ORIGINAL ARTICLE

# Effect of chromium and L-carnosine use on oxidative stress markers in experimental model of gestational diabetes mellitus

**Meltem Yardim, Suleyman Aydin, Tuncay Kuloglu**

Department of Medical Biochemistry, Yerkoy State Hospital, Yozgat, Selcuk, Namik Kemal Cd. No:2, 66900 Yerkoy/Yozgat, Turkey

## ABSTRACT

**Background/Aims:** To evaluate the effects of chromium, insulin and carnosine supplementation on serum and tissue levels of oxidative stress markers in streptozotocin (STZ)-induced gestational diabetes mellitus (GDM).

**Methods:** 132 Sprague-Dawley rats were divided into eight groups such as control, sham I, sham II, DM, DM+insulin, DM+chromium, DM+carnosine, and DM+chromium plus carnosine. The rats were sacrificed on day 17 of pregnancy to collect samples. Maternal and fetal levels of 8-OHdG, total antioxidant capacity (TAC) and total oxidant status (TOS) were measured. In addition, immunohistochemical staining was used to analyze different maternal tissues, such as the brain, kidney, liver, heart, ovary and pancreas.

**Results:** Increased 8-OHdG and TOS and decreased TAC levels were found in the DM group compared to controls (all, $p<0.05$). In addition, improvements in biochemical markers, 8-OHdG, TAC and TOS were observed in recipients of insulin, chromium, carnosine or chromium and carnosine (all, $p<0.05$).

**Conclusions:** Using chromium, insulin, or carnosine supplementation may alleviate GDM-related oxidative changes and could prevent oxidative stress triggered by glucose use.

**Keywords:** Experimental diabetes, pregnant rats, chromium, insulin, carnosine, oxidative stress

JMS: 2022; 25(4):37-46 DOI: https://doi.org/10.33883/jms.v25i4.1219

## INTRODUCTION

Gestational Diabetes Mellitus (GDM) is the most common metabolic disorder of pregnancy, and its prevalence varies between 1% and 28% according to the population.[1] In addition to causing hyperglycemia due to limited insulin response or pregnancy-induced insulin resistance,[2] GDM may lead to many adverse effects, including fetal death, macrosomia, polyhydramnios, and neonatal complications, such as hypoglycemia, respiratory distress syndrome (RDS), metabolic disorders, and hyperbilirubinemia.[3] The American Diabetes Association (ADA) recommends that all pregnant women between 24-28 weeks of gestation be applied a 75-gram glucose load test to assess GDM risk (oral glucose tolerance test; OGTT).[4] Women diagnosed with GDM and their offspring are at elevated risk for developing diabetes mellitus later in life.[5] Oxidative stress is one of the investigated mechanisms. Previous studies have found that hyperglycemia increases free oxygen radicals (FOR) and contributes to oxidative stress.[6] When FOR exceeds the antioxidant response level, DNA damage, lipid peroxidation, protein denaturation and cell necrosis may occur.[6] Although the relationship between GDM and oxidative stress has not been clarified. Recent evidence suggests that antioxidant molecules may be protective, limiting or partially reparative against GDM complications.[7]

**Access this article online**

**Website:** www.jmsskims.org

**DOI:** https://doi.org/10.33883/jms.v25i4.1219

**Correspondence:**
Meltem Yardim, MD
Department of Medical Biochemistry, Yerkoy State Hospital, Yozgat, Selcuk, Namik Kemal Cd. No:2, 66900 Yerkoy/Yozgat, Turkey
Telephone number: +905059064962
E-mail address: meltem_yardim@hotmail.com

**How to cite this article:** Yardim M, Aydin S, Kuloglu T. Effect of chromium and L-carnosine use on oxidative stress markers in experimental model of gestational diabetes mellitus. JMS 2022: 25 (4)37-46
Received: 2022-08-09 Accepted: 2022-12-20

This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Carnosine is an endogenic dipeptide composed of beta-alanine and L-histidine amino acids and is particularly abundant in mammalian skeletal muscle and neurons.[8] It involves many biological functions, including FOR scavenging, anti-ageing and anti-glycation activities.8 Carnosine contributes to recovery from muscle fatigue by neutralizing lactic acid produced during non-aerobic glycolysis,[9] reduces blood glucose concentration via regulatory effects on the autonomic nervous system, and ten and inhibits glucose oxidation and glycation of proteins.[11] Studies concerning carnosine's effects on oxidative stress in streptozotocin (STZ)-induced GDM are lacking.

Chromium is an essential mineral that plays a significant role in glucose metabolism by potentiating insulin signalling and improving insulin sensitivity.[12] Supplementation with chromium picolinate, a stable and highly bioavailable form of chromium, has been reported to decrease insulin resistance and alleviate the risk of type 2 diabetes mellitus and cardiovascular disease.[13] this study aimed to investigate the effects of chromium, insulin and carnosine supplementation on the oxidative stress biomarkers in the tissues and blood samples of pregnant rats (dams) with STZ-induced GDM. In addition to 8-Hydroxy-2'-deoxyguanosine (8-OHdG), total oxidant status (TOS) and total antioxidant capacity (TAC) were evaluated.

## METHODS

### Animal care and the study protocol

All experiments were conducted per the Guide for the Care and Use of Laboratory Animals. Approval was obtained from the Animal Care and Use Committee of Fırat University (2016/4). A total of 138 female Sprague-Dawley rats aged 34 months weighing 250 to 300 g and the fetuses of dams were enrolled in this study. Animals were housed in separate cages individually in air-conditioned rooms with 12-h light and dark cycles maintaining stable temperature (22 ± 2 °C) and relative humidity of 45%50% with ad libitum access to standard laboratory chow and tap water. The sexual cycle stages (synchronization) of rats were determined by the vaginal smear method. To achieve pregnancy, non-diabetic female and male rats were kept overnight in one cage with a ratio of 2:1 during the oestrous cycle. Spermatozoa in vaginal smear were evaluated under a light microscope each morning, and the morning on which a positive result was seen was identified as the 0th day of gestation. Female rats who could not conceive during this period were excluded from the study (n = 6).

In dams that were to be subject to STZ-induced DM, on day 13 of pregnancy, intraperitoneal administration of 60 mg/kg STZ (Santa Cruz Biotechnology Inc., USA) dissolved in sodium citrate buffer (0.1 M, pH 4.5) was performed.

132 rats in total were randomized into eight groups as follows:

1. Group 1 (Control group, n= 6): Normal dams were sacrificed on day 17 of pregnancy.
2. Group 2 (Sham I group, saline group; n = 18): Saline was administered instead of glucose by gavage on the 17th day of pregnancy.
3. Group 3 (Sham II group, 0.1M sodium citrate group; n = 18): On day 13 of pregnancy, 0.1 M sodium citrate (vehicle) without STZ was administered intraperitoneally.
4. Group 4, DM group (Diabetic controls; n = 18) (DM group): OGTT was performed on dams with DM.
5. Group 5, DM+INS group (Diabetic control + insulin group; n = 18): In dams with DM, 5 IU kg/day insulin was given by subcutaneous injection on the 16th day of pregnancy.
6. Group 6, DM+CH group (Diabetic control + chrome picolinate group; n = 18): In dams with DM, 80 microgram/kg chrome picolinate was given via gavage on the 16th day of pregnancy.
7. Group 7, DM+CAR group (Diabetic control + carnosine group; n = 18): In dams with DM, 100 mg/kg carnosine was applied via gavage on the 16th day of pregnancy.
8. Group 8, DM+CH&CAR group (Diabetic control + chrome picolinate + carnosine group; n = 18): In dams with DM, 80 microgram/kg chrome picolinate and 100 mg/kg carnosine were applied via gavage on the 16th day of pregnancy.

The development of DM was confirmed by fasting glucose level measurement at 72 hours after STZ administration and later with a demonstration of pancreatic necrosis via pathological analysis. All groups except the first underwent OGTT via gavage (with 2g/kg glucose) on the 17th day of pregnancy, and results for different subgroups for OGTT

time-points were recorded [pre-OGTT (n = 6), post-OGTT 1st hour (n = 6), and post-OGTT 2nd hour (n = 6)]. Before OGTT, rats fasted for 10 hours without water restriction. At the end of experimental procedures, dissection was performed for each animal in each group by anaesthetizing with ketamine /xylazine (90/10 mg/kg) on the 17th day of pregnancy after OGTT. Tissues of mothers and fetuses (brain, renal, liver, heart, ovarian and pancreatic tissues) were covered with aluminium foil and placed in 10% formaldehyde solution for preservation at -80°C until analysis. Blood samples of the mothers and fetuses were obtained separately and centrifuged at 4000 rpm for 5 min. The serum was stored at -80°C until measurements.

Tissue extraction and sample acquisition for measurements

Tissues excised from dams were blotted free of blood, cleaned with phosphate-buffered saline (PBS), and homogenization (10% w/v) in 0.1 M PBS solution was performed with the help of a Bullet Blender Homogenizer (BBY24M-CE Next Advance, USA). Homogenates were centrifuged at 4000 rpm at +4˚C for 20 min, and the supernatants were separated and stored.

**Biochemical Analyses**

Routine biochemical analyses of glucose, total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in the blood samples of dams and fetuses have been performed atFırat University Central Biochemistry Laboratory with an autoanalyzer (Siemens Advia 2400 Chemistry System, Siemens, Germany) using appropriate commercial kits. Low-density lipoprotein cholesterol (VLDL-C) levels were calculated using the Friedewald formula (VLDL-C =triglyceride/5). According to the manufacturer's instructions, insulin was measured with an ELISA kit for rat samples (Sunred Biological Technology Co., Ltd, Shanghai, China). The measurement range of the rat insulin kit was 0.2-40 mIU/L (sensitivity of 0.102 mIU/L), the Intra-Assay CV value was <10%, and the Inter-Assay CV value was <12%.To assess DNA oxidation, 8-OHdG levels were measured using an ELISA kit (Cayman Chemicals, USA). The measurement range of the rat 8-OHdG kit was 10.3-3000 pg/mL(sensitivity 30 pg/mL), the Intra-Assay CV value was 4.7-11.6%, and the Inter-Assay CV value was 4.5-10.7%.

Tissue and serum total antioxidant capacity (TAC) were measured using an automated method developed by Erel et al.14 Serum TAC levels were expressed as mmol Trolox equivalents/L. Total oxidant status (TOS) was determined in serum and tissues using a commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey). The assay was calibrated with hydrogen peroxide, and the results were expressed as the micromolar hydrogen peroxide equivalent per litre (µmol H2O2 equivalent/L).

**Immunohistochemistry**

All tissue samples were fixed in a 10% neutral buffered formaldehyde solution for histopathological examinations for 24 hours. Following washing, samples were embedded in paraffin after processing through graded ethanol (50% to 100%) and xylene, respectively. Serial sections of tissues (4-µm thickness) were stained for 3 min in hematoxylin and 30 sec in eosin. Two blinded pathologists examined sections under a standard light microscope (Leica DM500, Germany). Papanicolaou staining was used to evaluate the estrus cycle in vaginal smear samples according to Schaberg's assessment.[15]

The sections of 4-µm thickness taken from paraffin blocks were deparaffinized and dehydrated. Afterwards, sections were respectively incubated in 3% hydrogen peroxide (Hydrogen Peroxide Block, TA-125-HP, Lab Vision Corporation, USA) for 5 min, in PBS buffer (Invitrogen Corporation, USA), in Ultra V Block (TA-125-UB, Lab Vision Corporation, USA), in 8-oxo-dG antibody (Novus Biologicals, USA) for 60 min at room temperature, in Biotinylated Goat Anti-Polyvalent (Lab Vision Corporation, USA), and streptavidinperoxidase conjugate (Lab Vision Corporation, USA). As a chromogen, 3-amino-9-ethyl carbazole was used(AEC Substrate+AEC Chromogen, Lab Vision Corporation, USA), and then staining was performed with Mayer's hematoxylin staining. Sections were evaluated under the Leica DM500 microscope. Histoscore was established based on the extent and severity of immunoreactivity in staining.

**Statistical analysis**

All analyses were performed on SPSS v22 (SPSS Inc., Chicago, IL, USA) with p values of ≤0.05 accepted to demonstrate statistical significance. For the normality check, the Shapiro-Wilk test was used. Data are given as mean ± standard deviation. The Mann-Whitney U test was used to compare data between groups. For time-bound

(longitudinal) comparisons, paired tests were used concerning the fulfilment of parametric assumptions (one-way repeated measures ANOVA or the Friedman test).

## RESULTS

A total of 132 Sprague-Dawley dams aged 34 months and their fetuses (n=1445) were included in the study. During pregnancy, no rat mortality occurred before the planned sacrifice. Those with a fasting blood glucose level of 250 mg/dL(13.8 mmol/L) and above were considered diabetic. Detecting pancreatic necrosis on hematoxylin-eosin staining also confirmed the occurrence of diabetes in rats. The characteristics of damswere similar throughout pregnancy (all, $p>0.05$). The fetus weights of the GDM groups were found to be significantly higher compared to non-GDM groups ($p<0.05$) (data not shown).

### Biochemical parameters

When OGTT results on laboratory variables were evaluated, we found that serum glucose and triglyceride levels were lower in controls than in diabetic groups (all, $p<0.05$). Glucose levels were significantly lower in all intervention groups (INS, CH, CAR, and CH&CAR) compared to the group ($p< 0.05$). Insulin levels in the experimental groups were lower compared to the control group, except for those that received external insulin (DM+INS) ($p<0.05$). Increased levels of 1st- and 2nd-hour triglycerides were found in the DM, DM+INS, DM+CH and DM+CAR groups compared to controls($p<0.05$). Compared with the control group in terms of total cholesterol levels, an increase was observed in the DM and DM+CH groups and a decrease in the DM+CAR and DM+CH&CAR groups ($p<0.05$). It was determined that serum HDL-C levels were significantly higher in the DM+CAR and DM+CH&CAR groups compared to the control group ($p<0.05$). Interestingly, while LDL-C levels in the DM group were significantly elevated compared to controls ($p< 0.05$), all four interventional groups had similar values to the control groups. VLDL-C values were significantly increased in the diabetic groups compared to the controls ($p<0.05$). (Table 1).

### Oxidative stress markers in serum

Evaluation of oxidative markers in the serum showed that the development of diabetes was found to cause significant maternal and fetal increases in 8-OHdG and TOS levels and significant decreases in TAC values compared to control values ($p< 0.05$). When compared to the DM group, maternal serum 8-OHdG and TOS levels were lower, and TAC levels were higher in the DM+INS, DM+CAR, DM+CH, and DM+CH&CAR groups ($p< 0.05$). The highest maternal serum TAC values and the lowest TOS values were observed in the DM+CAR, DM+CH and DM+CH&CAR groups. These results were mirrored by fetal measurements, albeit to a lesser degree. The highest fetal serum TAC values were found in the DM+CH and DM+CH&CAR groups, and the lowest TOS and 8-OHdG values were found in the DM+CH and DM+CAR groups. However, fetal 8-OHdG levels did not significantly differ from either controls or the DM group (Table 2).

Within-group comparisons (to assess the effects of OGTT) showed that 2nd-hour 8-OHdG values in maternal and fetal serum were significantly elevated compared to baseline in the DM, DM+INS and DM+CAR groups ($p< 0.05$). No notable time-bound changes in TAC levels existed in any of the groups. However, 2nd-hour TOS values showed a significant increase from baseline in the maternal DM group and the fetal DM, DM+INS and DM+CH&CAR groups ($p< 0.05$)(Table 2).

### Histoscores and oxidative markers in maternal tissues

Inducing DM was found to cause increased levels of 8-OHdG and TOS and decreased levels of TAC in all tissues at almost all time points. Immunohistochemistry for 8-OHdG staining showed that histoscores were similar for all groups in brain, renal, liver and heart tissue comparisons; however, for ovarian and pancreatic tissue, histoscores were significantly lower in the DM+INS group compared to controls ($p< 0.05$). Additionally, there were no within-group histoscore changes in any tissue. Immunohistochemical expression of 8-OHdG in maternal rat pancreatic tissues concerning groups and OGTT time-points were shown in Figure 1.

When brain tissue results were evaluated relative to the DM group, 8-OHdG was lower, TAC was higher, and TOS was lower in the intervention groups ($p< 0.05$). Renal samples showed few significant differences in the DM versus intervention (INS, CH, CAR, CH&CAR) comparisons; however, the DM+INS group had significantly higher TAC and significantly lower TOS values compared to the DM group ($p< 0.05$). Liver tissue samples showed that the DM-CH, DM-CAR and DM-CH&CAR groups had substantially lower 8-OHdG and TAC levels and significantly higher TOS levels in almost all time points compared to the DM

**Table 1.** Biochemical parameters with respect to groups and OGTT time-points

| Parameter | Time (h) | Control | Sham I | Sham II | DM | DM+INS | DM+CH | DM+CAR | DM+CH&KAR |
|---|---|---|---|---|---|---|---|---|---|
| Glucose mg/dL | 0 h | 112.27 ±8.80 | 105.50 ±14.95 | 105.83 ±7.06 | **332.00 ±42.58** [a] | **163.67 ±24.40** [ad] | **279.17 ±34.65** [a] | **276.00 ±26.55** [a] | **290.67 ±28.58** [a] |
| | 1 h | | 120.50 ±13.92 | 119.67 ±16.80 | **381.50 ±36.12** [a] | **170.00 ±31.40** [ad] | **280.17 ±28.43** [ad] | **295.17 ±27.26** [ad] | **291.17 ±25.61** [ad] |
| | 2 h | | 125.67 ±12.50 | 119.67 ±17.94 | **411.50 ±28.56** [ab] | **171.50 ±31.98** [ad] | **290.17 ±31.75** [ad] | **301.00 ±26.10** [abd] | **300.67 ±27.51** [ad] |
| Insulin mIU/L | 0 h | 12.25 ±2.41 | 12.57 ±2.73 | 12.73 ±1.70 | **7.17 ±0.72** [a] | **12.54 ±4.91** [d] | **9.41 ±5.85** [a] | **9.85 ±0.83** [ad] | **8.52 ±0.83** [a] |
| | 1 h | | 12.25 ±8.27 | 12.69 ±5.51 | **8.38 ±0.54** [ab] | **13.65 ±4.99** [d] | **8.60 ±2.84** [a] | **9.36 ±0.90** [a] | **8.62 ±0.28** [a] |
| | 2 h | | 11.99 ±1.26 | 12.40 ±5.77 | **9.46 ±0.98** [ab] | **13.33 ±4.27** [d] | **8.42 ±3.42** [a] | **9.26 ±1.08** [a] | **9.12 ±0.89** [a] |
| Triglyceride mg/dL | 0 h | 159.20 ±34.17 | 160.80 ±15.76 | 150.20 ±24.87 | **285.60 ±11.68** [a] | **174.00 ±17.84** [d] | **187.00 ±18.85** [ad] | **252.00 ±17.39** [a] | **198.40 ±24.78** [ad] |
| | 1 h | | 168.40 ±18.99 | 155.00 ±25.82 | **312.20 ±36.71** [a] | **254.40 ±36.95** [abd] | **250.60 ±40.52** [abd] | **321.20 ±23.10** [ab] | **173.80 ±17.70** [d] |
| | 2 h | | 164.40 ±18.07 | 148.80 ±14.66 | **348.80 ±34.80** [ab] | **264.80 ±16.83** [abd] | **270.80 ±15.86** [abcd] | **357.80 ±17.44** [abc] | **155.40 ±15.56** [bd] |
| Total cholesterol mg/dL | 0 h | 71.80 ±5.56 | 65.80 ±6.31 | 69.00 ±7.78 | **96.80 ±8.37** [a] | **67.20 ±8.87** [d] | **84.20 ±6.35** [a] | **44.00 ±15.23** [ad] | **30.80 ±6.28** [ad] |
| | 1 h | | 70.80 ±6.31 | 63.00 ±6.20 | **98.60 ±7.61** [a] | **66.00 ±6.68** [d] | **95.80 ±10.46** [a] | **36.60 ±5.35** [abd] | **33.60 ±2.62** [ad] |
| | 2 h | | 64.80 ±5.17 | 62.40 ±5.82 | **105.20 ±8.29** [ab] | **69.20 ±6.42** [d] | **95.20 ±7.82** [a] | **35.80 ±6.51** [abd] | **30.20 ±4.18** [ad] |
| HDL-C mg/dL | 0 h | 32.26 ±4.23 | 33.46 ±8.75 | 38.46 ±6.76 | **15.34 ±2.57** [a] | **23.84 ±0.23** [ad] | **23.46 ±0.34** [ad] | **37.16 ±4.98** [ad] | **44.00 ±4.52** [ad] |
| | 1 h | | 34.62 ±7.63 | 37.84 ±5.76 | **13.48 ±3.77** [a] | **25.48 ±0.48** [ad] | **29.16 ±4.71** [d] | **33.06 ±4.27** [bd] | **44.98 ±5.67** [ad] |
| | 2 h | | 38.94 ±5.89 | 36.42 ±5.27 | **14.96 ±3.53** [a] | **24.06 ±0.38** [ad] | **27.26 ±0.57** [ad] | **44.72 ±5.32** [abcd] | **42.34 ±5.26** [ad] |
| LDL-C mg/dL | 0 h | 62.78 ±1.19 | 75.16 ±1.72 | 75.98 ±7.49 | **92.58 ±9.17** [a] | 66.44 ±1.56 | 63.92 ±6.13 | 64.22 ±6.98 | 63.18 ±6.10 |
| | 1 h | | 74.88 ±1.77 | 76.48 ±7.59 | **94.86 ±9.48** [a] | 66.20 ±1.80 | 64.22 ±6.16 | 64.68 ±6.49 | 64.44 ±6.27 |
| | 2 h | | 75.12 ±0.58 | 73.96 ±7.26 | **99.46 ±9.28** [a] | 62.80 ±1.52 | 73.74 ±6.14 | 63.86 ±6.46 | 63.12 ±6.27 |
| VLDL-C mg/dL | 0 h | 33.84 ±6.83 | 32.16 ±3.16 | 36.04 ±5.17 | **62.12 ±2.21** [a] | **40.88 ±7.13** [ad] | **49.40 ±4.77** [ad] | **60.40 ±6.34** [a] | **59.68 ±4.90** [a] |
| | 1 h | | 33.68 ±2.37 | 35.00 ±6.13 | **63.44 ±6.27** [a] | **42.96 ±6.20** [ad] | **50.12 ±6.18** [a] | **54.24 ±4.61** [abd] | **54.76 ±3.54** [ad] |
| | 2 h | | 36.88 ±4.21 | 29.76 ±3.17 | **69.76 ±7.27** [a] | **44.80 ±3.45** [ad] | **54.16 ±6.97** [a] | **61.56 ±3.15** [ac] | **51.08 ±3.19** [abd] |

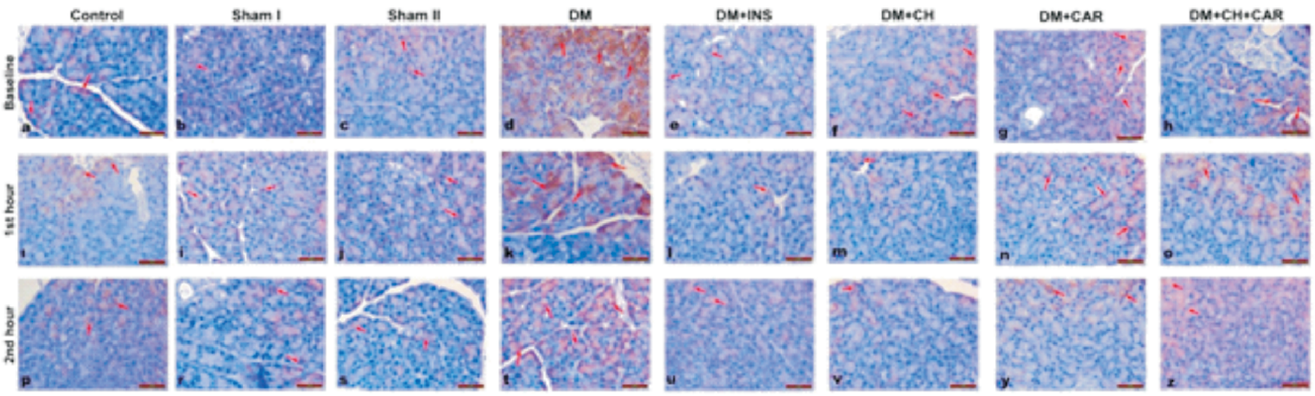
DM: Diabetes Mellitus, INS: Insulin, CAR: Carnosine, CH: Chrome, TAC: Total antioxidant capacity, TOS: Total oxidant status, 8 -OHdG: 8 -hydroxy 2 -deoxyguanosine. a: compared with the control group ($p<0.05$); b: compared with 0 hour ($p<0.05$); c: compared with 1 hour ($p<0.05$); d: compared with the DM group ($p<0.05$). Values are given as mean ± standard deviation.

**Table 2.** Serum 8-OHdG, TAC and TOS levels with respect to groups and OGTT time-points

| Parameter | | Time (h) | Control | Sham I | Sham II | DM | DM+INS | DM+CH | DM+CAR | DM+CH&CAR |
|---|---|---|---|---|---|---|---|---|---|---|
| Maternal | 8-OHdG pg/mL | 0 h | 430.17 ±30.57 | 420.51 ±30.42 | 460.73 ±80.22 | **920.00 ±120.54** [a] | **490.77 ±40.19** [d] | **760.67 ±160.94** [ad] | **620.07 ±90.71** [ad] | **580.28 ±20.75** [ad] |
| | | 1 h | | 400.70 ±60.87 | 450.08 ±110.5 | **980.83 ±110.08** [a] | **640.93 ±40.65** [abd] | **760.65 ±40.21** [ad] | **660.75 ±80.51** [ad] | **590.60 ±20.46** [ad] |
| | | 2 h | | 480.70 ±50.43 | 440.75 ±110.02 | **1100.27 ±100.59** [ab] | **700.77 ±20.68** [abcd] | **770.85 ±120.28** [ad] | **700.18 ±90.94** [abd] | **620.53 ±10.78** [ad] |
| | TAC mmol/L | 0 h | 1.37 ±0.43 | 1.30 ±0.18 | 1.35 ±0.11 | **0.75 ±0.31** [a] | **0.87 ±0.07** [ad] | **1.42 ±0.08** [d] | **1.80 ±0.24** [ad] | **1.74 ±0.40** [d] |
| | | 1 h | | 1.40 ±0.12 | 1.44 ±0.14 | **0.67 ±0.13** [a] | **0.84 ±0.03** [ad] | **1.51 ±0.30** [d] | **1.97 ±0.12** [ad] | **1.81 ±0.53** [d] |
| | | 2 h | | 1.44 ±0.16 | 1.52 ±0.32 | **0.65 ±0.49** [ab] | **0.84 ±0.03** [ad] | **1.61 ±0.34** [d] | **1.89 ±0.27** [ad] | **1.88 ±0.19** [ad] |
| | TOS µmol/L | 0 h | 74.56 ±6.99 | 69.12 ±6.96 | 66.82 ±8.00 | 82.39 ±7.06 | 67.39 ±7.10 | 69.05 ±6.66 | **65.89 ±10.94** [d] | **64.59 ±6.96** [ad] |
| | | 1 h | | 74.72 ±7.12 | 67.24 ±7.14 | **97.67 ±7.11** [ab] | **68.62 ±6.89** [d] | **69.21 ±9.04** [d] | **67.25 ±7.84** [d] | **67.84 ±9.71** [ad] |
| | | 2 h | | 76.00 ±7.37 | 71.85 ±7.10 | **98.38 ±8.14** [ab] | **69.36 ±6.15** [d] | **70.39 ±6.14** [d] | **68.07 ±8.48** [d] | **69.48 ±8.19** [ad] |
| Fetal | 8-OHdG pg/mL | 0 h | 660.77 ±80.39 | 660.10 ±70.54 | 580.13 ±70.46 | 730.33 ±90.40 | 600.07 ±40.95 | **450.03 ±20.31** [ad] | **510.82 ±40.84** [ad] | 640.43 ±140.96 |
| | | 1 h | | 650.13 ±80.36 | 660.80 ±50.34 | **770.57 ±40.70** [a] | **590.63 ±60.64** [ad] | **520.53 ±50.95** [abd] | **530.17 ±60.22** [ad] | 660.47 ±80.39 |
| | | 2 h | | 620.00 ±50.43 | 670.92 ±110.82 | **820.02 ±70.83** [ab] | **690.23 ±40.08** [bc] | **580.80 ±50.22** [abcd] | **610.75 ±60.10** [bd] | 690.60 ±50.70 |
| | TAC mmol/L | 0 h | 1.25 ±0.17 | 1.27 ±0.33 | 1.31 ±0.35 | **0.83 ±0.17** [a] | 1.20 ±0.25 | **1.31 ±0.17** [d] | **1.64 ±0.24** [ad] | **1.60 ±0.53** [d] |
| | | 1 h | | 1.30 ±1.45 | 1.37 ±0.39 | **0.77 ±0.22** [a] | 1.28 ±0.20 | **1.37 ±0.13** [d] | **1.71 ±0.37** [ad] | **1.61 ±0.13** [d] |
| | | 2 h | | 1.34 ±1.08 | 1.23 ±0.23 | **0.67 ±0.11** [ab] | **1.40 ±0.25** [d] | **1.48 ±0.24** [d] | **1.86 ±0.03** [abd] | **1.67 ±0.26** [d] |
| | TOS µmol/L | 0 h | 65.53 ±0.42 | 52.41 ±7.13 | 62.38 ±28.43 | **82.93 ±5.87** [a] | **62.14 ±3.14** [d] | **61.52 ±2.14** [d] | **58.03 ±8.07** [d] | **70.91 ±3.46** [a] |
| | | 1 h | | 59.24 ±0.86 | 60.19 ±15.89 | **89.64 ±4.54** [a] | **71.52 ±2.13** [abd] | **64.36 ±4.19** [d] | **63.80 ±7.49** [d] | **76.09 ±3.96** [abd] |
| | | 2 h | | 64.90 ±31.83 | 59.21 ±7.27 | **90.30 ±5.17** [ab] | **70.96 ±3.69** [abd] | **68.37 ±8.70** [d] | **66.39 ±9.97** [bd] | **76.05 ±2.38** [abd] |

DM: Diabetes Mellitus, INS: Insulin, CAR: Carnosine, CH: Chrome, TAC: Total antioxidant capacity, TOS: Total oxidant status, 8 -OHdG: 8 -hydroxy 2 -deoxyguanosine. a: compared with the control group ($p<0.05$); b: compared with 0 hour ($p<0.05$); c: compared with 1 hour ($p<0.05$); d: compared with the DM group ($p<0.05$). Values are given as mean ± standard deviation.

**Fig. 1.** Immunohistochemical expression of 8-OHdG in maternal rat pancreatic tissues with respect to groups and OGTT time-points.




group ($p< 0.05$). In heart tissue, there were only a few significant differences between DM-induced groups; most notably, pre-OGTT and post-OGTT 1-hour TAC values were significantly higher in the DM+INS, DM+CAR and DM+CH&CAR groups, and significant improvements in TAC and TOS values were observed in the DM+INS group, when compared to the DM group ($p< 0.05$). Ovarian tissue analyses showed that the 8-OHdG levels of the intervention groups were significantly lower compared to the DM group in terms of only post-OGTT 2-hour values ($p< 0.05$). In contrast, TAC and TOS values of the intervention groups demonstrated significant improvement in almost all time points compared to the DM group ($p< 0.05$). Finally, for pancreatic tissue, most differences were present in the DM+INS versus DM comparison except for TAC values which were most similar in the groups. Nonetheless, the TOS values of the DM+CH&CAR group were significantly lower relative to the DM group in both post-OGTT time points ($p< 0.05$) (data not shown).

We found the following results when data concerning only the DM-induced groups were briefly examined overall. In brain, renal and liver samples, the lowest 8-OHdG levels were observed in the DM+CAR and DM+CH&CAR groups. The lowest 8-OHdG levels were present in the DM+INS and DM+CH&CAR groups in the heart. The DM+INS and DM+CH groups demonstrated the lowest 8-OHdG levels in ovarian and pancreatic tissue. In almost all tissues (excluding the heart), the highest TAC values were observed in the DM+CAR group at all time points. Finally, TOS values were the lowest in the DM+INS group (renal, heart and ovarian tissue)and the DM+CH&CAR group (brain, liver, pancreatic tissue) (data not shown).

## DISCUSSION

The incidence of GDM is increasing worldwide in parallel with the increasing obesity epidemic.[16] The American Diabetes Association and the Diabetes in Pregnancy Working Group recommend performing OGTT in pregnant women between 24-28 weeks of gestation to determine whether there is gestational diabetes mellitus.4,17 Establishment of experimental diabetes with STZ application is one of the most widely used, easy-to-apply and inexpensive methods for the conduct of DM-related experimental studies.18 We demonstrated increased 8-OHdG and TOS and decreased TAC levels in the DM group compared to controls. The administration of insulin, carnosine, chromium, or chromium plus carnosine was demonstrated to improve 8-OHdG, TOS and TAC levels in collected samples. We confirmed the development of STZ-induced DM diagnosis by showing high glucose measurements in blood samples and necrosis in pancreatic tissues. Although beta cells of the pancreas are destroyed selectively and irreversibly by STZ administration, the reason for some level of insulin concentration in the DM-induced groups in our study may be due to the release of insulin remaining in the secretory vesicles.

Another source of insulin may be the salivary glands. It was reported that salivary glands also have immune reactivity of insulin hormone at low concentrations.[19] Another source of insulin in these diabetic rats may be related to the alpha and delta cells of the pancreas. In the absence of beta cells, alpha and delta cells can be reprogrammed to produce insulin at low concentrations.[20] The reason for the increase in the insulin level of the chromium-administered group

compared to the diabetes group in our study may be that chromium triggered insulin production. Previous studies have shown that chromium improves impaired glucose tolerance, and chromium deficiency causes an elevation in glucose, insulin, cholesterol, and triglyceride levels and decreases HDL levels.[21] Shinde et al. demonstrated in an experimental STZ-induced diabetes model study that chromium picolinate-containing drinking water improved blood glucose levels.[22] Sahin et al. showed that chromium administration to rats with experimental diabetes reduced blood glucose levels by 63% and total cholesterol levels by 9.7%, compared to a diabetes group without chromium administration.[23] Following previous studies, we found decreased glucose, insulin, total cholesterol, and triglyceride levels and increased HDL-C levels in chromium-administered DM rats. This may be related to the binding of chromium to a peptide called apo-DMACr (Apo-LC), increasing the activity of the insulin receptor and facilitating glucose uptake.

24 We observed that the combined administration of chromium plus carnosine to GDM rats further lowered glucose, insulin, cholesterol, and triglyceride levels and increased HDL levels compared to chromium alone. This positive effect may be due to the antioxidant property of carnosine. It has been reported in many studies that carnosine eliminates free oxygen radicals with its antioxidant effects[11]. In addition, the administration of carnosine alone to DM rats was found to reduce glucose, insulin, cholesterol, and triglyceride levels and increase HDL levels. Still, the combined administration of carnosine &chromium was more effective. This can be explained by the increased insulin sensitivity caused by chromium and the antioxidant properties of carnosine. Furthermore, it has also been reported that chromium is an agent that has a role in eliminating the adverse effects of oxidative stress.[21]

The oxidant-antioxidant balance is disrupted in favour of free radicals in diabetes mellitus, which leads to a decrease in the sensitivity of insulin receptors.[25] Free radicals also increase the oxidation of glucose in Langerhans island cells.[26] In maternal and fetal serum samples, 8-OHdG, TAC and TOS values all demonstrated significant improvements relative to the DM group, except for fetal 8-OHdG levels in the DM+CH&CAR group. We also found that tissue TAC values among the DM-induced groups were the highest in carnosine recipients, except for heart tissue. However, significant improvements in tissue TAC levels relative to DM values were also observed in almost all intervention groups. Concerning TOS, significant reductions were mainly observed in insulin and chromium plus carnosine recipients; however, statistically significant changes from DM were less frequently relative to those demonstrated for the TAC parameter. This situation suggests that the administration of intervention(s) for DM is relatively more effective in improving response to oxidation rather than directly reducing the level of oxidants in serum or tissues. However, rats were sacrificed within a short period after administering said interventions (24 hours), which may have limited the accuracy of interpretations in this respect. Notably, while the levels of 8-OHdG varied greatly depending upon the administered intervention, the highest TAC values were in carnosine recipients, and the lowest TOS values were observed in either insulin or chromium plus carnosine recipients. These findings may indicate that DM-related TAC decrease was alleviated with the administration of carnosine.

Additionally, insulin and chromium plus carnosine treatments prevent oxidant increase, as demonstrated by the lower TOS levels in these groups. Our results suggest that the oxidant-favouring changes in DM can be alleviated significantly by insulin and carnosine, thus preventing biological tissue damage. Our study also shows that carnosine is likely to be a potent antioxidant molecule itself and could be used to regulate glucose metabolism with or without chromium use.

8-OHdG is a highly sensitive marker for oxidative DNA damage in tissues and biological fluids.[27] The OH radicals formed by oxidative damage attack the 8th position of the guanine molecule and develop 8-OHdG, which is a reliable free radical marker of oxidation-modified DNA.28 The current study confirmed that the development of DM caused a significant elevation in 8-OHdG levels and that post-OGTT 2-hour values were relatively higher compared to baseline and 1st-hour values. Furthermore, in contrast to time-bound similarities in TAC and TOS values for most groups, we found a significant increase in 2nd-hour 8-OHdG values after OGTT. This indicates that increases in oxidation products until the 2nd hour following glucose loading may considerably contribute to the formation of OH

radicals, thereby leading to DNA damage and 8-OHdG elevation with the administration of OGTT. The increase in 8-OHdG observed at 2 hours was alleviated with the administration of interventions. The decrease in the amount of 8-OHdG with the administration of carnosine may be due to the antioxidant property of carnosine, which has been reported in previous studies that carnosine is a scavenger of superoxide, hydroxyl and nitrogen radicals as well as singlet oxygen molecules.[29] Although these results indicated significant efficacy for dam interventions, significantly elevated 8-OHdG levels compared to baseline were observed in fetal samples. This situation suggests that the amelioration of OGTT-related oxidative effects on DNA may not be present in fetuses. The beneficial effects are also evident in the evaluation of maternal tissue samples, which demonstrate a drastic decrease in 8-OHdG concentrations (brain, liver and pancreatic tissues) among recipients of the intervention. Most tissue results also appear to favour the chromium & carnosine intervention in terms of 8-OHdG levels when compared to chromium or carnosine alone (except for renal and ovarian samples). This may be due to the synergistic effect of chromium and carnosine on glucose metabolism and oxidative stress.

## CONCLUSION

In conclusion, our study suggests that the administration of chromium picolinate, insulin, and L-carnosine may alleviate DM-related adverse effects (DNA oxidation and oxidative parameters) and ameliorate the severity of oxidative stress triggered by OGTT in a rat model of STZ-induced DM. The application of these interventions, either alone or in combination, before OGTT may be protective, limiting or partially reparative against GDM complications and the oxidative injury caused by OGTT.

### Ethical approval

All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Approval was obtained from the Animal Care and Use Committee of Fırat University (2016/4).

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by the Firat University Scientific Research Projects Unit (project no: TF.16.20).

Authorship Contributions: MY designed, planned and conducted the study. All authors contributed to data interpretation and writing of the paper. MY, SA and TK wrote the first draft and MY prepared the final draft, which was approved by all authors.

## REFERENCES

1. Saeedi M, Cao Y, Fadl H, Gustafson H, Simmons D. Increasing prevalence of gestational diabetes mellitus when implementing the IADPSG criteria: A systematic review and meta-analysis. Diabetes Res Clin Pract. 2021;172:108642.
2. Pihelgas A, Prior A, Ospina MB, Jain V. Decreasing Insulin Requirements in Pregnancy With Pre-existing or Gestational Diabetes: Management Practices Across Canada. J Obstet Gynaecol Can. 2021;43:1180-1183.e1
3. Behboudi-Gandevani S, Bidhendi-Yarandi R, Panahi MH, Vaismoradi M. The effect of mild gestational diabetes mellitus treatment on adverse pregnancy outcomes: A systemic review and meta-analysis. Front Endocrinol (Lausanne). 2021;12:271.
4. Association AD. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes2018. Diabetes Care. 2018;41(Supplement 1):S13-S27.
5. Pathirana MM, Lassi ZS, Ali A, Arstall MA, Roberts CT, Andraweera PH. Association between metabolic syndrome and gestational diabetes mellitus in women and their children: a systematic review and meta-analysis. Endocrine. 2021;71(2):310-320.
6. Yaribeygi H, Atkin SL, Sahebkar A. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. J Cell Physiol. 2019;234(2):1300-1312.
7. De Mendonça ELSS, Fragoso MBT, De Oliveira JM, Xavier JA, Goulart MOF, De Oliveira ACM. Gestational Diabetes Mellitus: The Crosslink among Inflammation, Nitroxidative Stress, Intestinal Microbiota and Alternative Therapies. Antioxidants. 2022;11(1):129.
8. Artioli GG, Sale C, Jones RL. Carnosine in health and disease. Eur J Sport Sci. 2019;19(1):30-39.
9. Hue J-J, Kim J-S, Kim J-H, et al. Anti-glycemic effect of L-carnosine in streptozotocin-induced diabetic

mice. Korean J Vet Res. 2010;50(2):105-111.

10. Houjeghani S, Kheirouri S, Faraji E, Jafarabadi MA. L-Carnosine supplementation attenuated fasting glucose, triglycerides, advanced glycation end products, and tumor necrosis factorα levels in patients with type 2 diabetes: a double-blind placebo-controlled randomized clinical trial. Nutr Res. 2018;49:96-106.
11. Ghodsi R, Kheirouri S. Carnosine and advanced glycation end products: a systematic review. Amino Acids. 2018;50(9):1177-1186.
12. Khodavirdipour A, Haddadi F, Keshavarzi S. Chromium supplementation; negotiation with diabetes mellitus, hyperlipidemia and depression. J Diabetes Metab Disord. 2020;19(1):585-595.
13. Talab AT, Abdollahzad H, Nachvak SM, et al. Effects of chromium picolinate supplementation on cardiometabolic biomarkers in patients with type 2 diabetes mellitus: A randomized clinical trial. Clin Nutr Res. 2020;9(2):97-106.
14. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004;37(2):112-119.
15. Schaberg E, Jordan W, Kuyatt BL. Artificial intelligence in automated classification of rat vaginal smear cells. Anal Quant Cytol Histol. 1992;14(6):446-450.
16. Mitchell S, Shaw D. The worldwide epidemic of female obesity. Best Pract Res Clin Obstet Gynaecol. 2015;29(3):289-299.
17. Saravanan P, Magee LA, Banerjee A, et al. Gestational diabetes: opportunities for improving maternal and child health. Lancet Diabetes Endocrinol. 2020;8(9):793-800.
18. Singh R, Farooq SA, Mannan A, et al. Animal models of diabetic microvascular complications: Relevance to clinical features. Biomed Pharmacother. 2022;145:112305.
19. Gröschl M. The physiological role of hormones in saliva. Bioessays. 2009;31(8):843-852.
20. Chera S, Baronnier D, Ghila L, et al. Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers. Nature. 2014;514(7523):503-507.
21. Kooshki F, Tutunchi H, Vajdi M, et al. A Comprehensive insight into the effect of chromium supplementation on oxidative stress indices in diabetes mellitus: A systematic review. Clin Exp Pharmacol Physiol. 2021;48(3):291-309.
22. Shinde UA, Sharma G, Xu YJ, Dhalla NS, Goyal RK. Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus. J Trace Elem Med Biol. 2004;18(1):23-32.
23. Sahin K, Onderci M, Tuzcu M, et al. Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat. Metabolism. 2007;56(9):1233-1240.
24. Geyikli İ, Bayıl S. Kromun İnsülin Duyarlılığı İle İlişkisi. Gaziantep Tıp Dergisi. 2008;14:59-63.
25. Dos Santos JM, Tewari S, Mendes RH. The role of oxidative stress in the development of diabetes mellitus and its complications. J Diabetes Res. 2019;2019:4189813.
26. Wang J, Wang H. Oxidative stress in pancreatic beta cell regeneration. Oxid Med Cell Longev. 2017;2017:1930261..
27. Korkmaz KS, Butuner BD, Roggenbuck D. Detection of 8-OHdG as a diagnostic biomarker. J Lab Precis Med. 2018;3:95.
28. Cadet J, Douki T, Gasparutto D, Ravanat J-L. Oxidative damage to DNA: formation, measurement and biochemical features. Mutat Res. 2003;531(1-2):5-23.
29. Alkadi H. A review on free radicals and antioxidants. Disord Drug Targets. 2020;20(1):16-26.