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MYOPATHY IN TYPE II DIABETES: METABOLIC AND FUNCTIONAL STUDY IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: Diabetic myopathy is a common complication of diabetes mellitus (DM), yet experimental studies concentrated on insulin deficiency and motor neuropathy as the cause. C-peptide deficiency has recently been implicated in the pathogenesis of diabetic neuropathy. However, its effects on the contractile properties of skeletal muscles need investigation.

Objective: Testing the gastrocnemius muscle response to electric stimulation in normal and type II diabetic adult male albino rats with and without treatment by C-peptide or nitric oxide modulators and correlating it with the metabolic error.

Material and methods: Thirty six adult male albino rats of a local strain and average weight (160-170g) were equally divided into the following groups: **1- Control group (C):** normal non diabetic rats kept on normal pellet diet (NPD) without any treatment, **Diabetic groups;** Type II diabetes was induced by high fat diet (HFD), followed after 2 weeks by a single intraperitoneal injection of low dose streptozotocin (STZ) to induce partial pancreatic β-cell damage. One week after STZ injection, diabetes was verified by high serum glucose level (≥ 200 mg/dl), and diabetic rats were further classified into the following groups according to subsequent treatment; **2-Diabetic control** (no further treatment), **3-Diabetic+C-peptide**, **4-Diabetic+ N^G-L-Arginine Methyl Ester (L-NAME);** a nitric oxide synthase (NOS) inhibitor, **5-Diabetic+C-peptide+L-NAME**, and **6-Diabetic+L-arginine**; the substrate of NOS. Treatment continued for 4 weeks, during which rats were maintained on normal or HFD according to group. Under urethane anesthesia, the right gastrocnemius was exposed, subjected to direct electric stimulation with maximal low frequency stimulation. The peak force and the time till 50% fatigue (1/2 the peak force) were determined. The left gastrocnemius served as resting muscle. The experiment was then terminated by stopping stimulation and, immediately, rats were decapitated, blood samples were collected, and both gastrocnemius muscles were excised from origin to insertion and the liver was taken. Tissue and serum samples were subjected to biochemical analysis.

Results: HFD and STZ injection induced the metabolic error of type II diabetes in rats with diabetic myopathy picture characterized by significant lowering of peak muscle force, muscle weight, muscle and liver glycogen with a significant shortening of the time till 50% fatigue. The generalized metabolic error was manifested by hyperglycemia, hypoinsulinemia, increased insulin resistance indicated by a higher homeostatic model of insulin resistance (HOMA-IR), and dyslipidemia with higher atherogenic index. Oxidative stress was manifested by a significantly high malodialdehyde (MDA) and nitric oxide was significantly lowered. These errors were corrected with C-peptide treatment, but its beneficial effects were partially antagonized by L-NAME combination. L-arginine treatment partially corrected, while L-NAME treatment alone worsened the error.

Conclusion: Metabolic and functional deterioration occurred in the gastrocnemius muscles of type II diabetic rat model as a cause and effect of the generalized metabolic error present. C-peptide treatment acted as insulin mimetic and corrected to a significant level both errors. This effect was partially mediated by endogenous NO production. C-peptide could be a future substitution treatment for diabetic myopathy or chronic fatigue of the metabolic syndrome.

Key words: Diabetic myopathy, Type II diabetes, C-peptide, NO.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by insulin deficiency/insulin resistance, hyperglycemia with disturbed carbo-hydrate, fat and protein metabolism **(Coughlan et al., 2014).** Skeletal muscles represent 45 % of the body weight. Metabolically, they are essential for glucose and fatty acid oxidation, while protein synthesis is mandatory for formation of healthy muscle proteins. Functionally, they are essential for generating the muscle power to move, maintain posture and keep circulatory blood flow and energy homeostasis. The balance between the metabolic and functional role of skeletal muscle is disturbed in DM resulting in chronic fatigue syndrome and diabetic myopathy (**Carvalho et al., 2012 and Skovso, 2014).**

Diabetic myopathy turns on a vicious circle of slow mobility, sedentary life, obesity, insulin resistance, progressive muscle weakness, and more obesity **(Imam et al., 2012)**. Furthermore, diaphragmatic and respiratory muscle fatigue predispose to shortness of breath and hypoxia in diabetic patients with consequent development of a generalized oxidative stress condition **(Brotto et al., 2010).**

Type I and late type II DM are characterized by absolute insulin deficiency with complete burning out of the β-cell mass, while early type II is characterized by partial insulin deficiency with residual functioning β-cell mass and insulin resistance. Insulin is secreted in equimolar concentration with C-peptide and both are deficient in diabetes mellitus. In the last decade, C-peptide is no more considered as an inert molecule and its association with insulin in regulating most metabolic effects of insulin has been the subject of many researches **(Mohammad et al., 2013).** In addition, its role in ameliorating diabetic complications including neuropathy, nephropathy, retinopathy, and cardiovascular diseases was investigated by many researchers. Though motor nerve conduction defects were proved in diabetic rats and could be corrected with insulin and C-peptide **(Bughdadi and Attia, 2013).** Yet, the role of C-peptide in the correction of diabetic myopathy was not directly studied.

A diabetic type II rat model was recently developed, where rats were subjected to short term high fat diet for two weeks to induce insulin resistance followed by low dose intraperitoneal injection of the β-cell toxin; streptozotocin to produce partial pancreatic damage. This model mimicked type II DM in humans **(Samarghandian et al., 2012),** and was used in this study. The aim of the present work was to: 1) induce type II diabetes in adult male rats using HFD and low dose STZ, 2) study gastrocnemius skeletal muscle response to artificial electric stimulation in the diabetic rats as compared to control, 3) study the effects of C-peptide treatment for 4 weeks on the gastrocnemius muscle response, 4) study if NO is a mediator of C-peptide response, and 5) correlating these effects with the metabolic error present.

MATERIAL AND METHODS

Materials: The chemicals used in this study included STZ, L-NAME and L-arginine (Sigma, USA), C-peptide (Biorbyte, United Kingdom), normal

pellet diet (NPD) and high fat diet (HFD) constituents (El-Gomhoria Company, Cairo, Egypt).

Animals: Thirty six adult male albino rats of local strain were used in the present study. Their weights ranged between 160-170 grams at the beginning of the study. Rats were housed in groups of six in laboratory stainless steel cages that offered adequate space for free movement and wandering (40 cm x 40 cm x 25 cm) at room temperature with natural dark/light cycles, and allowed free access to water and commercial rat's diet (Nile Company, Egypt) for two weeks for acclimatization **(Naidoo and Islam, 2014)** in the laboratory of the Medical Physiology Department, Minia Faculty of Medicine. Experimental procedures and care of the rats were carried out according to the guidelines of the animal care and use committee of Minia Faculty of Medicine which coincided with international guidelines.

Rats were then divided into the following 6 equal groups:

- **Non diabetic control group:**

1. **Control group (C)** in which rats were maintained on the normal pellet diet (NPD) and received no treatment for 7 weeks **(Chaudhari et al., 2013)**.

- **Diabetic groups**:

Induction of type II diabetes (Chaudhari et al., 2013): Rats were fed HFD ad libitum for an initial period of 2 weeks to induce insulin resistance, followed by a single intraperitoneal (i.p.) injection of low dose STZ 35mg/kg body weight to partially damage the β-cells, and continued on HFD till the end of experiment. One week after STZ injection, the development of diabetes was verified by a blood glucose level ≥ 200 mg/dl in blood samples withdrawn from the retro-orbital sinus, using glucose-oxidase reagent strips (Accu-Chek, Roche). Treatment was then started for 4 weeks according to the group as follows:

2. **Diabetic non treated group** in which rats received no further treatment till the end of the experimental period **(Chaudhari et al., 2013)**.
3. **Diabetic+C-peptide treated group** in which rats received C-peptide (50 nmol/kg/day) by intraperitoneal injection for 4 weeks **(Rebsomen et al., 2006)**.
4. **Diabetic+L-NAME treated group** in which rats received L-NAME (20 mg/kg/day) by gavage for 4 weeks **(Maulood and Mahmud, 2013).**
5. **Diabetic + L-NAME + C-peptide treated group** in which rats received C-peptide and L-NAME as in groups 3 and 4 for four weeks **(Levi D'Ancona et al.,** 2010)**..**

6- **Diabetic + L-Arginine treated group** in which rats were given L-Arginine 200 mg/kg/day by gavage for 4 weeks **(Dumont et al., 2001).**

Diet composition (Srinivasan et al., 2005 and Chaudhari et al., 2013): Control rats received normal pellet diet composed of 5% fat [5%corn oil], 65% carbohydrates [15% corn starch and 50% sucrose], 20.3% proteins [20% casein and 0.3% DL-Methionine], 5% fiber, 3.7% salt mixture, and 1% vitamin mixture. It provided 3.0 kcal/g of diet. HFD for induction of diabetes was composed of 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal

which was 5.2 kcal/g of diet. Its composition (g/kg diet) was 365 g NPD, 310 g animal butter, 250 g casein, 10 g cholesterol, 60 g vitamins and minerals, 1 g yeast powder, 3 g methionine, and 1 g sodium chloride**.**

Electric stimulation of the right gastrocnemius muscle (MacIntosh & Gardiner, 1987 and MacIntosh et al., 2011): After 4 weeks treatment, and following an overnight fast, rats were anesthesized with urethane (1.5 g/kg; i.p.), and fixed to a dissection board. The gastrocnemius of both hind limbs were exposed from the calcaneous to the back of the knee. The right tendoachillis was tied with silk, cut and fixed to a force transducer (Bioscience, Holliston, MA, USA), avoiding severing the vascular supply of the muscle. Silver electrodes were directly applied to the right gastrocnemius muscle and connected to a stimulator (Bioscience, Holliston, MA, USA). Different stimulus intensities as well as muscle lengths (by moving the transducer) were tried to determine the lowest intensity and maximal length that produced maximal twitch force. This maximal length was measured with a tape (L cm). The voltage used was 0.5 V higher than the lowest voltage that produced maximal twitch amplitude (maximal voltage), and the pulse width was set at 0.5 ms. We used low frequency stimulus (10 Hz) to allow incomplete tetanus to simulate natural gait movements. The muscle was kept moist all the time with continuous drippling of saline at 37˚C. The force of contraction was recorded on a calibrated chart of the oscillograph (Bioscoence, USA). The force of contraction increased gradually to a maximum level, then decreased gradually thereafter. The experiment was terminated by cessation of electric stimulation when the force of contraction declined to 50% of the maximal force reached (50% fatigue). Immediately, rats were decapitated, and blood samples were collected. For glycogen assessment, the liver was taken and both gastrocnemius muscles were excised, weighed, labeled and placed in liquid nitrogen until biochemically assayed. The non-stimulated left gastrocnemius served to estimate the resting glycogen level. Previous studies have shown no significant differences between the right and left gastrocnemius muscles in resting glycogen content **(Le-Rumeur et al., 1993).**

- The maximal force (F) reached was determined from the calibrated chart.
- The time till 50% fatigue was determined from the start of stimulation till 50% fatigue using the speed of the chart recording.
- The cross sectional area of the muscle: This was done considering the muscle as a cylinder of measured length and an average density of 1.06 g/cm^3. The cross sectional area was calculated according to **Eu et al. (2003):**

Cross sectional area (A; cm^2) = muscle weight (g) / [density x length (L; cm)]

The force was calculated/unit surface area of the muscle by dividing F/A.

The blood samples were allowed to clot, centrifuged and serum was withdrawn in labeled Eppendorf tubes and stored at -20?C till assayed.

Biochemical analysis:

- **Fasting serum glucose and insulin levels:** Glucose level was measured

using glucose oxidase colorimetric kit ***(Spinreact, SPAIN***), while insulin level was assessed using rat insulin ELIZA kit ***(Calbiotech, USA***). Insulin level was assessed using rat insulin ELIZA kit ***(Calbiotech, USA***).

- **Homeostasis Model Assessment of Insulin Resistance (HOMA-IR):** It was calculated according to **Yada et al. (2008):**

  HOMA-IR = [fasting serum glucose (mg/dl) x serum fasting insulin (?U/ml)]/405]

- **Serum Lipid profile:** Triglycerides (TGs), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured using enzyme colorimetric commercial kits ***(Bio-systems SPAIN***), using spectronic 2000 (Baush and Lomb) spectrophotometer. The atherogenic index was calculated according to **Ikewuchi et al. (2014):**
  Atherogenic index of plasma = log [Triglyceride/HDL cholesterol]

- **Determination of total lipid peroxides:** We used the thiobarbituric acid method described by **Ohkawa et al. (1979),** which forms colored complexes with malondialdehyde (MDA) breakdown products of lipid peroxides that are extracted using n-butanol/pyridine and read at 532 nm using spectrophotometer. Concentrations were calculated from a standard curve made using 1,1,3,3-tetramethoxypropane (TMP).

- **Nitric Oxide:** We used a colorimetric nitrite assay kit (Bio-diagnostic, Egypt). Depending on the color intensity that develop following reaction of Griess reagent with nitrite. Its absorbance was read at 520 nm using spectrophotometer.

- **Muscle and liver glycogen:** It was determined according to sulfuric acid method adopted by **Ramu et al. (2016),** and based on the original method of **Kemp and Adrienne (1954)**. In brief, known weights of muscle or liver tissues were weighed, homogenized in 5 ml TCA reagent and transferred to a covered centrifuge tube, placed in a boiling water bath for 15 minutes then, cooled and centrifuged for 5 minutes to extract glycogen. 1 ml of the supernatant containing glycogen was boiled with 3ml of concentrated sulfuric acid in a water bath for 6.5 minutes then cooled again. The intensity of the pink color which was proportionate to glycogen concentration and read spectrophotometrically at 520 nm. Glycogen concentration was deduced from a standard curve prepared at the same time.

Statistical method: The results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis of the mean differences between groups was performed using one way ANOVA followed by Tukey-Kramer multiple comparison tests using In Stat, Graph Pad Software (version 4, San Diego, USA). Values of $P<0.05$ were considered significant.

RESULTS

❖ ***Gastrocnemius muscle response in control, diabetic and diabetic treated groups :***

The gastrocnemius of diabetic rats showed a significantly reduced peak force to electric stimulation associated with shortened time till 50% decline of peak force indicating accelerated fatigue. This effect was completely reversed with C-peptide treatment for 4 weeks. The levels were not statistically different from control. On the other hand, L-NAME treatment to diabetic rats significantly

worsened diabetic myopathy with a significantly lowered peak force and shortening of the time till 50% fatigue. L-NAME co-administration with C-peptide to diabetic rats antagonized but not completely the beneficial effects of C-peptide. The peak force was significantly higher and time till 50% fatigue was shorter than the diabetic group, but still significantly different from control. L-arginine improved muscle contraction in diabetic rats, but the peak force was significantly lower than that of C-peptide treatment and the time till 50%fatigue was also significantly shorter (Figure 1 and Table 1).

Table (1) also showed that gastrocnemius muscle weight was significantly lowered in the diabetic than the control groups, and the weight improved with both C-peptide and L-arginine treatment with insignificant difference from control. On the other hand, L-NAME treatment to diabetic rats significantly lowered muscle weight and, when co-administered with C-peptide, it significantly lowered its improving effect on muscle weight.

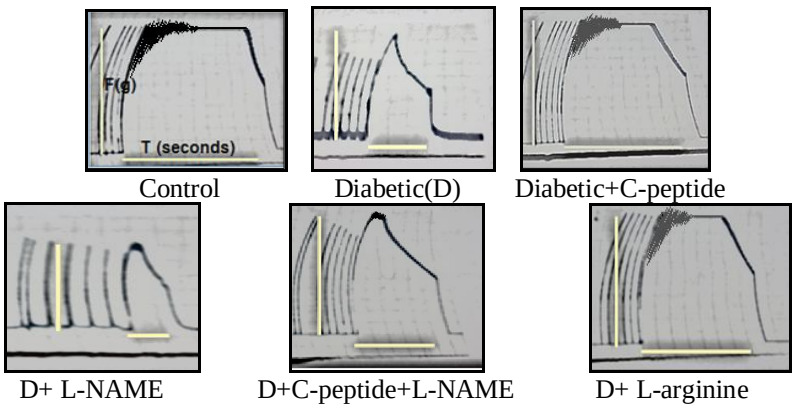

Figure (1): Effects of different treatments to diabetic rats on the peak force (F) of right gastrocnemius muscle contraction (represented by the vertical line; one big vertical square= 3 g tension), and on the time (t) till 50% decline from peak force (represented by the horizontal line; one big horizontal square=2 seconds).

Table (1): Gastrocnemius muscle response in control, diabetic and diabetic treated groups (Mean± SEM).

Groups / Parameters	Non-diabetic control (C)	Diabetic (D) on high fat diet (HFD)+ STZ injection				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
*Peak force (g/cm^2 area)	40.9 ±0.6^{a}	22.9±0.9^{d}	42.9±1.6^{a}	15.4 ± 2^{e}	29.9±1.8^{c}	34.5±1.2^{b}
*Duration(sec) till 50% fatigue	21 ± 0.85^{a}	7.5±1.25^{d}	19.8 ± 0.8^{a}	3.33±0.5^{e}	12 ±1.6^{c}	16 ±0.58^{b}
* Muscle weight (g)	2.16±0.15^{a}	1.63±0.13^{b}	2.22±0.18^{a}	1.5±0.17^{b}	1.83±0.15^{b}	2.17±0.17^{a}

Means in the same horizontal row with different superscripts $^{a, b, and\ c}$ were significantly different (P < 0.05). CP: C-peptide, N: L-NAME, CP+N: C-peptide+L-NAME, Arg.: L-Arginine.

❖ ***Gastrocnemius muscle and liver glycogen, serum glucose and HOMA-IR levels in the different groups :***

Table (2) showed that muscle glycogen concentration under both resting and stimulated conditions as well as liver glycogen levels were significantly lowered by diabetic induction than control levels, and the changes were completely prevented by C-peptide treatment where those levels were insignificant from control. On the other hand, L-NAME treatment produced more significant lowering of muscle and liver glycogen than the diabetic group and when given with C-peptide it significantly lowered these levels, but the levels were significantly higher than control indicating partial antagonism. The levels obtained with L- arginine treatment were higher than the diabetic but lower than the control groups indicating partial improvement.

Table (2): Gastrocnemius muscle and liver glycogen (mg/g tissue), serum glucose (mg/dl), insulin (μU/ml) and HOMA-IR levels in the different groups (Mean± SEM).

Groups / Parameters	Non-diabetic control (C)	Diabetic (D) on high fat diet (HFD)+ STZ injection				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
*Resting muscle glycogen	5.8 ± 0.14^{a}	2.7±0.2^{d}	5.4 ± 0.19^{a}	1.77 ±0.16^{e}	3.8 ± 0.22^{c}	4.6 ± 0.16
* Stim. muscle glycogen	4.69±0.23^{a}	1.69±0.2^{d}	4.14±0.23^{a}	0.86 ±0.09^{e}	2.63±0 .19^{c}	3.49 ±0.2^{b}
*Liver glycogen	34.66±0.92^{a}	18.04±1.04^{d}	31.37±1.49^{a}	12.71±1.57^{e}	26.57±1.94^{c}	27.7±1.92^{b}
*Fasting serum glucose	78.1±0.8^{e}	217.9±2.3^{b}	81.4±1.6^{e}	230 ±2.8^{a}	155.6±2.04^{c}	111.5±2.2^{d}
*Insulin	8.1±0.47^{a}	6±0.46^{c}	8.3±0.59^{a}	5.2±0.34^{c}	5.6 ±0.6^{c}	7.8±0.5^{ad}
*HOMA-IR	1.6± 0.11^{a}	3.53±0.28^{b}	1.69±0.09^{a}	2.95±0.22^{b}	2.29±0.17^{d}	2.25±0.18^{d}

Means in the same horizontal row with different superscripts $^{a, b,d \text{ and } e}$ were significantly different (P < 0.05). CP: C-peptide, N: L-NAME, CP+N: C-peptide+L-NAME, Arg.: L-Arginine.

Table (2) also showed that the diabetic group expressed the systemic metabolic error of carbohydrate in the form of a significantly higher serum glucose, HOMA-IR and lowered fasting serum insulin than the control group. These changes were completely reversed to control levels by C-peptide treatment, and incompletely by L-arginine treatment. The levels with L-arginine were significantly different from control group. Although L-NAME did not significantly change insulin concentration or HOMA-IR in the diabetic treated group from non-treated group, yet fasting glucose was significantly higher. Also, L-NAME co-administration with C-peptide significantly antagonized its hypoglycemic effects.

❖ ***Lipid profile, lipid peroxides, and nitric oxide levels in the different groups:***

The data in table (3) illustrated that:

- The diabetic group showed a diabetic lipid profile with significantly higher TG, LDL and atherogenic index with a lower HDL than the control group. These changes were completely prevented with C-peptide treatment. The balance turned towards anti-atherogenesis, and the atherogenic index was more significantly lower than the control. L- arginine improved lipid profile, but less than C-peptide. The HDL levels was significantly lower than C-peptide and the atherogenic index was significantly higher. On the other hand, L-NAME treatment produced the significantly highest atherogenic index and lipid profile and, when combined with C-peptide, it significantly antagonized its anti-atherogenic effects.
- MDA concentration as a measure of lipid peroxides and oxidative stress was significantly higher in the diabetic group than the control group, and this effect was completely prevented with either C-peptide or L-arginine treatments. However, L-NAME treatment alone had no significant effect and MDA was not significantly different from the diabetic group, but when combined with C-peptide it completely prevented its antioxidant effect.
- Serum NO levels were significantly lowered in the diabetic group than the control group. The least significant level was obtained in the diabetic group treated with L-NAME. Treatment of diabetic rats with either C-peptide or L-arginine significantly increased the NO levels with insignificant difference between them, but co-administration of L-NAME with C-peptide prevented the C-peptide effect.

Table (3): Serum Lipid profile, malondialdehyde (MDA), and nitric oxide (NO) levels in the different groups (Mean± SEM).

Groups / Parameters	Non-diabetic control (C)	Diabetic (D) on high fat diet (HFD)+ STZ injection				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
TG (mg/dL)	58.2±1.7^{c}	108.1±2.9^{b}	55.8±1.7^{c}	123.5±3.1^{a}	99.7±3.2^{b}	56.7±2.6^{c}
HDL (mg/dL)	63.8±1.2^{a}	47.9±1.3^{cd}	67.2±1.5^{a}	44.3±1.3^{d}	48.9±2^{c}	59.7±1.5^{b}
LDL (mg/dL)	32.7±0.7^{d}	73.6±2.1^{b}	33.1±0.8^{d}	78.8±2.1^{a}	56.1±1.4^{c}	34.6±0.5^{d}
Atherogenic Index (Aix)	- 0.06^{e} ±0.001	0.34^{b} ±0.007	-0.09^{f} ±0.003	0.44^{a} ±0.009	0.3^{c} ±0.008	-0.027^{d} ±0.003
MDA (nmol/ml)	9.1±0.4^{b}	17.8±0.4^{a}	8.8±0.5^{b}	16.7±0.5^{a}	15.8±0.5^{a}	8.6±0.4^{b}
NO (µmol/L)	33.1±1.2^{b}	15±0.6^{c}	35.7±1.0^{a}	8.5±0.54^{d}	13.7±0.3^{c}	34.8±1.0^{a}

Means in the same horizontal row with different superscripts $^{a, b, and\ c}$ are significantly different (P < 0.05).
CP: C-peptide, N: L-NAME, CP+N: C-peptide+L-NAME, Arg.: L-Arginine.

DISCUSSION

Diabetic myopathy, characterized by reduced physical capacity, strength, and muscle mass is a relatively understudied complication of diabetes mellitus, but is believed to directly influence the rate of its health hazards. This is based on the fact that skeletal muscle functions as the largest site for glucose uptake and, therefore, changes to skeletal muscle health can impact whole-body glucose homeostasis **(D'Souza et al., 2013).** In the present work, we aimed to study the effect of type II diabetic model on the functional and metabolic impairment of skeletal muscles as represented by the gastrocnemius muscle in adult male albino rats.

The gastrocnemius muscle was chosen because it is composed of mixed type 1 slow aerobic and type 2 fast glycolytic muscle fibers, and functionally it presents intermittent contractions of gait **(Cornachione et al., 2011).** Furthermore, direct electric stimulation of the muscle was performed to ensure that any effect of diabetes on force generation would be due to a decrease in the force generating capability of the contractile apparatus and not to effects on either nerve activity or the neuromuscular junction according to **Brotto et al. (2010).**

In the present study, HFD and low dose streptozotocin injection to adult male albino rats induced the diabetic picture of type II diabetes characterized by hyperglycemia, increased insulin resistance; manifested by a significant elevation of HOMA-IR with increased plasma atherogenic index due to a significant elevation of triglycerides and LDL with lowering of HDL. The impaired metabolic error was reflected on liver and muscle glycogen that was significantly reduced. Functionally, the peak force generated significantly reduced indicating a lowered muscle power, and fatigue was accelerated as indicated by a significant shortening of the time till half relaxation (50% of the peak force). Such reduced muscle power and easy fatigability were compatible with the picture of diabetic myopathy.

The changes observed were due to the fact that HFD increased the FFA load to different tissues including the muscle and liver. Skeletal muscles being the largest metabolic organ for carbohydrate and fat oxidation followed by the liver are affected first. FFAs compete with glucose for mitochondrial oxidation, hence sparing glucose and promoting lipid oxidation. In addition, lipid metabolites as long chain acyl~Co.A and diacyl glycerol (DAG) interfere with insulin function resulting in the development of insulin resistance. They block insulin-dependent mechanisms of glucose metabolism including glucose transporters, glucokinase and hexokinase activities and glycogenesis with stimulation of gluconeogenesis and glycogenolysis in the liver and muscle according to **Abel et al. (2012) and Skovso (2014).** Low dose STZ injection that induced partial β- cell damage explained the hyperglycemia associated with reduced liver and muscle glycogen and mimicks type II diabetes and was compatible with other studies **(Flanagan et al., 2008, Zhang et al., 2008 and Lark et al., 2012).** On the other hand, **i**nsulin resistance and deficiency stimulated hormone sensitive lipase and inhibited endothelial lipoprotein lipase; an effect that stimulates lipolysis and inhibits lipogenesis with consequent distorted

serum lipid profile and increases atherogenic index (**Forbes and Cooper, 2013).**

Furthermore, hyperglycemia increases the activity of mitochondrial NADPH oxidase and mitochondrial biogenesis with excess generation of reactive oxygen species (ROS). This oxidative stress state was manifested in the present work by the significantly higher MDA levels and was compatible with **Henriksen et al. (2010).** The shift in pro-oxidant/antioxidant balance towards oxidative stress inhibits nitric oxide synthase and decreases NO generation. In addition, the loss of NO in the process of neutralization of free radicals to form the reactive nitrogen species. Peroxynitrite can be responsible for the lower NO level in the diabetic group of this study and was compatible with **D'Souza et al. (2013).**

The functional impairment in skeletal muscle reduced force and accelerated fatigue which could be attributed to reduced ATP generation secondary to dysfunctional mitochondrial oxidation and reduced glycogen store. Failure of excitation-contraction coupling is due to oxidative damage of contractile proteins; actin and myosin as well as endoplasmic reticulum Ca^{2+} channel proteins with reduced availability of Ca^{2+} **(Allen et al., 2008).** In the present study, although the gastrocnemius muscle weight of the diabetic group significantly reduced, yet, the force was calculated per unit surface area to specify the results to the contractile machinery and exclude the effect of muscle weight variation. However, reduced muscle weight may be ascribed to reduced glycogen content and/or blood flow secondary to decreased NO and may indicate loss of some contractile sarcomeres by atrophy or fibrosis (**Russel et al., 2009).**

Skeletal muscle is metabolically and functionally flexible under normal condition and possesses both slow aerobic oxidative type 1 and fast glycolytic type 2 muscle fibers, and can modulate its fiber type to switch its metabolic functions according to the prevailing condition. Fast type 2 fibers fatigue more rapidly than type 1 fibers. In type II DM, as in the diabetic groups of this study, skeletal muscle have been reported to show morphological derangements in the form of increased glycolytic (type 2) /oxidative (type 1) fiber number, muscle atrophy **(Huang et al., 2010),** decreased capillary/fiber density **(Prior et al., 2009),** decreased inter-myofibrillar mitochondrial content and abnormal lipid deposition **(Nielsen et al., 2010 and Chomentowski et al., 2011).** Due to these morphological changes, the muscle becomes "metabolically inflexible" as it cannot oxidise efficiently carbohydrates or lipids with progressive intra-myocellular deposition of lipid droplets and deficient ATP resulting in progressive muscle fatigue **(D'Souza et al., 2013)** as found in the present work.

Functionally, C-peptide treatment to diabetic rats in the present study significantly increased the peak gastrocnemius muscle force and prolonged the time till 50% relaxation indicating improved muscle function. Metabolically, this was associated with both increased muscle weight and glycogen content of both the liver and muscle, decreased fasting glucose, increased fasting insulin and its sensitivity (decreased HOMA-IR) imp-

roved lipid profile with decreased atherogenic index, increased NO, and decreased oxidative stress as indicated by reduced serum MDA levels.

The results signified that C-peptide was no more an inactive peptide and raises the question; Is C-peptide an insulin mimetic? In cultutred myoblasts, C-peptide (0.3–3 nM) has been found to activate insulin receptor tyrosine kinase, tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1), glycogen synthase kinase-3 (GSK3), and glycogen synthesis **(Hills and Brunskill, 2008).** Evidences suggest that C-peptide mediates its own distinct signaling through a G-protein coupled receptor (GPCR), and there is some overlap between the signaling pathways activated by C-peptide and insulin, but crosstalk between GPCR activated signaling and insulin receptor tyrosine kinase activated pathways occurs by transactivation **(Bhatt et al., 2014).** The C- peptide' insulin independent mechanisms of improving skeletal muscle metabolic and functional state may involve activation of NOS activity, with consequent increase of NO, which increases blood flow and glucose uptake by different tissues including skeletal muscles **(Wilhelm et al., 2008).** In addition, C-peptide has been found to increase Na^+/K^+-ATPase activity which is depressed in DM and hence improved both renal and neuronal function **(Hills and Brunskill, 2008).** It is probable that this effect in skeletal muscle could improve membrane potential changes with better conditions for excitation-contraction coupling. Finally, the improved glycemic state reduces the formation of advanced glycation end products (AGEs) that contribute to oxidative stress, inflammation, insulin resistance and ionic disturbance specially the reduced Ca^{2+} availability for contraction (**D'Souza et al., 2013)**. Although no direct research work has been reported to study the effect of supplementing diabetic rats with C-peptide on diabetic myopathy, the above mechanisms reported with other diabetic complications can apply to our work supported by the findings of improved metabolic error, oxidative stress (lowered MDA), and increased NO, hand in hand with the contractile function.

NO is the endothelial derived relaxation factor and is endogenously produced from L-arginine by 3 isoforms of NOS; endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). It regulates metabolic functions through increased blood flow to different organs and as a cell signaling molecule, it increases glucose uptake and oxidation by facilitating glucose internalization through stimulating GLUT4, mitochondrial biogenesis and respiratory chain complexes. It also increases insulin release from beta cells and its sensitivity, and promotes glycogenesis, and decreases gluconeogenesis. In physiological concentrations, it is a strong antioxidant (**Dellamea et al., 2014).** That is why blocking NOS with L-NAME in the present study significantly lowered NO levels, and aggravated the metabolic error of diabetes whether hyperglycemia, dyslipidemia and oxidative stress. When L-NAME was given with C-peptide, it partially antagonized the antidiabetic effects of C-peptide including the restored gastrocnemius muscle mass, power, decreased fatigability and muscle glycogen. This indicates that part of the C-peptide induced effects are mediated by NO through stimulation of NOS activity.

In conclusion, skeletal muscles as represented by the rat gastrocnemius muscle of the present work are metabolically and functionally deteriorated in the diabetic type II rat model of the present work. This deterioration is a cause and effect at the same time of the generalized metabolic error found in this model. C-peptide treatment acted as insulin mimetic and corrected to a significant level both the muscular and generalized error of type II diabetes. These effects are partially antagonized with L-NAME indicating the mediating role of NO in C-peptide effects. C-peptide could be a future substitution treatment for diabetic myopathy or chronic fatigue of the metabolic syndrome.

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الوهن العضلى فى داء السكرى من النوع الثانى: دراسة أيضية ووظيفية فى ذكور الجرذان البيضاء البالغة

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خلفية البحث: يعد الوهن العضلى من المضاعفات الشائعة لداء السكرى، ومع ذلك ركزت الدراسات السابقة على مرض العصب الحركى المصاحب للسكرى سببا لهذا الوهن. ومع أن نقص الببتيد- س المتلازم مع نقص الإنسولين من البنكرياس ثبت دوره فى إحداث مرض العصب الحركى المصاحب للسكرى، إلا أن تأثير نقص الببتيد- س على الخصائص الإنقباضية للعضلات الهيكلية تحتاج إلى البحث.

الهدف من البحث: إختبار إستجابة عضلة بطن الساق (الجاستروكنيميس) الهيكلية للتنبيه الكهربى فى ذكور الجرذان البيضاء البالغة الطبيعية والمصابة بداء السكرى من النوع الثانى بدون علاج أو بالعلاج بالببتيد- س أو محورات إنتاج أكسيد النيتريك، وربط العلاقة بالخلل الأيضى

الطرق والمواد المستخدمة: إستخدم فى هذا البحث 36 جرذا من الذكور البيضاء البالغة قسمت إلى ست مجموعات متساوية كالتالى: **1- مجموعة ضابطة:** غير مصابة بالسكرى تتناول الغذاء الطبيعى دون أى علاج، **مجموعات الجرذان المصابة بالسكرى:** تم فيها إحداث السكرى من النوع الثانى بالتغذية عالية الدهون والحقن بجرعة واحدة صغيرة من الإستربتوزوتوسين وبعد التأكد منه بارتفاع نسبة جلوكوز الدم إلى 200مجم% أو أكبر تم تقسيمها إلى المجموعات التالية حسب العلاج: **2- مجموعة السكرى الغير معالجة، 3- مجموعة السكرى المعالجة بالببتيد- س ، 4- مجموعة السكرى المعالجة ب L-NAME** مثبط الإنزيم المخلق لأكسيد النيتريك، **5- مجموعة السكرى المعالجة بالببتيد- س + L-NAME ، 6- مجموعة السكرى المعالجة بالأرجينين المكون لأكسيد النيتريك**. واستمر العلاج لمدة أربعة أسابيع مع إستمرار نوع التغذية حسب المجموعات. وتم إختبار إستجابة عضلة بطن الساق بعدها حيث تركت اليسرى كضابطة فى حالة الراحة بينما تم تنبيه اليمنى مباشرة بالمؤثر الكهربى الأعلى وبتردد صغير ليحاكى إنقباض الخطوة، وتم تسجيل الإنقباض على جهاز راسم الذبذبات وحساب القوة العظمى والزمن حتى نصف التعب، حيث أنهيت التجربة بفصل الرأس وأخذ عينات مصل الدم وعضلتي بطن الساق والكبد للتحليل الكيميائى الحيوى.

النتائج: أظهرت الجرذان المصابة بالسكرى أعراض الوهن العضلى المتمثلة فى الإقلال ذو الدلالة المعنوية من القوة الإنقباضية العظمى ونقص الزمن اللازم للوصول إلى منتصف التعب مع نقص الوزن الكلى للعضلة ومحتواها من الجليكوجين سواء فى الراحة أو بعد التنبيه. وقد تمثل الخلل العام فى الأيض فى إرتفاع نسبة جلوكوزالدم والمقاومة للإنسولين، وكذلك نقص تركيزه، كما أحدث خللا فى دهون الدم مع إرتفاع معامل تصلب الشرايين. وقد تمثل إجهاد الأكسدة فى إرتفاع نسبة البيروكسيدات، كما نقص أكسيد النيتريك فى المصل. وقد تحسن هذا الخلل بالعلاج بالببتيد- س، بينما عارض تأثيره جزئيا إضافة **L-NAME**. وبينما صحح العلاج بالأرجينين جزئيا من هذا الخلل بينما أحدث العلاج المنفرد ب **L-NAME** زيادة للصورة سوءا.

الخلاصة: يحدث داء السكرى من النوع الثانى الوهن العضلى فى العضلات الهيكلية التى منها عضلة بطن الساق كسبب وعرض للخلل الأيضى الناتج. وقد شابه العلاج بالببتيد- س اللإنيولين فى تصحيح هذا الخلل، وكان ذلك جزئيا من خلال تنبيه تكوين أكسيد النيتريك الذى ثبت تأثير نقصه فى إحداث هذا الخلل، وهى نتيجة تشير إلى إمكانية استخدام الببتيد- س كعلاج مستقبلى تعويضى للوهن العضلى المصاحب للسكرى أو لمتلازمة الأيض.