: Plasma levels of amylase and insulin were found to be decreased and increased, respectively, after the treatment. When FHA patients were compared according to their baseline LH plasma levels, i.e. above (normo-LH) versus below (hypo-LH) 3 mIU/ml, the integrative treatment was found to be greatly effective in hypo-LH FHA subjects: LH and insulin increased, while amylase and cortisol decreased.

Conclusion: Stress-induced neuroendocrine impairments parallel the metabolic imbalance in FHA. The improvements observed after administering a combination of carnitines with anti-oxidants such as LArg, NAC, and vitamins E and C allow this approach to be considered a putative treatment option.

KEYWORDS

Functional hypothalamic amenorrhea, carnitines, N-acetyl cysteine, L-arginine, anti-oxidant.

Introduction

Functional hypothalamic amenorrhea (FHA) is a classic absence of menstruation that occurs as secondary amenorrhea but can also occur, not infrequently, in young girls heavily involved in activities involving intensive physical training, such as athletics, dance, and gymnastics [1]. FHA is reversible, since no anatomic or organic causes have been found. Indeed, the blockade is a form of defense which is triggered by the neuroendocrine control of the many hypothalamic nuclei that react to the excessive combination of stressors such as food restriction and weight loss, psychological distress and excessive exercise [2-6].

Various hormones, neurotransmitters and neuromodulators, such as prolactin (PRL), cortisol, opioids, noradrenaline, dopamine, etc. [3, 6], are involved in the control of gonadotropin-releasing hormone (GnRH), as well as gonadal steroids, such as estradiol (E2) [7] and weak estrogens, such as estriol, that exert specific priming functions, as recently reported [7, 8].

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Contact
Alessandro D Genazzani; algen@unimo.it
Department of Obstetrics and Gynecology, University of Modena and Reggio Emilia, Via del Pozzo 71, Modena, Italy

In addition, insulin and kisspeptin are known to be closely involved in the modulation of the neuroendocrine mechanisms of the reproductive axis since their plasma concentrations are severely impaired in FHA and show specific correlations with BMI [9]. This observation underlines the close link between metabolism and reproduction.

Various putative treatments have been suggested to restore the activity of the reproductive axis, ranging from estroprogestins and estriol [8] to neuroactive compounds such as pivagabine and naltrexone [10, 11]. Integrative approaches based on neuroactive compound, i.e. acetyl-L-carnitine (ALC) [12], have been

demonstrated to be capable of modulating hypothalamic functions as well as metabolic pathways, especially when combined with L-arginine (LArg) and N-acetyl cysteine (NAC) [13, 14]. Moreover, these latter compounds have a specific anti-oxidant effect that may be relevant in FHA, since reactive oxygen species (ROS) have been demonstrated to exert a negative modulation effect on hypothalamic functions as well as on cognition [15]. In fact, a regular diet and exercise affect mitochondrial energy production, which is important for maintaining neuronal excitability and synaptic function. In addition, the combination of certain diets and exercise can have additive effects on synaptic plasticity and cognitive function [15]. On the contrary, excess energy production caused by high caloric intake and/or strenuous exercise, quite frequent in FHA, results in the formation of excess ROS. When ROS levels exceed the buffering capacity of the cell, synaptic plasticity and cognitive function are compromised, probably owing to a reduction of the activity of signal-transduction modulators such as BDNF (Brain derived Neurotrophic Factor), and reproductive ability is affected [15, 16]. In addition, vitamins E and C have been demonstrated to be relevant in counteracting excess ROS-related negative modulation of the reproductive functions in females well as in males [16, 17].

On the above basis, we designed the present study to better evaluate the effects, on metabolic and hormonal parameters, of an integrative treatment based a combination of carnitines with LArg, N-acetyl-cysteine (NAC), and vitamins E and C, in a group of patients with FHA.

Material and Methods

Subjects

Among 65 amenorrheic patients attending the outpatient clinic at the Gynecological Endocrinology Center at the University of Modena and Reggio Emilia, Italy, 29 patients with a mean age of 23.5±1.5 years [mean±standard error of the mean (SEM)] were selected for participation in the study. All 29 patients were affected by FHA and had specifically requested not to be treated with hormonal or estro-progestin preparations. All of them gave their informed consent to participate in the study.

The patients were enrolled on the basis of the following criteria: 1) presence of amenorrhea for the last 6 months; 2) no metabolic diseases; 3) stable body weight for the last 6 months, within the normal range for their age and height (i.e. a body mass index [BMI] not below 19 kg/m^2); 4) history of emotionally stressful events preceding the onset of amenorrhea, such as problems within the family, at school, at work, or the presence of psychosocial stress (psychiatric diseases were excluded using DSM IV criteria [18]; 5) no intensive physical training; and 6) no adrenal, thyroid, or PRL disorders. All the patients underwent a gynecological examination, when possible (11 out of 29 were virgo intacta), and ultrasound examination of the pelvis. All of them reported normal pubertal development and normal development of sexual characteristics.

Nuclear magnetic resonance (NMR) of the hypophysis was performed in 23/29 patients before they attended our outpatient clinic, and all were negative for masses and/or adenomas, while the remaining 6 underwent NMR before undergoing the study (all negative for masses), as a routine investigation in patients with FHA.

All the patients were invited not to change their lifestyle and to undergo our center's standard hormonal evaluation procedure, to allow us to evaluate the hormonal aspects of their amenorrheic condition, before and after 12 weeks of integrative treatment with a combination of L-carnitine (500 mg/day per os) with ALC (250 mg/day per os), LArg (500 mg), NAC (50 mg), and vitamins E and C as antioxidants (Proxeed Women, Alfasigma, The Netherlands).

The hormonal evaluation procedure includes determinations of plasma levels of LH, FSH, PRL, E2, cortisol, DHEAS, androstenedione (A), testosterone, progesterone (P), insulin and amylase. Integrative treatment was continued at least up to the end of the second round of endocrine evaluations.

According to the levels of LH observed at baseline, the entire cohort of patients was subdivided into two groups: those with LH < 3 mIU/ml (hypo-LH, n=15) and those with LH ≥ 3mIU/ml (normo-LH, n=14). This subdivision was done as in previous reports [5-8, 10-13, 19, 20], and is based on the fact that it reflects the clinical evolution of the gonadotropin hormonal pattern in FHA, with patients recording LH levels below 3 mIU/ml being the ones most and/or longest affected by stressors.

The study was approved as an observational study by the Human Investigation Committee of the University of Modena and Reggio Emilia, Italy (Registration No. 181/12).

Assay

All samples from each patient were assayed in the same assay. Plasma LH and FSH, TSH, fT3 and fT4 concentrations were determined using an immunofluorimetric assay previously described elsewhere [8, 10]. The sensitivity of the assay, expressed as the minimal detectable dose, was 0.1 mIU/mL. The cross-reactivity with free α and β subunits of LH, FSH, and TSH was less than 2% [10]. Intra-assay and interassay coefficients of variation were 4.8% and 7.2%, respectively.

Plasma PRL, P, A, 17OHP, DHEAS and cortisol were determined by radioimmunoassay (Radim, Pomezia, Italy), as previously described elsewhere [7]. Based on two quality-controlled samples, the average within- and between assay coefficients of variation were 4.0% and 9.4%.

The serum concentration of E2 was measured by electrochemiluminescence immunoassay using the Elecsys E2 II reagent kit from Roche Diagnostics GmbH, Mannheim, Germany, with a sensitivity of 4.5 pg/ml. Plasma insulin was determined using an immunoradiometric assay (BioSource Europa S.A., Nivelles, Belgium). Based on two quality-controlled samples, the average within- and between-assay coefficients of variation were 4.3% and 11.4%, respectively.

Statistical analysis

Data are expressed as mean ± SEM. We tested data for significant differences between groups, after analysis of variance (one-way ANOVA), using Student's t-test for paired data. Pearson's index was computed to evaluate correlation coefficients between groups. A p-value of at least < 0.05 was considered significant.

Results

Table 1 summarizes the hormonal parameters observed for the whole group of patients before and after 12 weeks of integrative treatment. Interestingly, the only significant changes observed concerned plasma levels of insulin and amylase, which were increased and decreased, respectively, after the treatment. No modifications of any other hormonal parameter and no changes in BMI were observed (not shown).

On the contrary, when the patients were analyzed according to their baseline plasma levels of LH, significant differences were observed. Subjects with LH<3 mIU/ml (hypo-LH patients, n=15) (Table 2) showed significant improvement of hormonal parameters and a completely different hormonal pattern when compared with those showing normal LH levels (LH> 3 mIU/ml, n=14). Indeed, the hypo-LH patients had significantly lower plasma levels of LH, E2, fT3 and insulin, and a lower LH/FSH ratio, and higher plasma levels of cortisol

Table 1 Hormonal parameters of patients under study (n=29) at baseline and after 12 weeks of integrative treatment.

Baseline	fT4 pg/ml	fT3 pg/ml	TSH ßIU/ml	E2 pg/ml	P ng/ml	PRL ng/ml	A ng/ml	17-OHP ng/ml	Cortisol µg/dl	Amylase µU/L	Insulin µIU/ML	DEHAS µg/ml	LH mIU/ml	FSH mIU/ml	LH/FSH	17OHP/Cortisol
Mean	10.74	2.7	2.16	23.26	0.20	12.71	212.06	1.06	15.08	93.14	3.23	1.73	5.26	6.22	0.85	0.074
SEM	0.43	0.09	0.18	2.7	0.02	1.37	17.3	0.05	0.66	4.1	0.21	0.48	0.85	0.32	0.13	0.005
After treatment	**fT4 pg/ml**	**fT3 pg/ml**	**TSH ßIU/ml**	**E2 pg/ml**	**P ng/ml**	**PRL ng/ml**	**A ng/ml**	**17-OHP ng/ml**	**Cortisol µg/dl**	**Amylase µU/L**	**Insulin µIU/ML**	**DEHAS µg/ml**	**LH mIU/ml**	**FSH mIU/ml**	**LH/FSH**	**17OHP/Cortisol**
Mean	10.41	2.68	2.20	20.46	0.23	11.43	203.91	1.08	15.59	83.73	4.05	1.69	4.76	5.67	0.85	0.068
SEM	0.17	0.11	0.17	3.07	0.03	2.81	15.8	0.08	0.70	4.29	0.5	0.8	0.63	0.34	0.12	0.003
P level										0.002	0.05					

Table 2 Hormonal parameters at baseline and after 12 weeks of integrative treatment. Patients (n=29) were subdivided according to baseline LH levels (below or above 3 mUI/ml).

Hypo-LH N=15 Baseline	fT4 pg/ml	fT3 pg/ml	TSH ßIU/ml	E2 pg/ml	P ng/ml	PRL ng/ml	A ng/ml	17-OHP ng/ml	Cortisol µg/dl	Amylase µU/L	Insulin µIU/ML	LH mIU/ml	FSH mIU/ml	LH/FSH	17OHP/Cortisol
Mean	9.7	2.28	1.98	12.42	0.17	10.33	194.17	0.92	17.13	90.67	2.44	1.74	5.34	0.31	0.05
SEM	0.53	0.12	0.35	1.31	0.02	1.96	28.56	0.1	0.82	6.69	0.22	0.29	0.52	0.05	0.005
After treatment	**fT4 pg/ml**	**fT3 pg/ml**	**TSH ßIU/ml**	**E2 pg/ml**	**P ng/ml**	**PRL ng/ml**	**A ng/ml**	**17-OHP ng/ml**	**Cortisol µg/dl**	**Amylase µU/L**	**Insulin µIU/ML**	**LH mIU/ml**	**FSH mIU/ml**	**LH/FSH**	**17OHP/Cortisol**
Mean	10.55	2.55	2.27	11.45	0.15	9.15	174.44	0.98	14.32	79.45	3.20	2.99	5.67	0.53	0.07
SEM	0.22	0.09	0.34	1.68	0.02	2.76	12.88	0.08	0.70	5.24	0.34	0.72	0.72	0.15	0.007
P level									0.009	0.006	0.05	0.05		0.05	0.05
Normo-LH N=14 Baseline	**fT4 pg/ml**	**fT3 pg/ml**	**TSH ßIU/ml**	**E2 pg/ml**	**P ng/ml**	**PRL ng/ml**	**A ng/ml**	**17-OHP ng/ml**	**Cortisol µg/dl**	**Amylase µU/L**	**Insulin µIU/ML**	**LH mIU/ml**	**FSH mIU/ml**	**LH/FSH**	**17OHP/Cortisol**
Mean	11.22	2.92	2.30	28.76	0.22	76.77	228.1	1.13	14.4	95.8	3.7	7.36	6.59	1.18	0.08
SEM	0.6	0.09	0.22	3.71	0.04	66.37	22.50	0.06	0.90	5.56	0.29	1.17	0.39	0.19	0.007
P level vs hypo-LH		0.0008		0.001					0.05		0.02	0.000001		0.03	0.01
After treatment	**fT4 pg/ml**	**fT3 pg/ml**	**TSH ßIU/ml**	**E2 pg/ml**	**P ng/ml**	**PRL ng/ml**	**A ng/ml**	**17-OHP ng/ml**	**Cortisol µg/dl**	**Amylase µU/L**	**Insulin µIU/ML**	**LH mIU/ml**	**FSH mIU/ml**	**LH/FSH**	**17OHP/Cortisol**
Mean	10.16	2.9	2.19	27.07	0.25	12.91	221.54	1.18	16.47	88.62	4.93	6.14	5.85	1.07	0.07
SEM	0.33	0.26	0.19	4.91	0.05	4.00	25.92	0.15	1.17	7.20	0.80	0.92	0.32	0.16	0.005
P level										0.05	0.05				
P level vs hypo-LH		0.02		0.0009								0.006		0.03	

and a higher 17OHP/cortisol ratio (Table 2) than the normo-LH patients (Table 2). After the integrative treatment, hypo-LH patients showed significantly increased 17OHP/cortisol and LH/FSH ratios, and plasma levels of fT3, insulin and LH, as well as significantly reduced plasma levels of cortisol and amylase, which, however, remained significantly different from the levels recorded in the normo-LH patients (Table 2). Interestingly, the normo-LH patients showed no changes in any of the study parameters after the integrative treatment (Table 2).

To better understand the putative hormonal dynamics in patients with FHA, Pearson's correlation was computed between the hormonal parameters under study before and after the integrative treatment. On analysis of the whole group of patients (n=29) (Table 3), several parameters were found to be significantly correlated both at baseline and after the treatment (cortisol & LH, insulin & LH, fT3 & LH, LH & E2, LH & 17OHP, LH & 17OHP/cortisol ratio), while others were significantly correlated only after the treatment (LH & androstenedione, cortisol & androstenedione, androstenedione & E2, 17OHP & cortisol). Only the LH & 17OHP/cortisol ratio correlation was lost after the treatment.

A clearer picture emerged when Pearson's coefficient was computed on the two subgroups of patients: hypo- and normo-LH. Indeed, the hypo-LH patients demonstrated specific

Table 3 Pearson's correlation coefficient on the whole group of patients (n=29) before and after 12 weeks of integrative treatment.

BASELINE Pearson index			AFTER TREATMENT Pearson index		
P level			P level		
-0.339	0.05	cortisol & LH	0.534	0.01	cortisol & LH
-0.048		amylase & LH	0.149		amylase & LH
0.582	0.001	insulin & LH	0.707	0.0001	insulin & LH
0.539	0.001	fT3 & LH	0.364	0.05	fT3 & LH
0.316		LH & androstenedione	0.364	0.05	LH & androstenedione
0.204		cortisol & androstenedione	0.348	0.05	cortisol & androstenedione
0.737	0.0001	LH & E2	0.820	0.0001	LH & E2
0.259		androstenedione & E2	0.324	0.05	androstenedione & E2
0.456	0.01	LH & 17OHP	0.497	0.01	LH & 17OHP
0.623	0.0001	LH & 17OHP / cortisol	0.233		LH & 17OHP / cortisol
0.1999		insulin & amylase	0.199		insulin & amylase
0.112		LH & TSH	0.043		LH & TSH
0.191		17OHP & cortisol	0.758	0.0001	17OHP & cortisol

Table 4 Pearson's correlation coefficient on the hypo-LH group of patients (n=15) before and after 12 weeks of integrative treatment.

BASELINE Pearson index			AFTER TREATMENT Pearson index		
P level			P level		
-0.196		cortisol & LH	0.259		cortisol & LH
-0.271		amylase & LH	-0.059		amylase & LH
-0.012		insulin & LH	0.609	0.05	insulin & LH
0.333		fT3 & LH	0.580	0.05	fT3 & LH
0.099		LH & androstenedione	-0.187		LH & androstenedione
0.615	0.01	cortisol & androstenedione	0.110		cortisol & androstenedione
0.672	0.01	LH & E2	0.008		LH & E2
-0.119		androstenedione & E2	-0.034		androstenedione & E2
-0.097		LH & 17OHP	0.040		LH & 17OHP
-0.052		LH & 17OHP / cortisol	-0.060		LH & 17OHP / cortisol
-0.590	0.05	insulin & amylase	0.005		insulin & amylase
0.281		LH & TSH	0.030		LH & TSH
0.451	0.05	17OHP & cortisol	-0.150		17OHP & cortisol
0.236		17OHP & androstenedione	0.812	0.01	17OHP & androstenedione

Table 5 Pearson's correlation coefficient on the normo-LH group of patients (n=14) before and after 12 weeks of integrative treatment.

BASELINE Pearson index			AFTER TREATMENT Pearson index		
P level			P level		
-0.259		cortisol & LH	0.538	0.01	cortisol & LH
-0.161		amylase & LH	0.053		amylase & LH
0.476	0.05	insulin & LH	0.623	0.01	insulin & LH
0.363		fT3 & LH	0.004		fT3 & LH
0.311		LH & androstenedione	0.245		LH & androstenedione
0.049		cortisol & androstenedione	0.393		cortisol & androstenedione
0.667	0.001	LH & E2	0.799	0.001	LH & E2
0.322		androstenedione & E2	0.254		androstenedione & E2
0.486	0.05	LH & 17OHP	0.437		LH & 17OHP
0.522	0.01	LH & 17OHP / cortisol	0.221		LH & 17OHP / cortisol
-0.010		insulin & amylase	0.133		insulin & amylase
0.048		LH & TSH	0.164		LH & TSH
0.089		17OHP & cortisol	0.910		17OHP & cortisol
0.578	0.01	17OHP & androstenedione	0.230	0.001	17OHP & androstenedione

and more evident results than the normo-LH patients.

In fact, as shown in Table 4, the integrative treatment led to recovery of positive correlations between insulin & LH and between fT3 & LH. Conversely, the correlations between cortisol & androstenedione, LH & E2, insulin & amylase, 17OHP & cortisol disappeared after the treatment. A different pattern was observed in the normo-LH patients (Table 5) in whom the treatment had less impact on the correlations: indeed, after the treatment, the insulin & LH, LH & E2, and 17OHP & androstenedione ones were still present, while the cortisol & LH one appeared (Table 5).

According to the patients' individual diaries, only 4/29 patients (3 normo-LH and 1 hypo-LH patient) reported the occurrence of at least 1 spontaneous bleed.

Discussion

The present study reported that treatment with carnitines in combination with LArg and NAC positively modulate hormonal and metabolic parameters in patients with FHA, especially the hypo-LH subgroup.

FHA is triggered by a hypothalamic defensive blockade induced by combined negative modulation of various kinds of stressors (i.e. metabolic, psychological and physical) on hypothalamic functions [3, 19, 21]. None of these stressors acts singly; in general, at least two of them act in combination, affecting nuclei that control feeding, satiety, sleep and obviously those controlling the reproductive axis [6, 22].

Metabolic and nutritional factors are important issues in FHA since the restoration of normal feeding behavior and/or reduction of associated excessive energy consumption allows the restoration of many of the hypothalamic functions, thus permitting reactivation of the reproductive axis [8]. Although guidelines have been proposed [2, 23], up to now relatively specific treatments have been demonstrated to be effective in patients with FHA, such as naltrexone chlorydrate [10] and pivagabine [11], since these compounds act directly on some of the main casual factors of the neuroendocrine hypothalamic blockade. Moreover, the use of low doses of weak estrogens (i.e. estriol) [8] and the finding of very low plasma E2 levels [7] have clearly shown estrogen priming underlies a number of intracellular biochemical and biological events that support the chances of restoring normal neuroendocrine control of the reproductive axis.

However, an appropriate upload of nutraceutical compounds might be effective too, acting on the neuroendocrine mechanisms that regulate reproduction. Carnitines have been found to be greatly effective, both in animal models [24, 25] and in humans, especially in FHA [12, 20]. In fact, carnitines, especially L-acetyl carnitine, are reported not only to modulate beta-oxidative processes, but also to counteract the negative modulation of the opioidergic pathway on GnRH secretion [12].

Our present data confirm such previous reports [12, 20] and show the positive role of lower doses of carnitines (i.e. 750 mg) on LH secretion when combined with LArg and NAC [13]. In fact, our data confirm that this integrative combination is able to improve plasma gonadotropin levels, i.e. LH, mainly in hypo-LH FHA patients, with lower daily amounts of carnitines than when carnitines were administered alone at higher doses (1-2 gr) [12, 20].

As previously reported [13], this integrative combination was effective on specific metabolic parameters, since insulin and amylase levels changed both in hypo- as well as in normo-LH patients. Indeed, it is well known that elevation of amylase levels in plasma is classically due to pancreatitis, but in 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 plasma am-

ylase levels [27]. Our present observation clearly supports the argument that impaired metabolism might underlie maintenance of the neuroendocrine impairments of FHA, independently of plasma LH levels, and showed that this integrative combination acted specifically on these metabolic parameters. Further support is provided by our finding of improved fT3 levels only in hypo-LH patients characterized by low fT3 levels (low fT3 syndrome), given that these patients are the more metabolically impaired.

Our study also found that only hypo-LH patients showed significant reduction of plasma cortisol levels [13]. These patients were probably the ones with greater stress-induced adrenal hyperactivation.

On investigation of correlations between hormonal and metabolic parameters after the treatment, FHA patients did not show great changes: they continued not to show correlations between cortisol & LH, insulin & LH, fT3 & LH, and E2 & LH, for example. In fact, after the treatment, only LH & androstenedione, cortisol & androstenedione, and 17OHP & cortisol were found to be correlated. A completely different scenario emerged when FHA patients were analyzed according to their baseline plasma LH levels. In fact, the hypo-LH patients improved considerably after the integrative treatment, not only in terms of metabolic and hormonal parameters, as discussed before, but also in terms of their correlations. Indeed, these patients recovered the correlation between LH and fT3, a finding that reinforces the link between metabolism and reproduction.

After the treatment, the hypo-LH patients lost the correlation between cortisol and androstenedione. This can easily be explained by the fact that while most of the androstenedione in the baseline condition was adrenal in origin, after the integrative treatment, adrenal function was shown to be reduced, since cortisol levels decreased. This change in cortisol was at the root of the improved 17OHP/cortisol ratio that was observed in the hypo-LH patients and can be considered an index of reduced adrenal function. For the same reason, the 17OHP & cortisol correlation was lost after the treatment.

On the contrary, 17OHP and androstenedione were found to be correlated after the treatment. This finding suggests that, after the treatment, the measured 17OHP was mainly ovarian in origin, like the androstenedione. This suggestion is indirectly supported by the fact that the normo-LH subjects showed the correlation between 17OHP & androstenedione both at baseline and after treatment. Moreover, these patients showed higher (albeit not significantly) plasma E2 and androstenedione levels than the hypo-LH patients.

Even though a number of improvements were observed in the hypo-LH patients, LH and E2 showed no correlation after the treatment. Probably greater metabolic and neuroendocrine changes or a longer treatment interval are needed before ovarian activity is sufficiently evident to be expressed in significantly improved E2 production, associated with restored follicular recruitment. In fact, none of the patients in the study had menstrual bleeding during the observation period.

According to the present data, the integrative treatment approach described positively modulates hormonal and metabolic pathways mainly in hypo-LH FHA subjects. Dividing the patients into hypo- and normo-LH groups revealed two different populations: the normo-LH patients who showed improvement only of plasma insulin and amylase concentrations, and the hypo-LH patients, more deeply affected by stressors, who showed greater improvements after the treatment. This concept fits with what has been supposed in previous reports [10, 28, 29], in which opioidergic hypertone in FHA correlated with the presence or absence of LH response to opioidergic blockade (i.e. naloxone or naltrexone clorhydrate). These are subjects whose clinical state has not yet deteriorated as much as that of hypo-LH subjects. Nevertheless, the dominating feature in both groups is the deep involvement of the metabolic pathways, mainly represented by plasma concentrations of insulin and amylase.

It is well documented that administration of carnitines improved endogenous acyl-carnitine production with higher acyl-CoA and acetyl-CoA transportation inside the mythocondrium, thus improving metabolism and beta-oxidation [30]. Certainly, the combination of L-carnitine plus L-acetyl carnitine acted positively on the neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) secreting neurons that are the nuclei sensitive to nutrients, and are connected with the hypothalamic centers [30, 31]. According to recent studies, carnitines are the mediators of the actions of metabolically active hormones such as leptin, ghrelin and insulin on NPY and POMC secreting neurons [30, 31]. The positive actions on amylase and fT3, classically impaired in FHA patients similarly to anorectic patients [26], can be ascribed to the metabolic changes induced by administration of carnitines, as previously suggested [26, 27].

Additional important roles have also been played by LArg and NAC, here co-administered with carnitines, since these compounds act as metabolic and anti-oxidant inductors, together with vitamins E and C [32]. It is known that excessive dieting/altered feeding and/or excessive physical exercise induce ROS overproduction that leads to specific negative modulations at the central nervous system level, leading to impaired cognitive activities and reduced neuroendocrine signal transduction [15]. LArg and NAC administration can be assumed to positively interfere with ROS production in stress-induced FHA, improving the recovery of the endogenous anti-oxidant system, greatly represented by glutathione reactivation. This putative model has recently been proposed also for PCOS [14].

In conclusion, the present study showed that the magnitude of metabolic and stress-induced neuroendocrine impairments is greater in the presence of plasma LH levels of 3 mIU/ml or less. This evidence reinforces the clinical view that this plasma LH level represents a threshold with regard to the severity of the stressors' actions. The use of integrative carnitines in combination with LArg, NAC and vitamins E and C positively improved hormonal and metabolic impairments in patients with FHA, especially those with low plasma LH levels.

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