Plasma carnitine is associated with fatigue in chronic hepatitis C but not in irritable bowel syndrome

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Running Title: Carnitine and fatigue in hepatitis C

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Abbreviations: IBS: irritable bowel syndrome

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Abstract

Objectives Fatigue is an important determinant of altered quality of life in patients affected by chronic hepatitis C (CHC) or the irritable bowel syndrome (IBS). In this study, we aimed at determining the contributory role of plasma levels of leptin and carnitine on fatigue in CHC and IBS. ***Methods*** We enrolled 70 patients with CHC, 42 with IBS and 44 healthy subjects. Fatigue was evaluated using the Fatigue Impact Scale questionnaire. Body composition was assessed through impedance analysis. Plasma carnitine and leptin were measured. ***Results*** Fatigue scores were significantly more elevated in patients with CHC and IBS than in healthy subjects. Patients with CHC, but not with IBS, had significant lower plasma levels of total and free carnitine adjusted for fat mass compared to healthy subjects. In patients with CHC, and not with IBS, fatigue scores were negatively correlated with plasma levels of carnitine. Levels of free carnitine were significantly and independently associated with the severity of fatigue in patients with CHC (OR=2.019, p=0.02, CI 95% [1.01-1.23]).***Conclusions*** In patients with CHC, the severity of fatigue is associated with low level of carnitine, suggesting that an oral supplementation may be effective to relieve fatigue in CHC. The underlying mechanism of fatigue in IBS does not seem to involve carnitine.

Introduction

Fatigue is one of the most common complain reported by patients in primary care settings [1]. This persistent feeling of exhaustion and decrease capacity for physical and mental work is a ground state for many diseases and common symptom of various chronic digestive illness such as chronic hepatitis C (CHC) [2], primary biliary cirrhosis [3 1], and also functional bowel disorders, namely the irritable bowel syndrome (IBS) [4]. To date, no proper fatigue-related biochemical changes have been established, therefore identifying alterations of biochemical parameters would be helpful to understand the complexity of fatigue in the hope for future therapies.

As described by Swain and Maric [5 6], three major central changes have been implicated; (i) neuroendocrine causes, with abnormal function of the hypothalamic-pituitary-adrenal (HPA) axis; (ii) abnormalities in neurotransmission, particularly in the central serotonin pathway and (iii) alteration in immune activation and cytokines release, in particular the adipokine leptin. For example, in patients with chronic liver diseases, we have shown that the severity of fatigue was associated with high leptin levels in both primary biliary cirrhosis and CHC, in the latter, with a concomitant increase of the tumor necrosis factor α (TNF-α) levels, one major secretagogue of leptin [2]. We have also subsequently found a close association between the severity of fatigue and plasma leptin levels adjusted for fat-mass in a cohort of patients with IBS, suggesting a non-specific contribution of leptin in the complexity of fatigue associated with digestive and liver diseases [4].

L-carnitine is an endogenous compound absorbed in the intestine by a combination of active transport and passive diffusion [7]. The heart, skeletal muscle, liver, kidneys and epididymis have specific transport system for carnitine that concentrate carnitine within these tissues. Carnitine's primary mechanism of action is apparently attributable to its role as a cofactor in the transformation of free long-chain fatty acids into acyl carnitine for subsequent transport into the mitochondrial matrix [8]. The well-established roles in energy production and modulation of the mitochondrial coenzyme A (CoA)/acyl-Coa ratio in the skeletal muscle, suggests that carnitine supplementation might increase fatty acid oxidation, thus making more ATP available for mechanical work and delaying fatigue development [9]. In human, a serum acylcarnitine deficiency has been observed in certain types of diseases such as patients with chronic fatigue syndrome [10] and CHC [11]. However, plasma carnitine levels have never been evaluated in patients having IBS and fatigue. Interestingly, administration of carnitine to healthy elderly subjects has been shown to exert a favorable effect on fatigue and serum lipids [12]. Moreover, carnitine supplementation is relevant for the fatigue treatment of cancer chemotherapy [13], multiple sclerosis [14], fibromyalgia [15] haemodialysis [16], celiac disease [17] and also in patients with CHC who received interferon therapy [18]. Interestingly, experimental findings support that carnitine palmitotransferase-1 linked fatty acid oxidation is a key modulator of leptin expression [19]. In healthy ponies, carnitine supplementation has been associated with enhanced glucose tolerance and increase in leptin concentration [20].

In this study, we aimed at determining the contributory role of plasma levels of carnitine on fatigue in patients with untreated CHC and IBS.

Patients and Methods

Participants

Forty two patients with IBS (34F, 8M, mean-age 50.7±13.6 yrs) were included prospectively in the study during a 6-month recruitment period. The presence of IBS was determined according to the Rome II criteria following exclusion of organic disease. These criteria required the presence of abdominal pain or discomfort relieved by defecation or associated with change in stool form or frequency, together with at least two of the following symptoms: altered stool frequency (> 3 per day or < 3 per week), altered stool passage (straining/urgency), passage of mucus, bloating and/or abdominal distension. These symptoms had to be present more than 25% of the time. Patients were diagnosed with predominant constipation (IBS-C) if they reported abdominal pain or discomfort associated with a primary alteration in bowel function consistent with constipation (i.e. infrequent hard stools or increased straining to pass stools). Patients were categorized with predominant diarrhea (IBS-D) if they reported abdominal pain or discomfort associated with primary alterations in bowel function consistent with diarrhea (i.e. frequent loose or watery stools and urgency). Patients with alternating symptoms (IBS-A) of constipation and diarrhea were also included with at least one of the criteria required for the diagnosis of IBS-C or IBS-D.

The following exams were performed: laboratory blood tests (including erythrocyte sedimentation rate, C reactive protein, hemoglobin level, leukocyte count, electrolytes, liver function tests, pancreatic amylase, Human imunodeficiency and hepatitis C and B viruses, and thyroid function tests); a gastroscopy with biopsy specimen of the duodenum; an abdominal ultrasound or a computed tomography; and finally a fecal culture (also looking for

ova and parasites). A colonoscopy was performed in all patients and biopsy specimens were taken if diarrhea was predominant.

Eighty one patients admitted to our liver unit for compensated liver disease due to CHC (47F, 34M, mean age 43.7±12.3 years) were included in the study. The diagnosis of CHC was based on the association of: (a) elevation of serum alanine aminotransferase (ALT) above 40 U/l (upper normal limit) for six months or longer, (b) presence of anti-hepatitis C virus (HCV) antibodies, (c) presence of HCV viraemia, and (d) exclusion of other causes of chronic liver disease (alcoholism, chronic hepatitis B, Wilson's disease, hepatotoxic drugs, haemochromatosis, α1 antitrypsin deficiency, autoimmune chronic active hepatitis). A liver biopsy was performed in all patients, none of whom was cirrhotic. These patients had no evidence of dehydration or overhydration or any other acute or chronic disease suspected of causing hypermetabolism (including human immunodeficiency virus). Furthermore, none had renal failure, thyroid disease, or clinically apparent depression, and none was treated with beta blockers or had received antiviral therapy.

Forty-four healthy volunteers (34F, 8M, mean age 46.5±17.3 yrs) were recruited by advertisement during the same period and formed the control group. These subjects were considered normal on the basis of history, physical exam and biochemical blood screening and were enrolled if they did not have any digestive symptom. None of them experienced fatigue and depression that were excluded by a psychiatrist. All clinical studies were performed according to the declaration of Helsinki, and all participants gave their written informed consent.

Questionnaires

Participants were systematically asked for fatigue and were convened to complete a self-reported measure pertaining to the psychological state (e.g. fatigue). Fatigue was measured using the French version of the Fatigue Impact Scale (FIS) questionnaire which was used by us in patients with chronic hepatitis C [2] and IBS [21]. FIS is a self-report questionnaire consisting of 40 statements that describes possible manifestations of fatigue; these statements are divided in three categories (cognitive [n=10], physical [n=10], psychosocial [n=20]). Each item is rated on a five-point scale of distress, ranging from 0 (“no problem") to 4 ("extreme problem") with a maximum of 160 points. In addition to the 40 items, the FIS asks for the frequency of fatigue in terms of days per month and the usual duration of fatigue per day. The FIS also includes a visual analog scale (VAS) allowing evaluation of the "disabling effect" of fatigue on a scale of 1 ("no disabling effect") to 10 ("severe disabling effect").

Bioelectrical impedance analysis

Body composition was measured in the morning, after a 12-hours overnight fast (including alcohol and drugs and no smoking) in the Functional Explorations Unit of the department of Gastroenterology. Body composition was measured by bipolar bioelectrical impedance analysis (BIA) with an alternating electric current (50 μA) at two frequencies: 1 MHz and 5 kHz, as previously described and validated by Boulier et al [22]. Measurements were taken in the conditions described hereafter. The subjects were supine for 30 min, arms relaxed along their side but not touching the body. Body mass index (BMI) is expressed as weight per height squared (kg/m^2).

Plasma biochemical analysis

All plasma samples were collected at 08:00 AM in both IBS and controls and were stored in duplicate at -80°C until assayed. Plasma cortisol, ACTH and TSH levels were used to evaluate basal HPA axis as well as thyroid functions. Serum leptin levels were measured using a specific radioimmunoassay for human leptin (Linco Research, St. Charles, MO, USA) with a limit of detection of 0.5 ng/mL. The intra- and inter-assay coefficients of variation were less than 8%. Acylcarnitines samples were prepared for analysis as following: 10µL of plasma sample is extracted with 100 µl of methanolic solution containing stable isotope-labeled acylcarnitines internal standards (Cambridge Isotope Laboratories, Inc. #NSK-B) for 20 min at ambient temperature.

The methanolic eluates were dried under nitrogen, and reconstituted with 100 µl of mobile phase (water/acetonitrile v/v, 0.02% formic acid) prior to analysis.

Electrospray MS/MS was performed on an API3000 model tandem mass spectrometer (Applied Bioystems, Foster City, CA). Acylcarnitine standards including free, acetyl, propionyl, butyryl, hexanoyl, octanoyl, decanoyl, dodecanoyl, tetradecanoyl, palmitoyl and stearoyl carnitines were purchased from Dr. HJ ten Brink (VU Medical Center, Amsterdam, The Netherlands) and allowed to establish standard curves for quantitation.

Statistical analysis

Quantitative data were expressed as mean ± SD and were compared using the Mann-Whitney U-test. The Chi-2 test was used to compare qualitative data. The independent predictors of fatigue were determined using a multiple logistic regression model. A p value < 0.05 was considered significant.

Results

Characteristics of the study population

The characteristics of the study populations are presented in Table 1. The percentage of female was significantly (p=0.0002) more elevated in patients with IBS (80.9%) than in patients with CHC (42%) or controls (52,2%). Patients with CHC were significantly younger than IBS patients (43.7±12.3 vs 50.7±13.6, years p=0.03). Patients with IBS had significantly higher BMI than patients with CHC or controls (27±5 vs 24±4 and 23±4 kg/m^2, p=0.008 vs CHC and p=0.007 vs controls, respectively).

Evaluation of fatigue

Overall, 46% of patients with CHC and 55% of patients with IBS verbally expressed fatigue and rated more than 4 on the visual analog scale. Among IBS patients, 20% considered that fatigue was the worst symptom of their disease whereas 45% observed that it was as disabling as their digestive symptoms. The total score of fatigue was significantly higher in IBS patients (68.4±32.9) and CHC patients (56.0±39.8) than in controls (15.5±11.6, p<0.0001 vs IBS and CHC). Fatigue scores were not different between IBS subtypes. Table 2 presents fatigue scores according to the sex of both patients and controls. There were no significant differences between patients with CHC or IBS among each subscale of fatigue (Figure 1).

Carnitine and leptin assessments

Due to the sex dependency of body composition, leptin and carnitine biological findings are presented in Table 3 in both male and female patients and controls.

Absolute plasma leptin levels were significantly higher in IBS patients (32.8±26.2 ng/ml) than in patients with CHC (15.0±19.4 ng/ml, p<0.0001 vs IBS) and controls (7.4±5.2 ng/ml, p<0.0001 vs CHC). When adjusted for fat mass or BMI, leptin levels were significantly higher in IBS (1.8±2.8 ng/mL.Kg^{-1} and 11.6±9.4 ng/mL.Kg/m^2respectively) than in CHC (0.6±0.6 ng/mL.Kg^{-1} and 5.9±7.0 ng/mL.Kg/m^2 p<0.0001 and p=0.0002 respectively) or in CHC than in controls (0.5±0.4 ng/mL.Kg^{-1} and 3.0±2.1 ng/mL.Kg/m^2, p=NS and p=0.03 vs CHC respectively).

Absolute plasma levels of total carnitine were similar among patients with CHC (44.7±10.1 ng/mL), IBS (47.0+10.1ng/mL) and controls (47.1±10.1 ng/mL, p=NS). Similarly, absolute plasma levels of free carnitine were not significantly different among patients with CHC (36.0±8.5 ng/mL), IBS (36.7±8.9 ng/mL) and controls (36.7±8.9 ng/mL). When adjusted for fat mass, total and free carnitine levels were significantly lower in CHC (2.7±1.6 and 2.2±1.3 ng/mL.Kg^{-1}) than in controls (4.5±4.3 and 3.5±3.6 ng/mL.Kg^{-1}, p=0.02 and p=0.02 respectively). In IBS patients, there were a statistical trend favor to lower total and free carnitine levels compared to controls (2.9±4.7 and 2.4±4.6 ng/mL.Kg^{-1}, p=0.08 and p=0.12 respectively) (Figure 2).

Correlation analysis

In patients with CHC, the total score of fatigue was significantly correlated with leptin (r=0.30, p=0.009), leptin adjusted for fat mass (r=0.31, p=0.01). The total score of fatigue

also correlated with total (r=-0.41, p=0.0003) and free (r=-0.43, p=0.0001) carnitine levels even when adjusted for fat mass (r=-0.23 p=0.03 and r=-0.22, p=0.04) (Figure 3). There were no significant relationship between plasma levels of carnitine and the degree of fibrosis and/or inflammation observed in liver biopsy specimens.

In patients with IBS, the total score of fatigue was significantly correlated with leptin (r=0.31, p=0.04), leptin adjusted for fat mass (r=0.25, p=0.03), but not with total or free carnitine levels even when adjusted for fat mass.

In CHC patients, the following parameters were included in a multiple logistic regression model: METAVIR score activity and fibrosis, leptin, BMI, fat mass, age, sex, and free carnitine levels. Only free carnitine levels were significantly and independently associated with the severity of fatigue (OR=2.019, p=0.02, CI 95% [1.01-1.23]).

Discussion

In the present study, we showed that fatigue, evaluated with the Fatigue Impact Scale questionnaire, altered equally patient's quality of life in both CHC and IBS. We found that plasma leptin was associated with the severity of fatigue in both CHC and IBS. Moreover, our findings revealed a carnitine deficiency in patients with CHC, but not with IBS, and that carnitine levels were negatively correlated with fatigue scores in CHC only. Whether carnitine is specifically related to fatigue in CHC remains to be further elucidated.

Because chronic fatigue is commonly reported in more than 20% of people [23], it is necessary to study what cause fatigue and to develop treatments for it. To date, no specific fatigue related changes have been clearly established, leading to major limitations to determine the influence of fatigue on the disease process. Thus, identifying alterations of biochemical parameters would enable to understand the underlying mechanisms of fatigue. In the present study, the Fatigue Impact Scale questionnaire allowed an evaluation of the perceived impact of fatigue on patients' - lives, the factors that affect patients' perceptions of fatigue, and the affect of fatigue on the mental and general health of patients. As previously reported, we found that the severity of fatigue was markedly increased in both CHC and IBS patients, particularly in females, involving all three domains exploring quality of life (i.e. cognitive, physical, and psychosocial) [4 2 24]. We also retrieved significant positive correlations between plasma leptin levels adjusted for fat mass and the total score of fatigue in both patients with CHC and IBS further arguing for the role of the adipokine leptin in the sequence leading to fatigue.

In the current study, patients with CHC, but not with IBS, had significant lower levels of total and free carnitine adjusted for fat mass as compared to healthy subjects. These findings are in accordance with those of Kuratsune et al. [11] who found low levels of serum acylcarnitine (e.g. carnitine deficiency) in chronic fatigue syndrome and CHC, but not in other diseases. In our study, all patients were evaluated in same conditions, completing questionnaires in the morning and having no major changes of their daily activities (e.g. physical & psychosocial) and/or dietary habits in the past 4 weeks. Although the mechanism involved in the occurrence of carnitine deficiency in CHC has not been investigated in the present study, it could be cautiously speculated that virus C infection may alter the physiological process of carnitine biosynthesis in the liver.

In our study, we found significant negative correlations between circulating carnitine levels (e.g.total and free) and fatigue scores in patients with CHC but not in IBS. Moreover, in a logistic model of regression, free carnitine was significantly associated with fatigue, independently from age, sex, fat mass, METAVIR score (both activity and fibrosis), BMI and leptin. In patients with CHC, similar strength of associations between carnitine levels and each domain of fatigue were observed (data not showed), thus arguing for a non-specific contributory role of carnitine in the development of both mental and physical fatigue. Although our study cannot address further the mechanism underlying fatigue in CHC, it is tempting to speculate on the relationship between carnitine and liver inflammation. Indeed, Selimoglu et al. [25] found that plasma carnitine levels were lower in children with chronic hepatitis B compared with healthy ones and that carnitine was inversely correlated with liver portal inflammation and fibrosis scores. In the present work, although significant lower levels of carnitine adjusted for fat mass were observed in CHC patients, we found no relationship between carnitine and the degree of liver fibrosis and/or inflammation. In fact, we believe that

this should be interpreted with caution since blood samples were not concomitant to liver biopsy (e.g. up to 8 months), liver contents of carnitine were not assessed and only 13% of CHC patients had a METAVIR score activity up to 2.

Our study has also other limitations. In the present work, we did not evaluate numerous other biological candidates that could play a role in the development of fatigue in human [26]. Moreover, a more marked effect of carnitine on physical fatigue was not explored since all patients and controls were not evaluated while on physical tasks [26]. We did not perform specific dynamic challenges of the HPA axis and repeated circulating levels of both leptin and carnitine which would have been helpful to identify more subtle biological disturbances [27]. Moreover, we did not analyze liver biopsy specimens, which would have also been relevant to consider the relationship between immune activation, plasma cytokines and carnitine. Finally, increasing the sample size of patients would have probably unmask significant higher levels of carnitine in male patients in particular in the IBS group.

The present findings have important and practical clinical consequences in the hope to reduce fatigue and increase adherence to antiviral therapy in CHC. Indeed, there are few available effective therapies for fatigue associated with CHC. We have previously documented that the 5-hydroxytryptamine receptor type 3 antagonist ondansetron had a significant positive effect on fatigue in CHC. However, its use may be impinged by a relative high rate of constipation that occurred in patients who received active drug [28]. Given the fact that emerging evidence from an uncontrolled study [18] has suggested that CHC patients treated with interferon in association with carnitine (2g daily) showed a marked and early significant reduction of fatigue levels, our findings provide good reasons to pursue larger trials on the possible benefit of carnitine supplementation in CHC patients reporting fatigue.

In conclusion, we have shown that fatigue alters equally patient's quality of life in both CHC and IBS. Our findings suggest that carnitine may play a contributory role in the occurrence of fatigue associated with CHC but not in IBS. Whether the relationship between fatigue and carnitine involves a specific pathway in CHC requires further investigations. Finally, the benefit of carnitine supplementation may be evaluated in future randomized trials aimed at reducing fatigue and increasing adherence to available antiviral therapies.

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Figure legends:

Figure 1: Analysis of the total score and each domain of fatigue patients with chronic hepatitis C (CHC), irritable bowel syndrome (IBS) and controls. [a] $p<0.001$ vs CHC and IBS

Figure 2: Comparisons of circulating levels of free and total carnitine adjusted for fat mass between patients with chronic hepatitis C or irritable bowel syndrome and healthy subjects.

Figure 3: Correlation between the total score of fatigue and absolute plasma levels of total and free carnitine (left of the panel) and total and free carnitine adjusted for fat mass (right of the panel) in patients with chronic hepatitis C.

Table 1: Characteristics of patients with hepatitis C, irritable bowel syndrome and controls.

	Hepatitis C	IBS	Controls	P value
Sex (F/M)	34/47	34/8	23/21	<0.001
Age (years)	43.7±12.3[a]	50.7±13.6	46.5±17.3	[a] 0.03 vs IBS
BMI (Kg/m^2)	24±4	27±5[a]	23±4	[a] 0.008 vs hepatitis C and 0.007 vs controls
Fat mass (Kg)	20.3±9.6 [a]	25.2±11.6 [b]	16.3±9.2	[a] 0.04 vs controls and 0.02 vs IBS [b] 0.0002 vs controls
Fat-free mass (Kg)	49.4±11.3	46.9±9.1	50.8±9.4	NS

Table 2: Fatigue scores in patients with chronic hepatitis C, irritable bowel syndrome and controls according to the sex.

	CHC			IBS			Controls		
	F	**M**	***p***	**F**	**M**	***p***	**F**	**M**	***p***
Total score	77.1±38.4	42.6±36.2	0.001	71.1±34.1	56.0±26.3	0.2	15.9±10.7	15.9±12.6	0.8
psychosocial	36.7±19.3	19.5±19.0	0.001	35.2±18.6	23.0±17.3	0.2	7.4±7.5	6.1±5.4	0.3
physical	21.8±9.6	12.8±9.7	0.002	19.3±9.5	17.4±7.7	0.5	4.5±3.5	4.9±4.7	0.8
cognitive	18.6±11.5	10.3±8.8	0.002	18.4±9.4	12.4±8.3	0.1	3.9±3.9	4.8±4.4	0.5

Table 3: Plasma levels of leptin and carnitine in patients with chronic hepatitis C, irritable bowel syndrome and controls according to the sex.

	CHC			IBS			Controls		
	F	**M**	***p***	**F**	**M**	***p***	**F**	**M**	***p***
Leptin (ng/mL)	24.6±23.9	7.8±10.9	0.0002	38.0±26.2	9.2±3.5	0.02	9.4±4.3	5.4±5.4	0.02
Leptin/BMI (ng/mL Kg/m^2)	9.0±6.7	2.9+3.7c	0.0001	13.8±9.6	3.4±1.5	0.02	4.1±2.0	2.0±1.7	0.005
Leptin/FM (ng/mL.Kg^{-1})	1.0±0.6	0.3±0.4	0.0001	2.1±3.0	0.4±0.1	0.2	0.6±0.5	0.4±0.3	0.04
Total carnitine (ng/mL)	42.7±12.3	46.2±8.0	0.1	46.4±10.3	49.7±9.6	0.09	43.1±5.8	49.1±12.3	0.07
Free carnitine (ng/mL)	33.8±9.9	37.7±6.9	0.06	37.2±7.7	41.7±6.3	0.2	33.6±5.5	39.8±10.5	0.04
Total carnitine/ FM (ng/mL.Kg^{-1})	**2.3±1.4**	**3.0±2.3**	**0.05**	**2.9±1.2**	**2.9±5.2**	**0.9**	**2.7±1.3**	**6.0±5.6**	**0.01**
Free carnitine/FM (ng/mL.Kg^{-1})	**1.8±1.1**	**2.4±1.3**	**0.05**	**2.4±4.5**	**2.5±1.1**	**0.8**	**2.1±1.1**	**4.9±4.7**	**0.01**

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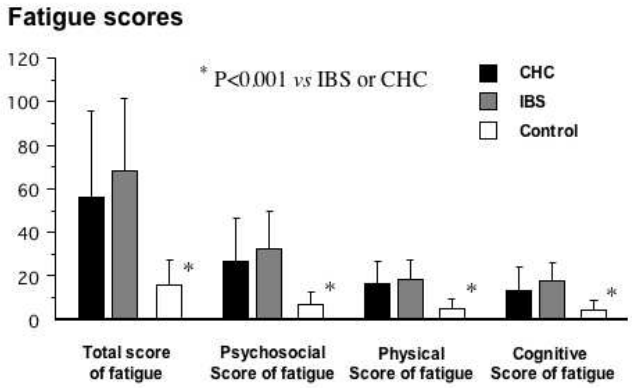

Figure 1

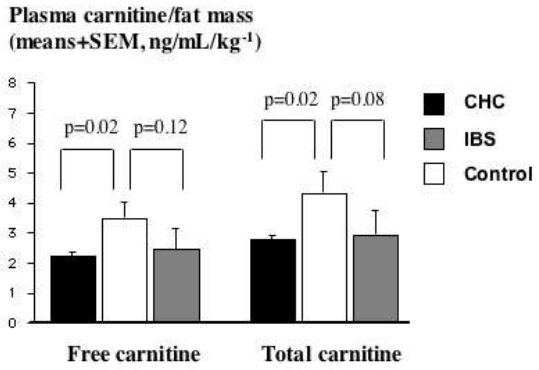

Figure 2

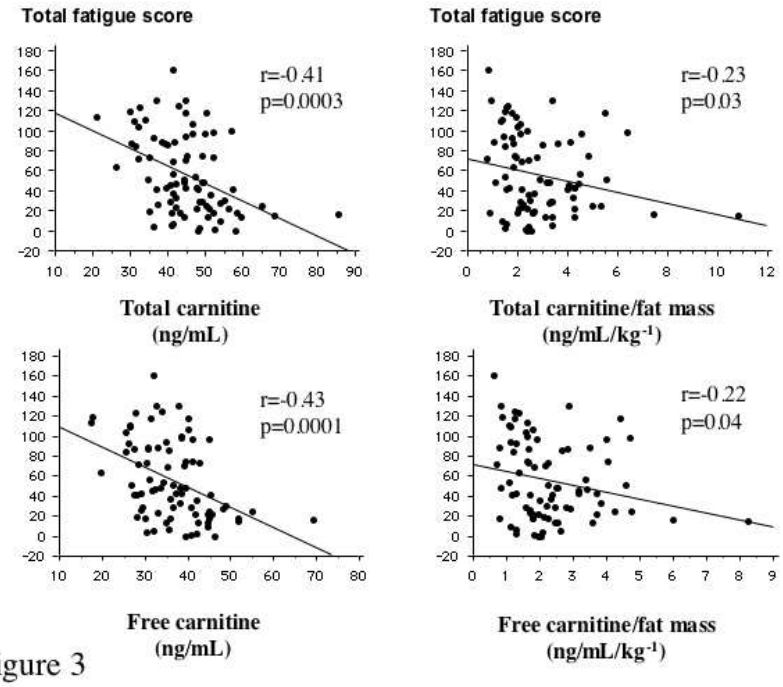

Figure 3