Research Article

The Role of C-peptide and Nitric Oxide in the Metabolic Changes of Type II Diabetes in Adult Male Albino Rats

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Abstract
The C-peptide; co-secreted, in equimolar concentrations, with insulin from pancreatic β-cells is no more considered a silent molecule, but suggested to be involved in the diabetic metabolic error**.** The aim of this study therefore was to; **١)** induce experimental type II diabetes in adult male albino rats, **٢)** study the effects of C-peptide treatment on the metabolic changes induced, and **٣)** investigate the role of nitric oxide (NO) as a mediator by *in vivo* modulating its production. Sixty adult male albino rats (١٨٠–٢٠٠g) were divided into ٦ equal groups: **١-control** (non-diabetic on normal diet) and **five diabetic groups**. Type II diabetes was induced by feeding rats with high fat diet (HFD) for ٢ weeks, followed by a single intraperitoneal (i.p.) injection of low dose streptozotocin (STZ). One week after STZ injection, diabetes was verified by high serum glucose level (≥ ٢٠٠ mg/dl), and diabetic rats were further classified into the following groups according to further treatment; **٢-Diabetic control** (no further treatment), **٣-Diabetic+C-peptide**, **٤-Diabetic+ N^G-L-Arginine Methyl Ester (L-NAME);** a nitric oxide synthase (NOS) inhibitor, **٥-Diabetic+C-peptide+L-NAME**, and **٦-Diabetic+L-arginine**; the enzyme substrate of NOS. Treatment continued for ٤ weeks, during which rats were maintained on normal or HFD according to group, then the experiment was terminated by rats' decapitation after an overnight fast. Serum samples were subjected to biochemical analysis. HFD and STZ injection induced the metabolic error of type II diabetes in rats with significant hyperphagia, decreased body weight gain, hyperglycemia, hypoinsulinemia, increased insulin resistance indicated by a higher homeostatic model of insulin resistance (HOMA-IR), and dyslipidemia in the form of higher triglycerides (TG), and low density lipoproteins (LDL) with lower high density lipoproteins (HDL) as compared with control rats. The metabolic error was corrected with C-peptide treatment, but its beneficial effects were partially antagonized with L-NAME combination. L-arginine treatment partially corrected, while L-NAME treatment worsened the error. It was **concluded** that, both NO and C-peptide deficiencies contributed to the metabolic error of diabetes mellitus (DM). Substituting type II diabetic rats with C-peptide could correct the metabolic error and could be considered a future line of management in such patients. Furthermore, NO partially mediates the beneficial effects of C-peptide and NO precursors partially improved the error, hence could be combined with traditional anti-diabetic drugs.

key words: Diabetes, HFD, Streptozotocin, C-peptide, NO

Introduction
Diabetes mellitus is a group of chronic metabolic disorders characterized by hyperglycemia resulting from defective insulin secretion, decreased insulin sensitivity or both. It is frequently associated with body weight changes and defects in pancreatic β-cell mass and function[(١)].

C-peptide is secreted from pancreatic β-cells in equimolar concentrations with insulin and its lack with insulin is the main feature of type I and late stages of type II DM and signifies absolute loss (burn out) of the pancreatic β-cell mass. However, in early type II DM with development of insulin resistance, a compensatory hyperinsulinemia and increased C-peptide release is present[(١)].

C-peptide plays an important role in the biosynthesis of insulin and formation of the proper disulphide bridge between the **α** - and **β** -chains of insulin and hence, its

bioactivity. After separation from pro-insulin in the pancreatic β-cells, C-peptide is released into the blood stream with insulin, but unlike insulin, C-peptide is not catabolized in the liver that is why its systemic blood level is relatively higher. Furthermore, it has a plasma half-life about ٣٥ minutes while that of insulin is only ٥ minutes. It is catabolized mainly in the kidney, so its blood level is high in DM with nephropathy [٢]. C-peptide has long been regarded as an inert compound; however, several studies suggested that C-peptide has physiological functions in various tissues. C-peptide stimulates nitric oxide (NO) generation and Na^+/K^+-ATPase activation in different tissues[٣]. C-peptide also has a role in improving the complications caused by type II DM, including nephropathy and neuropathy[٤] .

Since the incidence of DM is rapidly rising worldwide, there is an urgent need for more effective treatments and therapeutic regimens. To date, glycemic control is not sufficient to prevent diabetic complications; including obesity, microvascular; retino-pathy, neuropathy and nephropathy, nor macrovascular complications as cardio-vascular diseases[٥].

An example of an experimentally-induced animal model of type II diabetes is the HFD/STZ rat model. This model involves a combination of a diet high in fat, and in some cases sugar, to bring about insulin resistance and hyperinsulinemia followed by treatment with low dose of the β-cell toxin STZ, which results in a reduction in functional β-cell mass. This model mimics the pathophysiology of type II diabetes on a shorter time scale than found in the human condition[٦].

Based on the new concept of a patho-physiologic triad mechanism for diabetes pathogenesis and complications; hyper-glycemia, insulin deficiency, and C-peptide deficiency[٥], this study was designed to: **١)** induce experimental type II diabetes mellitus by combining STZ and HFD in adult male albino rats, **٢)** study the effects of C-peptide treatment on the developed metabolic error, and **٣)** investigate the role of NO as a mediator of C-peptide effects by *in vivo* modulating its production; either by blocking it synthesis using the nitric oxide synthase (NOS) inhibitor; N^G-L-Arginine Methyl Ester (L-NAME), or by increasing its production by treatment with the enzyme substrate; L-Arginine.

Materials and methods

Materials

STZ, L-NAME and L-arginine were purchased from Sigma, USA. C-peptide was purchased from Biorbyte, United Kingdom. Normal pellet diet (NPD) and HFD constituents were purchased from El-Gomhoria Company, Cairo, Egypt.

Animals:

Sixty adult male albino rats from the local strain were used in the present study. Their weights ranged between ١٨٠ and ٢٠٠ grams at the beginning of the study. They were housed at room temperature with natural light\dark cycles for one week for acclimatization to the lab environment prior to inclusion in the study. All rats were provided with commercially available normal rat pellet diet (NPD) and tap water *ad libitum* prior to the dietary mani-pulation[٧]. During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat. The rats were randomly divided into the following ٦ groups of ١٠ rats each:

- ***Non diabetic control group (C):*** in which rats were fed a commercially available NPD and received no treatment for ٧ weeks[٨].
- ***Diabetic groups***:

The rats of these groups were subjected to induction of diabetes type II as follows: Rats were given HFD *ad libitum* for an initial period of ٢ weeks followed by a single intraperitoneal (i.p.) injection of low dose STZ ٣٥ mg/kg body weight in ٠.١ M citric acid buffer, pH ٤.٥. Rats were maintained on HFD for another week after STZ injection. The development of diabetes was then verified by evaluating glucose levels in blood samples withdrawn from the retro-orbital sinus using glucose-oxidase reagent strips (Accu-Chek, Roche). Rats having blood glucose level ≥ ٢٠٠ mg/dl were considered to be diabetic and selected for the subsequent treatment according to

the group[٨]. These rats were maintained on the HFD for an additional ٤ weeks during which the treatment was given[٩].

The diabetic rats were classified into the following equal groups (١٠ rats each) according to type of treatment:

١- ***Diabetic control non treated group:*** in which rats received no further treatment till the end of the experimental period[٩].

٢- ***Diabetic+ C-peptide treated group:*** in which rats received C-peptide ٥٠ nmol/kg/day by intraperitoneal injection for ٤ weeks [١٠].

٣- ***Diabetic + L-NAME treated group:*** in which rats received L-NAME; ٢٠ mg/kg/day by gavage for ٤ weeks [١١].

٥- ***Diabetic + L-NAME + C-peptide treated group:*** in which rats received C-peptide and L-NAME as in groups ٣ and ٤ for ٤ weeks[١١,١٢].

٦- ***Diabetic + L-Arginine treated group***: in which rats were given L-Arginine ٢٠٠ mg/kg/day by gavage for ٤ weeks [١٣].

Diet composition[١٤,٨]**:**

Control rats received normal NPD composed of Fat ٥٪ [corn oil ٥٪], carbohydrates ٦٥٪ [corn starch ١٥٪ and sucrose ٥٠٪], proteins ٢٠.٣٪ [casein ٢٠٪ and DL-Methionine ٠.٣٪], fiber ٥٪, salt mixture ٣.٧٪, and vitamin mixture ١٪ and provided ٣.٠ kcal/g of diet. HFD for induction of diabetes was composed of ٥٨٪ fat, ٢٥٪ protein and ١٧٪ carbohydrate, as a percentage of total kcal which was about ٥,٢٠٠ kcal/g of diet. Its composition (g/kg diet) was ٣٦٥g NPD, ٣١٠g animal butter, ٢٥٠g casein, ١٠ g cholesterol, ٦٠g vitamins and minerals, ١g yeast powder, ٣g methionine, and ١g sodium chloride**.**

Rats were followed during the experimental period by:

- **Daily food intake measurement**
- **Body weight and weight gain:** Rats were weighed using electronic balance (FY ٢٠٠٠) initially, after ٢ w, ٣ w (one week after STZ injection) and at the end of the experiment (٤ w later). Body weight gain at each time interval was calculated from the previous measure.
- **Body mass index (BMI):** Body length (nose to anus length) was measured in all rats under light ether anesthesia using a measuring tape from the base of the central lower incisor to the anus from the ventral surface. The BMI was calculated using the formula; BMI = body weight (g)/ length^{٢} (cm^{٢})[١٥]**.**

After ٤ weeks of treatment, rats of all groups were fasted for overnight and then decapitated. Blood samples from the jugular vein were collected, left to clot at room temperature, and then centrifuged at ٣٠٠٠ rpm for ١٥ min in a cooling centrifuge (Hettich centrifuge). The serum layer was then withdrawn into identified eppendorf tubes and stored at - ٢٠°C till the time of assay**.**

Serum was assayed for:

- **Lipid profile:**

Triglycerides (TGs), Low-density lipo-protein (LDL), and High-density lipo-protein (HDL) were measured using enzyme colorimetric commercial kits ***(Bio-systems SPAIN***), using spectronic ٢٠٠٠ spectrophotometer.

- **Fasting glucose level:**

Glucose level was measured using glucose oxidase colorimetric kit ***(Spinreact SPAIN***) and spectronic ٢٠٠٠ spectrophotometer.

- **Fasting insulin level:**

Insulin level was assessed using rat insulin ELIZA kit ***(Calbiotech USA***).

- **Homeostasis Model Assessment of Insulin Resistance (HOMA-IR):**

It was calculated from the following formula[١٦]**:**

HOMA-IR = [fasting serum glucose (mg/dl) x fasting serum insulin (μU/ml)]/٤٠٥

Statistical analysis

The results are expressed as mean ± standard error of the mean (M±S.E), and differences between all six groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post-hoc correction for multiple comparisons. A value of $p \leq ٠.٠٥$ was considered statistically significant.

Results

- **Changes in B.W. and weight gain in the different studied groups:**

Data presented in Table (١), illustrate that the mean of the initial body weights (IBW) were not significantly different among all groups.

- **B.W. and weight gain changes in the diabetic non treated group (Tables ١, ٢):**

HFD feeding for ٢ weeks resulted in a significantly higher body weight and weight gain when compared with NPD-fed control rats.

Injection of STZ (٣٥mg/kg, IP) after ٢ weeks of HFD resulted in a significant lowering in the mean weight gain, however, the change in body weight during the week following injection (٣rd week of dietary manipulation) was not statistically significant and remained significantly higher as compared with the control rats.

At the end of experiment, weight gain was significantly lower than the control group, while body weight showed insignificant difference from control group.

- **B.W. and weight gain changes in the diabetic treated groups (Tables ١, ٢):**

Except for the C-peptide treated group which showed a significantly higher B.W. during the study duration than the control and diabetic non-treated groups till the end, all diabetic treated groups of rats exhibited a significantly higher body weight in the initial weeks, but at the end of the experiment there was insignificant difference with the control group.

Diabetic group treated with C-peptide showed a significantly higher weight gain than all groups, while L-NAME treatment significantly lowered it as compared with both control and diabetic non-treated groups. L-NAME Coad ministration with C-peptide to the diabetic group partially antagonized the increased weight gain induced by C-peptide. L-arginine treatment, on the other hand, prevented the drop in weight gain due to diabetes. The levels were not significantly different from control group.

Table (١): Body weight changes in the different studied groups (M±SE)

Time	Groups					
	Control (C) on normal diet (NPD)	Diabetic (D) on high fat diet (HFD)				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
IBW (g)	١٩٦.٥±٥.٨	١٩٦.٧±٦	١٩٧.٢±٥.٤	١٩٨.٤± ٤.٨	١٩٨.٢±٤.٥	١٩٦.٧±٥.٦
After ٢ weeks	٢٢٧.٤^{b}±٦.٨	٢٦٦.٢^{a}±٦.٢	٢٦٤.٢^{a} ±٧.٧	٢٦٨.٦^{a}±٩.٣	٢٦٨.٨^{a}±٧	٢٦٣.٩^{a}±٤.٧
		Streptozotocin (STZ) injection				
One week after STZ	٢٣٦.٣٠^{b}±٦.٥	٢٥٧.٤^{a}±٥.٩١	٢٥٧.٢^{a} ±٦.٨	٢٥٩.٩ ^{a} ±٩	٢٥٧.٦٠ ^{a} ±٥.٧	٢٥٦.٧^{a}±٥
		Start of treatment for ٤ weeks				
Final B.W.	٢٧٨.٦^{cd}±٥.٤	٢٨٣^{bcd} ±٦.٦	٣١٩.٢ ^{a} ±٨.٦	٢٧٥.٧^{d} ±٩.٤	٣٠٤.٩^{ab}±٨	٣٠٣.٣^{abc}±١١

Means in the same horizontal row with different superscripts $^{a, b, c \text{ and } d}$ are significantly different ($P \leq ٠.٠٥$). CP: C-peptide, N: L-NAME, CP+N: C-peptide + L-NAME, Arg.: L-Arginine, IBW: Initial body weight, sig: significance.

Table (٢): Body weight gain (g) changes in the different studied groups (M±SE)

Time	Groups					
	Control (C) on normal diet (NPD)	Diabetic (D) on high fat diet (HFD)				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
After ٢ weeks	٣٠.٩^{b}±٢.٢٢	٦٩.٥^{a}±٤	٦٧^{a}±٤.٤٨	٧٠.٢^{a}±٥.٣٨	٧٠.٥ ^{a}±٥.٦٠	٦٧.٢ ^{a}±٢.٥
		Streptozotocin (STZ) injection				
One week after STZ	٨.٩^{a}±٠.٩٩	-٨.٨^{b}±١.٠٣	-٧ ^{b}±٢.٣٣	-٨.٧^{b}±٠.٥	-١١.٢^{b}±١.٦٨	-٧.٢^{b}±٠.٩٨
		Start of treatment for ٤ weeks				
Final BWG	٤٢.٣^{b}±٣.٤	٢٥.٣^{c}±٣	٦١.٩^{a}±٣.٧	١٥.٧^{c}±١.٦	٤٧.٣^{b}±٣.٦	٤٦.٦^{b}±٧.٤

Means in the same horizontal row with different superscripts $^{a, b, c \text{ and } d}$ are significantly different ($P \leq ٠.٠٥$). CP: C-peptide, BWG: body weight gain, N: L-NAME, CP+N: C-peptide + L-NAME, Arg.: L-Arginine, sig: significance.

- **Changes in daily food intake in the different studied groups:**

Table (٣) shows the following:

The initial food intake following the acclimatization period was insignificantly different between all groups.

All rats of the diabetic groups showed a significantly higher food intake as compared to the control group till the start of treatment. Rats of the diabetic nontreated group showed significantly higher food intake till the end of experiment.

Both C-peptide and L-arginine treatment to diabetic groups lowered the diabetic increase in food intake to control levels.

L-NAME treatment to diabetic rats had no significant effect on the diabetic increase in food intake, but when coadministered with C-peptide, it partially antagonised the lowering effect of C-peptide on food intake. The levels were significantly lower than C-peptide alone, but higher than control rats.

- **Changes in body mass index (BMI) in the different studied groups:**

As shown in table (٤), the initial body mass index (IBMI) was not significantly different among all groups. Induction of diabetes without any treatment produced insignificant change, however, treatment with C-peptide significantly increased the final BMI than the control group. Neither L-arginine, nor L-NAME produced any significant change and even coadministration of L-NAME with C-peptide failed to produce significant lowering of BMI produced by C-peptide.

Table (٣): Changes in food intake (g/day) in the different studied groups (M±SE)

Time	Groups					
	Control (C) on normal diet (NPD)	Diabetic (D) on high fat diet (HFD)				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
IFI (g/day)	١٤±٠.٤	١٤.٥±٠.٤	١٤.٧±٠.٥	١٤.٩±٠.٣	١٤.١±٠.٥	١٤.٢±٠.٣
After ٢ weeks	١٤.٩^{b}±٠.٣	١٦.٧^{a}±٠.٤	١٦.٨ ^{a}±٠.٢	١٦.٨^{a}±٠.١	١٦.٨^{a}±٠.٢	١٦.٩^{a}±٠.٢
		Streptozotocin (STZ) injection				
One week after STZ	١٥.٧^{b}±٠.٢	١٨.٨^{a}±٠.٢	١٨.٨ ^{a}±٠.٢	١٨.٩^{a}±٠.٢	١٨.٧^{a}±٠.٢	١٨.٨^{a}±٠.٢
		Start of treatment for ٤ weeks				
Final F.I.	١٦.١^{c}±٠.٢	٢٠.٤^{a}±٠.٤	١٥.٤^{c}±٠.٣	٢١.٥^{a}±٠.٤	١٧.٥^{b}±٠.٤	١٥.٧^{c}±٠.٢

Means in the same horizontal row with different superscripts a, b, and c are significantly different (P ≤ ٠.٠٥). CP: C-peptide, IFI: initial food intake, Final F.I.: final food intake, N: L-NAME, CP+N: C-peptide + L-NAME, Arg.: L-Arginine, sig: significance.

Table (٤): Changes in body mass index in the different studied groups (M±SE)

Parameter	Groups					
	Control (C) on normal diet (NPD)	Diabetic (D) on high fat diet (HFD)				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
IBMI	٠.٥٤±٠.٠٢	٠.٥٤±٠.٠١	٠.٥٥±٠.٠١٣	٠.٥٤±٠.٠١٢	٠.٥٥±٠.٠١٦	٠.٥٤±٠.٠١٥
Final BMI	٠.٦٩^{cd} ±٠.٠١٣	٠.٧١^{bcd}±٠.٠١٦	٠.٧٩^{a}±٠.٠٢١	٠.٦٨^{d}±٠.٠٢٣	٠.٧٦^{ab}±٠.٠٢٠	٠.٧٥^{abc}±٠.٠٢٧

Means in the same horizontal row with different superscripts a,b,c and d are significantly different (P ≤ ٠.٠٥). NS: not significant. CP: C-peptide, IBMI: initial body mass index, Final BMI.: final body mass index, N: L-NAME, CP+N: C-peptide + L-NAME, Arg.: L-Arginine, sig: significance.

- **Changes in fasting serum glucose, insulin, HOMA-IR and lipid profile in the different studied groups:**

Table (٥) shows that:

The diabetic non treated group showed significantly higher fasting serum glucose, higher HOMA-IR, and lower insulin levels as compared to rats of the control group. While the lipid profile showed a significantly higher serum TG, LDL and lower HDL levels. These effects were completely reversed with C-peptide treatment. L-arginine treatment partially corrected the diabetic changes; significantly reduced blood glucose and HOMA-IR levels, but were still significantly higher than control rats. Insulin levels also significantly increased. While TG and LDL were normalized, HDL was partially improved; being significantly higher than diabetic but lower than control levels.

Administration of L-NAME to diabetic rats did not significantly alter the insulin or HOMA-IR levels as compared with the diabetic group. However, the hyper-glycemia of diabetes was worsened as indicated by a significantly higher blood glucose level. The lipid profile worsened with a significant higher TG and LDL levels than the diabetic group. When L-NAME was coadministered with C-peptide, it significantly but not completely antagonized the beneficial hypoglycemic effects of C-peptide. It also antagonized its dyslipidemic correcting effects.

Table (٥): Changes in fasting serum glucose, insulin, HOMA-IR, and lipid profile in the different studied groups (M±SE)

Parameter	Groups					
	Control (C) on normal diet (NPD)	Diabetic (D) on high fat diet (HFD)				
		D	D+ CP	D+ N	D+CP+N	D+Arg.
Glucose (mg/dL)	٧٧.٣^{e}±٠.٧٦	٢١٩.٩^{b}±٢.٣١	٨٢.٤^{e}±١.٦٢	٢٢٩.٥^{a}±٢.٧٨	١٥٧.٣^{c}±٢.٠٤	١١٤.٥^{d}±٢.١٩
Insulin (µIU/ml)	٨.٤^{a}±٠.٤٧	٦.٥^{c}±٠.٤٦	٨.٣^{a}±٠.٥٩	٥.٢^{c}±٠.٣٤	٥.٩^{c}±٠.٦٨	٧.٩٧^{ab}±٠.٥١
HOMA-IR	١.٦^{d}± ٠.١١	٣.٥٣^{a}±٠. ٢٨	١.٦٩^{d}±٠.٠٩	٢.٩٥^{b}±٠.٢٢	٢.٢٩^{c}±٠.١٧	٢.٢٥^{c}±٠.١٨
TG(mg/dL)	٥٥.٢٥^{c}±١.٧١	١٠٤.٩٣^{b}±٢.٩٣	٤.٧٧^{c}±١.٦٧	١٢٣.٤٩^{a}±٢.٧٨	٩٨.٧٠^{b}±٣.١٩	٥٦.١٠^{c}±٢.٥٧

HDL(mg/dL)	٦٣.٨٥^{a}±١.١٥	٤٧.٩٧^{cd}±١.٣١	٧.٢١^{a}±١.٤٥	٤٤.٢٨^{d}±١.٢٨	٤٨.٩٣^{c}±٢.٠٣	٥٩.٦٧^{b}±١.٤٨
LDL(mg/dL	٣٢.٧٥^{d}±٠.٦٩	٧٣.٦٥^{b}±٢.٠٦	٣٣.٠٧^{d}±٠.٨٤	٧٨.٨٣^{a}±٢.١٠	٥٦.١٢^{c}±١.٤١	٣٤.٦١^{d}±٠.٤٦

Means in the same horizontal row with different superscripts $^{a,b,c\ and\ d}$ are significantly different ($P \leq ٠.٠٥$). TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein, CP: C-peptide, N: L-NAME, CP+N: C-peptide + L-NAME, Arg.: L-Arginine, sig: significance.

Discussion

The results of the present study showed that feeding rats with HFD for ٢ weeks followed by STZ injection (HFD+STZ) resulted in a significant increase in blood glucose level accompanied with significant insulin deficiency and increased insulin resistance as marked by a significantly higher HOMA-IR when compared to the normal control rats. This picture is compatible with type II diabetes with residual pancreatic function as described by[١].

Many studies have reported that rats fed with HFD develop insulin resistance but not frank hyperglycemia or diabetes[١٧,١٨,١٩]. HFD is the better way to initiate insulin resistance which is one of the important features of type II diabetes, so it was given for two weeks first, while STZ is widely used as β -cell toxin, inducing β cell death through alkylation of DNA[٢٠], so, it was used in the present work, as a single low dose injection to produce only partial β -cell mass loss. The residual mass remaining is responsible for the fasting insulin level observed in the diabetic group of this study, thus simulating early, but not late type II or type I DM, with absolute insulin deficiency. HFD induces insulin resistance by increasing free fatty acids (FFA) available for β-oxidation, with consequent sparing of glucose which is shuttled to lipogenesis, thus saturating the adipose tissue store and depositing in other organs. These changes disturb adipokine secretion resulting in hyperleptinemia and hypoadiponectinemia; both effects oppose insulin function. Furthermore, lipid metabolites specially long chain acyl~Co.A and diacyl glycerol (DAG) block insulin-dependent mechanisms of glucose metabolism including glucose transporters, hexokinase activity, and glycogenesis with stimulation of gluconeogenesis in the liver and muscle according to[٢١,١].

In the present study, HFD feeding for two weeks resulted in a significant increase in body weight, body weight gain, and food intake. STZ was not yet given, and the availability of insulin was adequate to stimulate lipogenesis and weight gain. Insulin is known to suppress hormone-sensitive lipase; the key enzyme for lipolysis and stimulate lipoprotein lipase; that provides precursor substrates for lipogenesis in adipose tissue [٢٢], hence excess caloric intake is directed to the anabolic pathway. Furthermore, the palatability of dietary fat could stimulate appetite and increase food intake. This is aided with time by the gradual development of insulin resistance leading to hyperphagia due to decreased anorectic effect of insulin [٢٣]. However, when STZ was given, and both insulin level and sensitivity decreased together, the catabolic effects dominated. Increased lipolysis explains the significantly higher TG and LDL levels with lower HDL levels in the diabetic group of this study. Decreased body weight and weight gain to control or lower levels respectively, occurred in spite of the fact that food intake remained significantly higher. It is probable also that the relative insulin deficiency uncouples oxidation and phosphorylation mechanisms with loss of the high energy intake as waste heat. The body mass index as a marker of obesity did not significantly change at the end from the control group due to enhanced catabolism and/or short duration of experiment.

These results are consistent with those of Nordquist et al.,[٢٤], Samarghandian et al.,[٦] and Nithya and Subramanian[٢٥] who reported that HFD with low dose STZ induced diabetic animals with hyperglycemia, polyuria, dehydration, and increased water and food intake, which closely resembles human type II diabetes.

The results obtained in the present study demonstrated that administration of C-peptide for ٤ weeks significantly lowered blood glucose, HOMA-IR, and food intake that were increased by diabetes, while it increased the lowered insulin level to control levels. Body weight, body weight gain and body mass index became significantly higher than the control levels. These results reflect the improved anabolic state of diabetic rats under the effect of C-peptide treatment. C-peptide improved insulin resistance, and increased insulin levels. This was reflected by normalization of fasting blood glucose, and increased weight gain, body weight and body mass index in spite of disappearance of hyperphagia. The high caloric value of diet overbalanced the decreased food intake and resulted in a degree of obesity as indicated by the significant increase in BMI.

Studies in the past decade have shown that C-peptide is much more than a byproduct of insulin synthesis and has biological role in metabolism and its deficiency with insulin in DM predisposes to the metabolic error. By binding to insulin through charge interaction, C-peptide prevents insulin aggregation to form polymeric inactive forms, hence keeping the monomeric biologically active insulin[٢٦]. C-peptide increases glucose utilization in muscles and peripheral tissues by increasing translocation of glucose transporter ٤ (GLUT٤) to cell membranes to facilitate glucose uptake. Furthermore, C-peptide can activate insulin signaling pathways by activating tyrosine kinase and phosphorylating insulin receptor substrate (ISR), thus, increasing its sensitivity, in addition to improving organ blood flow by stimulating endothelial nitric oxide synthase (eNOS) with increased NO and vasodilatation [٥]. These effects can explain the positive energy balance observed with C-peptide treatment to diabetic rats and its normalizing effects on blood glucose level seen in the present study that agrees with Wu et al. [٢٧]. However, other studies have reported C-peptide hazardous effects in type II DM, with increased recruitment of inflammatory cells in subendothelial layer of blood vessels and stimulation of smooth muscle proliferation which predispose to atherosclerosis[٥]. Differences in doses, animal species, duration of treatment and experimental protocol may explain such variations in responses.

In the present work, we investigated the role of NO in mediating C-peptide hypoglycemic effect by giving L-NAME; the nonselective blocker of NOS, either alone or combined with C-peptide to diabetic rats. L-NAME worsened the metabolic error produced by diabetes with production of a significantly higher blood glucose level and more disturbed lipid profile; with significantly higher serum TG and LDL levels. On the other hand, when combined with C-peptide, L-NAME partially antagonized the positive energy balance induced by C-peptide, but completely prevented its correcting lipid profile effect, apart from partial lowering of LDL. These results are compatible with those of Wu et al.,[٢٧] who reported that L-NAME was able to block about ٨٥٪ of the C-peptide-induced increase in glucose disposal rates, suggesting that the C-peptide stimulation of glucose utilization is mediated by NO.

NO, is produced in nearly every tissue of the body from the amino acid L-arginine by the three isoforms of NOS (nNOS, eNOS and iNOS). It plays an important role in regulating metabolic functions in all tissues under physiological conditions. It is formed in the hypothalamus and increases food intake[٢٨], increases glucose uptake and oxidation by facilitating blood flow to increase glucose availability, increases glucose internalization by stimulating GLUT٤, and its oxidation by stimulating mitochondrial biogenesis and respiratory chain complexes, increases insulin release from beta cells and its sensitivity, and promotes glycogenesis, and decreases gluconeogenesis[٢٩]. However, in DM, oxidative stress is present due to: the stimulatory effect of glucose on mitochondrial NADPH oxidase, generating excess superoxide ions. Inducible NOS is stimulated by inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and interleukins; -١ and -٦ resulting in excess NO formation which reacts with superoxide ions to form the highly toxic peroxinitrite.

Reactive oxygen and nitrogen species (ROS/RNS) further destroy membrane phospholipids, metabolic enzymes, and insulin receptors decreasing both insulin release and sensitivity. On the other hand, ROS/RNS could also oxidize and nitro-sylate NOS decreasing its activity and producing a vicious cycle of reduced NO, reduced blood flow, ischemia and more ROS generation. This could contribute to diabetic pathogenesis and compli-cations[٣٠,٣١]. According to these effects of NO, blocking NOS with L-NAME in the present work did not significantly alter the metabolic changes already present in diabetic rats since NOS activity is already deteriorated by diabetes. On the other hand, it partially attenuated the beneficial effects of C-peptide indicating that part of the effects of C-peptide are mediated by stimulating NO production.

A G-protein coupled C-peptide receptor has been identified and its stimulation induces NOS activation, and through increased NO production C-peptide can produce vasodilatation of pancreatic vessels with enhanced function of β-cells and increased insulin secretion. In the present study, the higher levels of insulin in the diabetic group treated with C-peptide and the lower levels found with L-NAME treatment supports this role of NO according to Bhatt et al.,[٥]. This could occur by its antioxidant effect which could protect both NOS and insulin. These results are consistent with the biochemical findings of Tekin et al.,[٣٢] in STZ-induced diabetic rats treated with L-NAME.

In the present work, and going with the beneficial effects of NO in regulating body metabolism, insulin secretion and function, administration of L-arginine; the substrate of NOS, to diabetic rats significantly, but partially corrected the disturbed serum levels of fasting glucose, insulin and insulin sensitivity as well as the dyslipidemic profile. These results are in line with those of Claybaugh et al.,[٣٣] and with Miczke et al.,[٣٤] who ascribed the beneficial effect of L-arginine to enhancing the regenerative capacity of pancreatic *β*-cells in diabetic rats.

In conclusion, HFD with low dose STZ injection to rats induced diabetic picture similar to early type II diabetes in human. HFD induced insulin resistance, while STZ produced partial degeneration of pancreatic *β* -cell mass, both resulting in decreased insulin secretion and sensitivity with its metabolic consequences. Both NO and C-peptide deficiencies contributed to the metabolic error, since C-peptide treatment corrected completely this error by promoting the residually functioning *β*-cells, while L-arginine treatment partially corrected it through enhancing NO synthesis. The beneficial effects of C-peptide were partially prevented by L-NAME coadministration indicating that NO partially mediates the effects of C-peptide. The results open the way for C-peptide trials in treating type II diabetes with residual pancreatic mass. Trials of C-peptide with insulin in type I diabetes with absolute insulin and C-peptide loss should be a subject of future research.

author that, despite its limitations and the wide variety of both the high-fat fed regimen and the STZ treatment, the HFD/STZ is a reasonable animal model of type ٢ diabetes mainly representing the later stage of the disease depending on the amount of residual b-cell mas inally and most importantly, it is the opinion of this author that, despite its limitations and the wide variety of both the high-fat fed regimen and the STZ treatment, the HFD/STZ is a reasonable animal model of type ٢ diabetes mainly representing the later stage of the disease depending on the amount of residual b-cell mas

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