Ain Shams University
Girls College for Art, Science and Education
Department of Zoology

Effects of L-Carnitine Treatment On Heart Functions in Diabetic Rats

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By

Amal Mahmoud Hafz Shalaby

B.Sc.Degree in Science
Department of Zoology
Girls College For Art, Science And Education ,Ain Shams university
Lab. Staff,
Ain Shams Specialized Hospital

Supervised By

Dr. Mohamed IBrahim Rady
Associate Professor of Cytology and Histochemistry
Zoology Department, Faculty of Science El-Azhar University

Dr. Afnan Moustafa Ahmed Amer
Associate Professor of Physiology,
Zoology Department, Girls College for Art , Science and Education
Ain Shams University

Dr. Nadia Hussen Esmail
Lecturer at Zoology Department, Girls College for Art , Science and Education
Ain Shams University

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Amal Mahmoud Hafz

Name: Amal Mahmoud Hafez Shalaby

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Superveriors : Dr/ Mohamed Ibrahim Rady

Dr/ Afnan Moustafa Ahmed Amer

Dr/ Nadia Hussein Esmail

ABSTRACT

This study carried to investigates the effect of L-Carnitine on the diabetic male albino rats using physiological histological and histochemical tools.

Rats divided to 4 groups, the first group cited as control group, the second group was alloxan-diabetic; the third group received L-Carnitine by dose of 60 mg/kg body weight orally day by day for six weeks. The fourth group was alloxan-diabetic plus L-Carnitine (60 mg/kg body weight) day after day for six weeks).

The emerged results revealed that, glucose level in the alloxan-diabetic rats was highly significantly reduced after treatment with L-Carnitine. Lowering lipids, both of the triglyceride, fatty acids, cholesterol and LDL were found. The present results proved that L-Carnitine treatment improved significantly the high density lipoprotein in alloxan-diabetic group of rats. Also, the atherogenic index exhibited highly significant reduction in alloxan-diabetic rats after treatment with L-Carnitine.

L-Carnitine did not improve the value of sodium, as the Na^{+} level was insignificantly increased. The level of K^{+} ions decreased in diabetic group after treatment by L-Carnitine. The level of Ca^{++} exhibited highly significant change in diabetic rats after L-Carnitine administration.

L-Carnitine significantly improve Na^{+},K^{+} ATPase, ,but L-Carnitine did not improve Mg^{2+} ATPase. The histological study revealed slight improvement in the cardiac muscle architecture after treatment with L-Carnitine. Also, the treatment with L-Crnitine insignificantly affected the general carbohydrates and total protein in the cardiac muscle of alloxan-diabetic rats.

CONTENTS

AIM OF THE WORK

The present study aims to determine the changes occurred in rats after induction of diabetes by intraperitoneal injection of alloxan through measuring the changes of some major physiological parameters as:

Serum glucose − Insulin − Lipid profile and Atherogenic index.

Also, measuring of some serum electrolytes: Sodium − Potassium − Chlorine as well as estimating Na^+ -K^+ ATPase and Mg^{2+} ATPase in heart tissue. The study also aims to determine the histological changes occurred in heart muscle structure due to diabetes.

Then evaluation the effect of L-Carnitine supplementation on these changes by following up the previous variations in addition to performing histochemical investigation, mainly general carbohydrates and total protein content, in heart muscle of alloxan-diabetic rats and compare with control as well as with diabetic rats after treatment with L-Carnitine.

INTRODUCTION

The discovery of L-Carnitine occurred at the beginning of the last century when an important feature of the embryonic science of biochemistry was a descriptive one, entering primarily on the characterization and distribution of the main chemical components of the human body. L-Carnitine plays a crucial role in fat and carbohydrate metabolism and is required for the proper functioning of heart and muscle. In 1905, L-Carnitine was isolated for the first time from muscle tissue and its structure was established in 1927. L-Carnitine was shown to be an essential nutrient for a meal worm (Tenebrio molitor) and was therefore called vitamin BT (**Bremer, 1983**).

In animals and man, L-Carnitine is synthesized mainly in the liver from where it is transported into mucle tissue. About 98% of the body pool is localized in skeletal and heart muscle; In addition, it can be taken up from food and therefore L-Carnitine in the body is always a mixture from both sources biosynthesized and supplied in the diet. The biosynthesis requires lysine, methionine, niacin (vitamin B3), vitamin B6, vitamin C and iron. Dietary sources of L-Carnitine are mainly meat products, while plant foods contain considerably lower levels (**Costell and Grisolia ,1993**).

A too low intake of one of these nutrients will lead to a diminished L-Carnitine synthesis and consequently to muscle fatigue. Due to this dependence on micronutrients, L-Carnitine can be considered as a vitamin like substance similar to choline or lipoic acid (**Brass *et al.* 1993** and **Berardi *et al.* 2000**). Therefore, an incomplete diet, physiological stress situations and also some clinical cases can create a need for external L-Carnitine supplementation in the form of functional food or dietary supplements (**Treem *et al.* 1988**; **Brass *et al.* 1993**; **Costell and Grisolia 1993** and **Berardi *et al.* 2000**).

Carnitine is a physiological substance of the intermediary metabolism which is synthesised in the human organism. 98% of the body Carnitine is found in skeletal and heart muscle. Normal adult Carnitine concentrations are reached in this compartment at about 6 months of age. The capacity of Carnitine synthesis is strongly influenced by the availability of vitamin C and divalent iron (Fe2+), which explains the inadequate Carnitine availability in situations of iron or vitamin C deficiency. It is a characteristic of Carnitine synthesis that it is slow and does not readily keep up with fast changes of the metabolic requirements (**Friolet *et al.* 1994**).

The function of Carnitine is classically described to support the transport of long chain fatty acids across the inner mitochondrial membrane to the place of their oxidative degradation. This Carnitine fatty acid (acylcarnitine) shuttle system is strongly regulated at the Carnitine palmitoyl transferase I (CPT I) level. This transfer system is needed to prevent an uncontrolled mitochondrial fatty acid influx. In the cytoplasm free fatty acid are transferred to acyl CoA (the active form) by the action of acyl CoA synthetase enzyme. As the mitochondrial membrane is impermeable to CoA and its derivatives, so, the Carnitine shuttle plays an important role in entering acyl CoA to the mitochondrial matrix. In the mitochondrial matrix, the fatty acids are reactivated to acyl CoAs. The ratio of intramitochondrial acyl-CoA/free CoA is in a dynamic equilibrium with that of acyl-carnitine/free carnitine (**Brass and Hoppel 1980** and **Harris *et al.* 1987**).

A normal acyl-carnitine and free Carnitine (AC/FC) ratio is about 0.4 in the fed state. Ratios 10.4 are indicative of a certain degree of a limited availability of free Carnitine, which defines a state of Carnitine insufficiency. In patients with coronary heart disease tissue hypoxia leads to an inhibition of fatty acid oxidation and hence to an increase of acyl-CoA and consecutively also of acyl-carnitine. The resulting increase of the AC/FC ratio indicates

an insufficient availability of free Carnitine (**Ramsay and Arduini 1993**).

Carlin *et al.* (**1990**) reported that there are 4 main commercial forms of L-Carnitine on the market they are:

1. L-Carnitine free base
2. L-Carnitine L-tartrate
3. L-Carnitine magnesium citrate
4. Acetyl-L-carnitine.

L-Carnitine free base (USP 23) is the basic form and the starting material for all of Lonza's L-Carnitine derivatives. Lonza's L-carnitine USP is a crystalline, white powder and is highly soluble in water. L-Carnitine USP is extremely hygroscopic and therefore suitable for all liquid formulations (syrups, sport drinks, infant formula, clinical nutrition, ampoules), but is not recommended for solid dose formulations.

L-Carnitine L-tartrate (US Pat 5,073,376 and other international patents) is the market's favorite and most used form of L-Carnitine. It is a 100% stable, white crystalline, free-flowing salt of L-Carnitine (68%) and natural GRAS tartaric acid (32%) and contains the highest L-Carnitine concentration of all available salts. L-Carnitine L-tartrate has a pleasant citric taste; it is non-hygroscopic and therefore is the optimal form for all solid products including capsules, tablets, bars, etc. It perfectly fulfills the needs of contract manufacturers (absolutely free-flowing, non-hygroscopic, no dust) and marketing companies (bright white crystals, long-term stability, pleasant taste, odorless, etc.).

L-Carnitine magnesium citrate (US Pat 5,071,874 and other international patents) is another non-hygroscopic salt containing L-Carnitine (43%), citric acid (51%) and magnesium ions (6%). It is a white granulated powder which is perfect for effervescent tablets and powder drink mixtures. L-Carnitine magnesium citrate combines the benefits of L-Carnitine and magnesium. Active people have an increased need for both of these nutrients and L-Carnitine magnesium citrate supplies both in an ideal ratio. Acetyl-L-Carnitine (ALC) hydrochloride is a natural physiological active metabolite of L-Carnitine and plays an important role in the nervous system. ALC is widely used in so called brain food products.

With respect to carnitine supplementation the following points can be brought to attention:

(1) Carnitine is a natural compound of the organism.

(2) There is only a slow and inadequate adaptation of carnitine synthesis to rapidly changing metabolic requirements.

(4) The plasma AC/FC ratio is a sensitive tool to indicate carnitine insufficiency.

(5) The amount of carnitine to be supplemented is orientated towards the normalization of the AC/FC ratio.

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin

secretion or decreased sensitivity of the tissues to insulin. There are two general types of diabetes mellitus; type I diabetes, also called insulin dependant diabetes mellitus (IDDM) is caused by lack of insulin secretion. Type II diabetes, is caused by decreased sensitivity of target tissue to the metabolic effect of insulin. This reduced sensitivity to insulin is often referred as insulin resistance (**Guyton and Hall 2000**).

Cardiovascular diseases are the most common serious complication of diabetes mellitus. Coronary atherosclerosis and cardiomyopathy occur as a result of the metabolic abnormalities associated with diabetes (**Grundy *et al.,* 1999**).

Clinical and experimental studies have established that cardiomypathy occurs during diabetes (**Regan *et al.,* 1992** and **Rodriguses *et al.,* 1988**). Although a number of factors have been implicated in the development of this disease state (**Rodriguses *et al.,* 1995**), increasing evidence suggests that a metabolic derangement in fuel supply and use by cardiac myocytes could be a primary biochemical lesion in the pathogenesis of diabetic cardiomypathy under normal aerobic conditions. Cellular energy in the form of ATP is obtained via the oxidation of various substrate, with free fatty acids being the preferred energy substrate used by heart muscle (**Van der Vusse *et al.,* 1992**).

In diabetes, energy production is almost entirely via β-oxidation of free fatty acids, a process that may have delirious effect on myocardial function. This results from the abnormally high requirements for oxygen during free fatty acid metabolism, an intracellular accumulation of potentially toxic intermediates and a free fatty acid induced inhibition of glucose oxidation (**Randle *et al.*, 1992**).

REVIEW OF LITERATURE

Dhalla *et al.* (**1985**) reported that severe cardiac complications occur in both patients and animal models of chronic diabetes mellitus. They also added that various disorders in subcellular organells also occur during diabetes but their contributions to the overall depression of heart function remain ill defined.

Broderick *et al.* (**2000**) found that postischemic diabetic; rat heart function can be improved following chronic propionyl L-Carnitine (PLC) treatment. This beneficial effect of PLC can be explained by an improvement in the oxidation of glucose and palmitate.

Irat *et al.* (**2003**) suggested that the beneficial effects of L-Carnitine treatment partially improve vascular reactivity and antioxidant property beyond its reduction of plasma lipids and it may have an important therapeutic approach in the treatment of diabetic vascular complication.

Usyal *et al.* (**2005**) studied the effect of L-Carnitine on diabetogenic action of streptozetocin in rats. Their results suggested that L-Carnitine exerts antioxidative

effect in expiremental diabetes.

The effects of L-Carnitine on lipid metabolism have been documented by several authors such as **Rupp *et al.*** (**1994**)**; Sirtori *et al.*** (**2000**)**; Sayed–Ahmed *et al.*** (**2001**) and **Muller *et al.*** (**2002**).

Rupp *et al.* (**1994**) found that diabetes is associated with elevated plasma levels of free fatty acids (FFAs) and a marked increase in their oxidation. This excessive utilization of FAAs by the diabetic myocardium could represent a signal for the defects in sub-cellular organelles.

Sirtori *et al.* (**2000**) reported that L-Carnitine offers a potentially useful therapeutic agent for atherogenic conditions characterized by high lipoprotein levels, also in view of the excellent tolerability and essential lack of major side effects.

Sayed–Ahmed *et al.* (**2001**) remarked that L-Carnitine prevents the progression of atherosclerotic lesions by its antioxidant and lipid lowering effects. They also found that endogenous Carnitine depletion and or Carnitine deficiency should be viewed as an additional risk factor in

atherogenesis.

Brandsch and Eder (**2002**) published that L-Carnitine supplementation can influence the lipid metabolism in some species and can also affect body composition of growing animals. But L-Carnitine did not show a positive effect on weight loss and body composition of rats fed on energy deficient diet. The animal's endogenous Carnitine synthesis was obviously adequate to ensure efficient β–oxidation of fatty acids during the catabolic phase

Muller *et al.* (**2002**) demonstrated that oral L-Carnitine supplementation results in an increase in long-chain fatty acid oxidation in vivo in subjects without L-Carnitine deficiency or without prolonged fatty acid metabolism.

Derosa *et al.* (**2003**) recorded that after 3 and 6 months, L-Carnitine significantly lowered the plasma lipoprotein level compared with placebo in selected hypercholesterolemic patients with newly diagnosed type II diabetes mellitus.