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Impact of vigorous exercise on serum levels of L-carnitine in prisoners in Colombia

Impacto del ejercicio vigoroso sobre los niveles séricos de L-carnitina en prisioneros de Colombia

Ángel F. Acevedo-Pabón, Fred G. Manrique-Abril[1,2] y Juan M. Ospina-Díaz

1 Universidad Pedagógica y Tecnológica de Colombia. Tunja, Boyacá, Colombia. angel.acevedo@uptc.edu.co; fred.manrique@uptc.edu.co; juan.ospina@uptc.edu.co
2 Facultad de enfermeria. Universidad Nacional de Colombia. Bogotá. fgmanriquea@unal.edu.co

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ABSTRACT

Objective To assess the effect of a program of vigorous physical exercises on the serum concentration of free and total L-carnitine, in male inmates at a prison in Boyacá, Colombia.
Methods Pre-post intervention population-based study. 44 male prisoners with overweight and/or obesity, from a jail in Boyacá, Colombia were randomly assigned into two groups: an intervention group and a control group. The intervention consisted in participating in a vigorous exercise program over twelve weeks. Anthropometric measures and levels of free and total L-carnitine were every four weeks.
Results There were significant increases in serum levels of free and total L-carnitine in the intervention group compared to the control group. Concurrently, in this group there was a reduction in body mass index (BMI), while in the control group there were no changes.
Conclusion In overweight and/or obese patients, the routine practice of vigorous exercise plus caloric restriction offers significant benefits in reducing body fat volumes through the mechanisms of energetic consumption of long chain fatty acids.

Key Words: Obesity, physical exercises, carnitine, lipid metabolism (*source: MeSH, NLM*).

RESUMEN

Objetivo Evaluar el efecto de un programa de ejercicio físico intenso sobre las concentraciones séricas de L-Carnitina libre y total, en varones recluidos en una prisión de Boyacá, Colombia.
Métodos Estudio de intervención antes – después, de base poblacional. 44 internos con sobrepeso y/o obesidad, de una prisión en Boyacá, Colombia, fueron aleatoriamente asignados a dos grupos: Uno de intervención y uno de control. La

intervención consistió en un programa de práctica sistemática de ejercicio intenso, durante doce semanas continuas. Cada cuatro semanas se realizaron mediciones antropométricas y se determinaron los niveles séricos de L-Carnitina libre y total.
Resultados Hubo un incremento significativo en los niveles séricos de L-Carnitina libre y Total en el grupo de intervención, comparado con lo registrado en el grupo control; simultáneamente, en el grupo de intervención se registró disminución en el índice de masa corporal (IMC), mientras que en el grupo control no se registraron cambios.
Conclusión En presencia de sobrepeso y/o obesidad, la práctica rutinaria de ejercicios físicos intensos además de la restricción calórica, ofrece significativos beneficios en la reducción del volumen de grasa corporal por el mecanismo de consumo energético de los ácidos grasos de cadena larga.

Palabras Clave: Obesidad, ejercicios físicos, carnitina, metabolismo de los lípidos (*fuente: DeCS, BIREME*).

Overweight and obesity, have been gradually increasing around the world; in 2008 it was estimated that there were around one billion people living in conditions of overweight and/or obesity (1). In the province of Boyacá, this situation became a public health problem, especially in rural areas, with averages of Body Mass Index (BMI) above 25, especially among women (2). Simultaneously, technological development has led to a gradually reduction in the amount of physical activity that these people do and to an increase in the consumption of processed foods without fiber. Moreover, in the assessment of cardiovascular risk, there are percentages of the population that do not exercise on a routine basis of more than 63 % (3).

Scientific research seeking less expensive alternatives to improving prevention strategies in non-transmissible chronic diseases (NTCD) includes vigorous physical exercise as an appropriate method in the solution of this problem.It is thought that this "burns" fats as a natural physiological process by stimulating mitochondria and helps in the process of weight loss in the case of overweight or obese patients. In addition, we believe that the exercise practiced in altitudes above 2 500 m. may increase this process. In this sense, research efforts are aimed at establishing the mechanisms that allow the entry and subsequent utilization of long-chain fatty acids as fuel to generate energy for skeletal muscle function.

L-carnitine is recognized as an important link in the process by facilitating the oxidation of lipids and by transportation of long-chain fatty acids into the mitochondria within the region where they undergo β-oxidation (4).

Scientists at the Nottingham University (UK), have shown that carnitine plays a dual role in skeletal muscle fuel metabolism that is exercise intensity dependent. Specifically, they have shown that increases in the total carnitine volume in muscle reduces carbohydrate use during low intensity exercise, consistent with an increase in muscle lipid utilization (5). This finding is particularly important in clinical cases in which excessive fat deposits, particularly in the abdomen, may significantly increase cardiovascular risk.

Carnitine was discovered in 1905 by Russian researchers Gulewitsch and Krimberg in mammalian muscle flesh (6). It was postulated then that it was absolutely necessary for the biochemical functioning of muscle cells. Its name derives from the Latin carnis (flesh). The chemical structure was enunciated in 1927. In 1952, Fraenkel discovered that it is a necessary nutritional component for life and named it vitamin B_T (7). Studies on the mealworm *Tenebrio molitor* showed that if the organisms are growing in a state of L-carnitine deficiency, they accumulate excessive amounts of fat in their cells and yet seem to die of starvation. This suggested that L-carnitine might play a role in the oxidation of fat. The function of L-carnitine in mammals, however, remained a mystery for some time. In 1955 Fritz discovered that adding L-carnitine to muscle extracts stimulated the oxidation of palmitate. This discovery led to the postulate of the mitochondrial function as carrier of L-carnitine and its important role in the burning of free fatty acids (8).

In mammals, L-carnitine is synthesized primarily in the liver, kidney and in some species, in the testes and the brain, from the essential amino acids lysine and methionine. The amino acids act as precursors to the synthesis of trimethyl-lysine (TML). Bioavailability of this intermediate step limits the biosynthesis of trimethyl-lysine carnitine. Then, most of bodily TML reserves are found in skeletal muscle proteins. Consequently, the replacement of skeletal muscle proteins is considered to be the speed-limiting step in the biosynthesis of carnitine (9).

Although there are two forms of carnitine —L-carnitine and D-carnitine— differentiated by their spatial structure, in the human body only L-Carnitine is synthesized. Furthermore, the D-form does not provide any of the benefits of the L-form.

Jail environments, because of their regulations, represent a risk factor for cardiovascular disease because of the inactivity that prisoners are forced to undergo, a condition that is exacerbated when the detention lasts longer (10). The aim of this study was to determine the effect of a program of vigorous physical exercise on the serum concentration of free and total L-carnitine among inmates in a prison in Boyacá, Colombia.

MATERIALS AND METHODS

Study Design. This research was designed as a pre-post intervention, prospective population-based study. The study population was defined as overweight and/or obese male prisoners living in a high-medium security prison, located in Boyacá, Colombia. The altitude is approximately 2 800 m above sea level.

Clinical records of 257 inmates were assessed, then, 51 subjects were classified as eligible since they met basic inclusion criteria (age between 20-65 years, BMI >25 and <40, without mental disease, imprisoned for at least two months, without diagnosis of any chronic non-transmissible disease, and willing to participate voluntarily in the study). All prisoners were widely informed about the purposes of the study and were invited to sign a written informed consent form, in accordance with the criteria of the bioethical regulations concerning good scientific research practices. Finally, 44 subjects were randomly assigned to two groups of 22 inmates. An intervention group and a control group. During the monitoring period, all subjects of both groups were fed according to a standardized and homogenous diet, under the supervision of a trained professional in nutrition and dietetics.

Description of the intervention. Inside the prison, rules strictly limit entry of materials such as gym equipment into the courtyards. For this reason, for the intervention group, we designed a program of vigorous physical exercise (PVPE), consuming more than 6 METs in each session, one MET is defined as the energy cost of sitting quietly and equivalent to a consumption of 1 kcal/kg/h. More than 6 METs occurs when we perform vigorous physical activity (11). Exercise sessions included activities such as walking briskly up and trotting down stairs, aerobics, sports and competitive games (micro football, basketball and volleyball). Also included were work activities such as digging trenches with a pick and shovel, floor exercises and moving loads exceeding 20 kg (Table 1).

The exercise routine was carried out by all the subjects of the intervention group, every day for twelve weeks. Previously, blood samples were obtained the day before starting exercises and routinely every 4 weeks on the same day and at the same time according to a previously standardized protocol designed by professional nurses. Blood was taken from the veins of the left arm. The samples were then centrifuged to obtain the serum and to measure total and free levels of L-carnitine in the intervention and control groups. Additionally, anthropometric measures —including weight and height— were taken to determine the body mass index (BMI). Subsequently a blood sample for the serum measure of free and total L-carnitine was taken weekly. Also, additional anthropometric measures were taken weekly.

Table 1. Vigorous exercise program for recluses: Start: May 02; Ending September 01, 2011. Boyacá – Colombia

Phase	Exercises	Duration
Heating	Stretching, flexibility, calisthenics, slow walking or jogging	5-10 minutes
Aerobic–oxidative stimulus	Fast walking, jogging, running, aerobic dancing, jump quick, up and down stairs one by one and two by two steps	15-30 minutes
Cooling	Progressively reduce intensity aerobic exercise, stretching, relaxation activities	5-10 minutes

Anthropometric measurements were performed with a weight scale floor device "Tanita®" BC-578. This device, in addition to low frequency bio-impedance, calculates the average body fat and composition. Height measurements were performed using Kent's height rod, which has a scale of 0 to 210 cm.

Serum samples were processed in a spectrophotometer RA-50, triple quadruple tandem mass spectrometer (Micromass UK®), equipped with a spray ionization source (ESI), and data analysis system "Micro MassLynx mass." The introduction of the source samples was done with an automatic device made by Jasco AS980, coupled to the Jasco pump PU980HPLC.

Baseline data, from week 0 until weeks 4, 8 and 12 were recorded in an Excel spreadsheet. Later, data were analyzed in the SPSS statistical program, version 15. Initially, a descriptive analysis of the behavior of the main variables was carried out. Percentages and means were compared in both groups of independent samples. We recorded results with the corresponding confidence intervals at 95 % and standard deviation. We used the T-test and the chi square test to compare the groups, with a significance alpha equal to 0.05. An alternative research hypothesis was that there is a statistically significant difference between serum concentrations of L-carnitine in the two groups.

RESULTS

Comparability between the groups. Baseline comparative between groups didn't show the existence of statistically significant differences in the anthropometric variables and serum levels of L-Carnitine, totally and free. The results of the comparative exercise and the corresponding hypothesis test are shown in Table 2.

Table 2. Baseline comparison between groups

Variable	Intervention (n=22)			Control (n=22)			ρ
	Mean	SD	Range	Mean	SD	Range	
Age	39,3	11,1	23,7-61,6	38,2	13,4	20,3-64,6	0,75
Initial Weight	82,6	11,0	61,9-100,3	78,1	11,2	56,9-102	0,18
Tall	1,7	0,1	1,4-1,9	1,7	0,1	1,5-1,8	0,30
Initial BMI	28,5	2,8	25,6-35,3	27,7	2,4	25,5-35,8	0,28
Initial free L-carnitine	296,8	9,1		294,5	15,5		0,55
Initial Totally L-carnitine	379,9	4,9		379,4	9,6		0,81

Starting in the fourth week, the levels of L-carnitine in the intervention group increased significantly, whereas in the control group they remained stable. From that moment the existence of statistically significant differences between the two groups was evident. Serum levels of L-carnitine taken every 4 weeks are shown in Table 3 and Figure 1.

Table 3. Measurement of free and total L-carnitine serum levels (μMol/L). (Intervals of 4 weeks)

	Grupo						
	Control (n=22)		Intervention (n=22)		ρ	Total (n=44)	
	Mean	SD	Mean	SD		Mean	SD
LcL basal	294,55	15,47	296,85	9,15	0,55	295,70	12,61
LcT basal	379,43	9,63	379,97	4,90	0,81	379,70	7,56
LcL S4	294,50	10,82	338,78	2,49	<0,0001	316,64	23,70
LcT S4	380,06	10,30	410,28	1,79	<0,0001	395,17	16,94
LcL S8	293,90	16,52	340,80	2,41	<0,0001	317,35	26,43
LcT S8	378,17	6,84	436,77	3,63	<0,0001	407,47	30,13
LcL S12	295,86	14,41	340,05	4,62	<0,0001	317,96	24,73
LcT S12	377,64	13,59	438,56	6,66	<0,0001	408,10	32,57

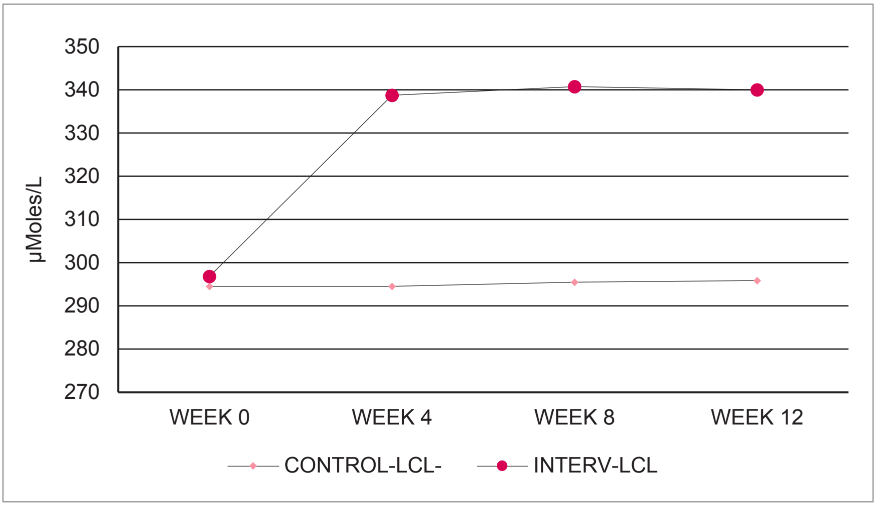
LcL=Free L-carnitine; LcT=Total L-carnitine; S# =number of week; SD=Standard Deviation

In terms of the average BMI values, in the control group, there was no significant variation from week 0 to 12 (27.7 Vs 27.5; p=0.79), while in the control group there was a marked reduction: It dropped from 28.6 to 25.7 (p=0.0011).

DISCUSSION

The main result of this study is the evidence that vigorous routine exercise increases quantities of L-carnitine in muscle cells. The process of fat combustion requires small amounts of L-carnitine to function. It is not really consumed and is available for this function over and over again during the process. L-carnitine promotes the burning of fat and inhibits lipogenesis. In body cells, such as muscle, fatty acids must be transported for combustion and energy generation into the mitochondria where they are burnt initially by β-oxidation and later in the citrate cycle. This assumption is corroborated by the scientific certainty that in situations shown as deficient in L-carnitine, one significant manifestation is the accumulation of fat in muscle tissue in the form of lipid drops (myolipidosis) (12).

Figure 1. Serum levels of free L-carnitine



While exogenous administration of L-carnitine has been used in some experimental studies to achieve the reduction of body fat, it is evident that satisfactory results can only be achieved by adding the performance of intensive physical exercise (13), because fatty acids (FAs) as fuel for energy during exercise originate from different sources: FAs transported in the circulation bound to albumin or as triacylglycerol (TG), or carried by very low density lipoproteins. In addition, FAs come from the lipolysis of TG in the muscle deposits. Despite a high rate of energy expenditure during high intensity exercise, the total (FA) oxidation is suppressed to below that observed during moderate intensity exercise. Although this has been known for many years, the mechanisms behind this phenomenon are still not fully elucidated (14).

Additionally, the effects of moderate to intense exercise in overweight and obese individuals have been studied in different environments. In most it has been concluded that physical activity favors the activity of lipoprotein lipase, which increases the catabolism of chylomicrons and VLDL while also reducing LDL. These effects result in a decrease in circulating levels of triglycerides and LDL cholesterol while HDL increases, improving the lipid profile of patients performing physical exercises (15).

However, other studies carried out in obese and postmenopausal women, which compare a combination of caloric reduction and moderate to intense exercise to caloric reduction alone, evaluated the effect on the reduction of body fat in the two groups. They found no statistically significant differences in body fat loss percentages (16). This phenomenon could be due to the fact that the practice of exercise involves an improvement in the quality of the muscle, which become stronger and may increase in volume and weight, thus masking the beneficial effect of exercise —better quality muscle tissue.

Where the studies agree is in the significant changes in levels of biochemical components: cholesterol, triglycerides, low density lipoprotein and areas of insulin and glucose in addition to oxygen absorption. These results support the beneficial effect of exercise on the biochemical metabolism of muscle tissue.

One phenomenon which has been explained as a cause of cellular aging refers to the exposure of biological systems to oxidative stress, a process which results in a loss of exercise capacity and impaired metabolic integrity. Meanwhile, it has been shown that exogenous administration of L-carnitine and lipoic acid has the potential to prevent oxidative stress and macromolecular damage in skeletal muscle cells (17).

Faced with the phenomena of chronic inflammation, insulin resistance and cellular oxidative stress often present in overweight and obese individuals, some studies have shown beneficial effects in the application of caloric restriction and Mediterranean-type diet on the inflammatory state. In a test performed in 180 patients (99 men and 81 women) with metabolic syndrome, where half of them followed a Mediterranean-style diet and half a regular diet (50-60 % carbohydrate, 15-20 % protein and <30 % fat), it was shown, after two years of follow up, patients who consumed the Mediterranean style diet, compared with the control group, had significantly reduced plasma concentrations of CRP, interleukin-6, interleukin-7, interleukin-8, insulin resistance and improved endothelial function (18).

Studies of the metabolism of L-carnitine acquire special importance for the understanding of the phenomena associated with heart muscle disease, which is ultimately expressed as the failure of the pump to meet the functional circulatory needs of the organism. Different findings show that the caloric restriction plus the routine practice of vigorous exercise substantially improves substantially heart energy usage and optimizes the functionality of this organ (19).

In overweight and/or obese individuals, the routine practice of vigorous exercise plus caloric restriction offers significant benefits in reducing body fat volumes through mechanisms of energetic consumption of long chain fatty acids. Simultaneously the cell damage is attenuated by reducing hypoxia generated during intense exercise. Also minimized is the generation of free radicals produced by mechanical stress and increased metabolic demand. It also optimizes hormonal function in muscle cells by maintaining the integrity of receptors for testosterone, insulin and growth hormone ♦

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Conflict of interest: None.

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