

Research Article

Analysis of effect of Levocarnitine on Muscle Glycogen Content in Type 2 Diabetes in Pakistan

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a long term metabolic disorder that leads to various complications resulting in decreasing life expectancy by about 10 years. This condition is associated with a derangement in metabolic flexibility, i.e., a derangement in transition from fatty acid to glucose oxidation in response to insulin. The glycogen stores in skeletal muscles are considerably reduced in T2DM. The most initial and essential strategy in the management of diabetes is exercise, but the low levels muscle glycogen content in skeletal muscles in diabetics affects the duration of physical activity, owing to easy fatigue. *Levo-carnitine* is a synthetic form of naturally occurring nutrient that has been found to improve the glycogen stores in healthy subjects. In this study, we have observed the effects of levocarnitine in T2DM by estimating the glycogen stores of skeletal muscle. **Methods:** This laboratory based experimental study was conducted in Physiology Department of Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. The study involved 30 healthy male Sprague-Dawley rats. Serum creatine phosphokinase (CPK) levels were measured to exclude any skeletal muscle disorder. Rats were fed high fat diet (2 weeks) followed by intra-peritoneal injection of streptozocin (35 mg/kg). On 21st day, after confirmation of type 2 diabetes by measuring plasma glucose and TG/HDL ratio, rats were divided into 2 equal groups; group I (Diabetic) and group II (Carnitine). Group II was administered l-carnitine (200mg/kg) for 6 days. The extensor digitorum muscle (EDL) of rats of both groups were dissected for estimation of muscle glycogen content. The serum free carnitine (FC) levels were also measured. **Results:** Carnitine treated rats resulted in increase in skeletal muscle glycogen stores ($p < 0.001$). Serum free carnitine levels were also found to be higher in carnitine groups. **Conclusion:** Levocarnitine increases the glycogen stores in type 2 diabetic rats.

Keywords: type 2 diabetes, levo-carnitine, muscle glycogen store

INTRODUCTION

Glucose and lipid metabolism are inter related phenomena in the human body. The number of people with T2DM is spiking worldwide, thus efficient management is required

to improve the quality of life of diabetics. Initial management of T2DM consists of weight reduction, regular exercise and controlled diet. Exercise plays a dominant role in controlling

hyperglycemia by way of increase in peripheral insulin sensitivity, strengthening insulin bonding and minimizing obesity.¹

Both glucose and free fatty acids (FFA) are consumed by skeletal muscles as sources of fuel for energy production. During the fasting state, FFAs provide major source of energy production, as skeletal muscle glucose uptake is considerably low. After uptake of glucose, insulin secretion from the beta cells of pancreas is stimulated leading which lowers the rate of lipolysis leading to a reduction in plasma FFA levels. Simultaneously, there is a rise in the rate of glucose oxidation in muscle.² This transition from fatty acid oxidation to glucose oxidation is called metabolic flexibility. After transfer of glucose into the muscle cells through of GLUT-4 transporter (glucose transporter 4), it is phosphorylated by hexokinase, and then either oxidized by glycolytic pathway or stored as glycogen. As the insulin levels rise, glycogen synthesis rate also improves, i.e; about 70% of glucose is converted to glycogen.

About 90% of total glycolytic flux undergoes glucose oxidation, whereas anaerobic glycolysis accounts for the remaining 10 percent. Pyruvate dehydrogenase, an insulin regulated enzyme, plays indispensable role in glucose oxidation.³ In T2DM, increased FFA suppresses PDH activity, thereby impairing glucose oxidation. Insulin also activates glycogen synthesis by stimulating a series of phosphorylation-dephosphorylation reactions which result in increased activity of protein phosphatase and reduced activity of glycogen synthase kinase. During insulin-stimulated state, skeletal muscle is the major tissue of the body that utilizes glucose; hence it is also a major site of impaired insulin action in T2DM.⁴ The combined effect of decreased muscle glucose uptake and the deranged activity of glycogen synthase activity results in about 20% reduction in skeletal muscle glycogen in T2DM, as compared to skeletal muscle of healthy individuals.⁵ During exercise, fat oxidation is

regulated at the entry of long-chain fatty acid (LCFA)- coenzyme A into the mitochondria of skeletal muscle cell.⁶ This entry of LCFA is initiated by transfer of acyl moiety to carnitine (L-3-hydroxy

trimethylaminobutanoate, synthesized from the essential amino acids methionine and lysine). This mechanism is in turn dependant on carnitine palmitoyltransferase 1 (CPT-1), which is allosterically inhibited by malonyl-CoA, an intermediate in the synthesis of fatty acids. Thus, this is a likely mechanism by which fat oxidation is suppressed by high glucose availability.⁷ CPT-1 activity is also affected by availability of carnitine in the cytosol and more than 95 % of total body carnitine is found in the cytosol of skeletal muscle. Thus, carnitine is a necessary co factor required for the enzymes that transfer the long chain fatty acids across the mitochondrial membranes for beta oxidation.⁸ Carnitine also buffers acetyl-CoA, hence modifying the intra-mitochondrial acylCoA/CoA ratio.^{9,10}

L-carnitine is the available form of carnitine present in the body and in food. Animal products such as meat, milk, poultry and fish are rich in L-carnitine. Levocarnitine is a synthetic form of carnitine, available in oral as well as injectable forms. Levo carnitine has been used in various diseases like cardiomyopathies, metabolic nerve diseases, senile dementia, HIV infection, tuberculosis, myopathies, renal failure and anemia.¹¹

Various studies have shown that *L-carnitine* activates pyruvate dehydrogenase complex (PDHC), improves glucose oxidation and increases insulin sensitivity¹². Skeletal muscle contain higher concentrations of carnitine as compared to plasma. Serum carnitine levels have also been found to be low in T2DM.¹³ Previous studies have shown that levocarnitine improves the muscle glycogen content in healthy individuals.^{14,15} As glycogen is the main source of energy for physical activity and exercise is one of the essential strategies in management of T2DM,

the present study was designed to determine the effect of this supplement on skeletal muscle glycogen content in type 2 diabetes.

MATERIAL AND METHODS

This laboratory based experimental study was conducted Physiology department of Army Medical College, Rawalpindi, in collaboration with NIH, Islamabad. Thirty healthy male Sprague Dawley rats were used. Serum creatinephosphorkinase (CPK) levels were measured to exclude skeletal muscle disorder. The rats were induced type 2 diabetes by administering single dose of streptozotocin (35mg/kg, intraperitoneally),¹⁶ after feeding them 2 weeks high fat diet.¹⁷ A week later, diabetes was confirmed by measuring plasma glucose (>200 mg/dl)¹⁷ and insulin resistance (TG: HDL ratio >1.8)¹⁸ by drawing tail vein blood.¹⁹ Rats were then subjected to 24 hours fast in order to decrease glycogen content⁸. Rats were then divided into two equal groups, group I (Diabetic group) and group II (Carnitine group). Group II rats were administered levo-carnitine for 6 days, at a dose of 200 mg/kg intra-peritoneally.¹⁶The EDL muscle of all rats was assessed for glycogen content, using an anthrone based method.²⁰ Serum free carnitine levels of all the rats were measured from terminal intracardiac blood using L-carnitine assay kit.

Data Analysis

Data was analysed using SPSS 17. Mean and standard deviation were calculated for all values.

Table3.3 Serum free carnitine levels and muscle glycogen content of groups

Variables	Group I (Diabetic) n = 15	Group II (Carnitine) n = 15	Group I v/s II
Carnitine levels (nmol/μl)	0.109 ± 0.014	0.312 ± 0.158	p < 0.001
Glycogen content (mg per 100gm muscle)	82.55 ± 10.30	124.20 ± 17.78	p < 0.001

All values are expressed as Mean ± SD

DISCUSSION

Our observation showed that *levo-carnitine* increased the muscle glycogen content in T2DM.

Statistical significance of difference between the subgroups was determined by applying independent samples t-test. (p≤0.05 was considered significant).

RESULTS

The average body weight (174.80 ± 14.42 gm), fasting serum plasma glucose level (97.08 ± 7.19 mg/dl), fasting serum TG (101.38 ± 7.15mg/dl), fasting serum HDL (79.65 ± 5.11mg/dl) and TG:HDL ratio (1.27) in healthy rats were documented initially.After induction of diabetes mellitus, the same parameters were repeated. Statistical analysis showed significant(p <0.001) increase in weight (268.40 ± 14.65 gm), fasting serum plasma glucose level(404.05 ± 59.65 mg/dl), fasting serum TG (132.17 ± 6.25mg/dl), fasting serum HDL (62.28 ± 1.25mg/dl) and TG:HDL ratio (2.12) due to high fat diet and injection of streptozotocin.²¹

After development of type 2 diabetes, rats were divided into group I and II. Group II rats were administered levo-carnitine for 6 days, at a dose of 200 mg/kg intra-peritoneally.The serum free carnitine was measured by terminal samplingand glycogen content of the EDL muscle of both groups were calculated.An increase in glycogen contentwas observed in carnitine group II (124.2±17.78 mg/100 gm muscle) as compared to diabetic group I (82.55±10.30 mg/100 gm muscle).The serum free carnitine levels showed a significant rise in carnitine group as compared to diabetic group.

The fast twitch muscles (type II) were used because they have comparatively more glycogen stores than the slow twitch muscle. Also, the

lower levels of muscle glycogen in T2DM has been observed to be higher in type II muscle fibers as compared to type I muscle fibers.²² Muscle glycogen is an important fuel for moderate to severe intensity exercise, and exercise is one of the initial regimens for management of T2DM.²³ Studies have shown that *l*-carnitine increases insulin sensitivity in T2DM, thereby improving glucose uptake by all tissues upto 8% of the basal glucose uptake level.^{12,24}

Carnitine has also been shown to improve PDH activity, resulting in reduction of plasma and skeletal muscle lactate levels by improving glucose oxidation.^{12, 25} Studies have also proved that increase in plasma carnitine levels results in increased storage of skeletal muscle glycogen.^{26,27} In present study, *l*-carnitine administration has shown to raise the plasma free carnitine levels 3 fold as compared to the diabetic group. Studies by Tamamogullari *et al.* had determined the levels of total, free and esterified carnitines in humans and observed that the levels of these carnitines were decreased in diabetic patients²⁸. Free carnitine levels were found to be lower in diabetics while esterified carnitines were found higher in diabetics. *L*-carnitine supplementation in the carnitine group of our study probably enhanced the PDH activity by buffering the excess acetyl CoA.²⁹ This was supported by the presence of higher muscle glycogen content after *l*-carnitine administration.

CONCLUSION

Levo carnitine increases the glucose uptake by the skeletal muscle and hence improves the skeletal muscle glycogen stores in type 2 diabetes mellitus.

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