

Original Research Article

Dapagliflozin and metformin combination therapy modulates lipid metabolism and oxidative stress in a sex-dependent manner

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Abstract

Purpose: To investigate the effect of dapagliflozin combined with metformin on metabolic, oxidative and endothelial factors, with a particular focus on sex-specific variation.

Methods: A total of 32 patients (18 males, 14 females) were recruited from Al-Salam Teaching Hospital in Mosul city, Iraq. The patients received metformin monotherapy (1000 mg/day) from October 2024 to April 2025. Thereafter, dapagliflozin (10 mg/day) was administered as an adjuvant therapy for 8 weeks. Serum samples were collected before and 8 weeks after dapagliflozin therapy, and HbA1c, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), malondialdehyde (MDA), total antioxidant status (TAS), vascular and intercellular cell adhesion molecule 1 (VCAM-1 and ICAM-1) were measured using enzyme-linked immunosorbent assay (ELISA).

Results: Dapagliflozin therapy significantly reduced glycated haemoglobin (HbA1c), cholesterol, LDL, and atherogenic index, with a significant reduction of TG in males compared to females ($p < 0.05$). Serum MDA was significantly reduced in both sexes ($p < 0.01$), while TAS was significantly higher in females compared to males ($p < 0.001$). Furthermore, VCAM-1 and ICAM-1 did not significantly change in either sex.

Conclusion: Treatment with dapagliflozin following metformin therapy induced favourable hypolipidemic and oxidative benefits with sex-related differences, which highlighted the importance of considering biological sex when evaluating antidiabetic agents.

Keywords: Dapagliflozin, Lipid profile, Metformin, Oxidative stress, Type II diabetes

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INTRODUCTION

Type II diabetes mellitus (DM) is a metabolic disorder characterized by reduced tissue responsiveness to insulin together with relative impairment in insulin secretion. Patients with DM suffer from dyslipidaemia with enhanced oxidative stress and endothelial damage. In addition to glycaemia control, treatment is

directed towards controlling accelerated atherosclerosis and improvement in vascular function [1]. Dapagliflozin inhibits glucose reabsorption by enhancing glucose excretion. It encourages lipolysis with subsequent enhancement in ketogenesis. Ketone bodies play important roles in metabolism as they enhance mitochondrial biogenesis, mitigate oxidative damage and potential cellular stress [2].

Metformin (a biguanide) induces a modest energy shift in hepatic metabolism, inhibits gluconeogenesis by mitochondrial inhibition with subsequent activation of adenosine monophosphate-activated protein kinase (AMPK) and fatty acid oxidation [3]. Such a mechanism leads to a small physiological rise in ketone body production without significant effect on glucagon and insulin secretion. Accordingly, combined treatment of dapagliflozin with metformin may offer a complementary additive mechanism to improve oxidative status and overall vascular function. It has been suggested that sex-based biological variation may affect metabolic and oxidative status with collective vascular response in type II diabetes. Females showed more antioxidant oestrogen-mediated protective mechanisms with enhanced endothelial nitric oxide protection. On the other hand, males have more visceral adiposity associated with higher triglyceride levels and basal oxidative stress, which may result in diminished response to antioxidant therapy [4].

Vascular cell adhesion molecule 1 (VCAM-1) is a key early indicator of endothelial injury, which is an important step in the development of visible atherosclerotic plaques. By contrast, intercellular cell adhesion molecule-1 (ICAM-1) maintains lymphocyte adhesion during the latter inflammatory steps. Thus, elevated levels of VCAM- 1 and ICAM- 1 reflect cardiovascular risk in patients with diabetes, and serve as useful, non-invasive indicators of vascular inflammation [5].

This current study investigated the effects of dapagliflozin in combination with metformin on glucose levels, lipid profiles such as LDL, HDL, TG, and AI; oxidative markers such as MDA and TAS; and endothelial inflammatory markers such as VCAM-1 and ICAM-1 in both males and females with type II diabetes mellitus.

METHODS

Study settings

A total of 32 diabetic patients (18 males and 14 females) aged 31 to 71 years were recruited from Al-Salam Teaching Hospital in Mosul city, Iraq (<https://maps.app.goo.gl/znMKs6oA59D1h7d86>).

Selection criteria

Inclusion criteria

Diabetic patients who have received metformin monotherapy for 6 months from October 2025 to April 2025.

Exclusion criteria

Pregnant and breastfeeding diabetics, patients who utilized numerous medications, presence of other disorders other than diabetes, and history of substance abuse.

Treatment

All enrolled patients received oral dapagliflozin 10 mg/day (Divinus, Hikma® Jordan) as an adjuvant therapy after oral metformin monotherapy 1000 mg/day (Glucophage®, MERCK, Germany) for at least 6 months. Treatment with dapagliflozin lasted for 8 weeks. Written informed consent was provided by each patient to take part in this research.

Ethical consideration

Ethical approval was obtained from the Medical Research and Ethics Committee of the College of Pharmacy at the University of Mosul, Iraq (CCMRE phA-25-13), and complied with the Declaration of Helsinki [6].

Evaluation of parameters/indices

Serum TC and TG levels

Venous blood (5 mL) was collected, centrifuged at 3000 rpm for 15 min, and serum TC and TG levels were measured with the total cholesterol and triglycerides BIOLAB kits (France) following the enzymatic colorimetric method. All measurements were done in triplicate, and according to the manufacturer's instructions, before dapagliflozin administration and 8 weeks after dapagliflozin treatment [7].

Serum HDL

Venous blood (5 mL) was collected, centrifuged at 3000 rpm for 15 min, and levels of HDL-C were measured with the HDL-C BIOLABO kit (France) following the precipitation technique. Determination was done in triplicate before dapagliflozin administration and 8 weeks after dapagliflozin treatment.

Serum LDL

Venous blood (5 mL) was collected, centrifuged at 3000 rpm for 15 min and serum LDL-C and Atherogenic Index (AI) were calculated using Friedewald Equations 1 and 2.

$LDL-C(mmol/L) = (TC - HDL-C - TG) / 2.2 \dots (1)$ [8]

AI = TC/HDL (2) [9].

Glycated haemoglobin (HbA1c)

Glycated haemoglobin (HbA1c) was measured using the HbA1c ELISA Kit (Antibodies-online) [10].

Total antioxidant status (TAS)

Total antioxidant status (TAS) was measured using the kit provided by Elabscience Laboratories (China) following previous method [11].

Serum MDA, VCAM-1 and ICAM-1

Serum MDA, VCAM-1, and ICAM-1 were measured using a kit provided by Elabscience Laboratories (China) following the thiobarbituric acid (TBA) assay [12].

Statistical analysis

Data collected were analysed using GraphPad Prism (Ver. 11.5, USA). Data were expressed as

mean $\pm$ standard deviation (SD). A two-sample t-test was used for comparing 2 groups (males and females), while paired t-test was used for the same group before and after dapagliflozin administration. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical data

There was a significant difference in the duration of diabetes mellitus between males and females ($p < 0.001$; Table 1).

Effect of dapagliflozin on TG and LDL levels

Treatment with dapagliflozin following 6 months of metformin administration resulted in significantly lower serum TG (A) and LDL (D) in males compared to females (B and E, respectively; $p < 0.05$). Furthermore, males exhibited greater mean reduction in TG (C) and LDL (F) compared to females using unpaired analysis ($p < 0.05$).

Table 1: Clinical data of patients (mean $\pm$ SD)

Parameter	Males (n=18)	Females (n=14)	P-value
Age (years)	48.1 $\pm$ 9.8	50.6 $\pm$ 10.6	0.48
BMI (kg/m ²)	26.9 $\pm$ 2.1	27.7 $\pm$ 1.5	0.22
HbA1c (%)	8.02 $\pm$ 0.91	7.76 $\pm$ 0.94	0.44
Duration of diabetes (months)	8.6 $\pm$ 1.0	9.9 $\pm$ 0.7	< 0.001

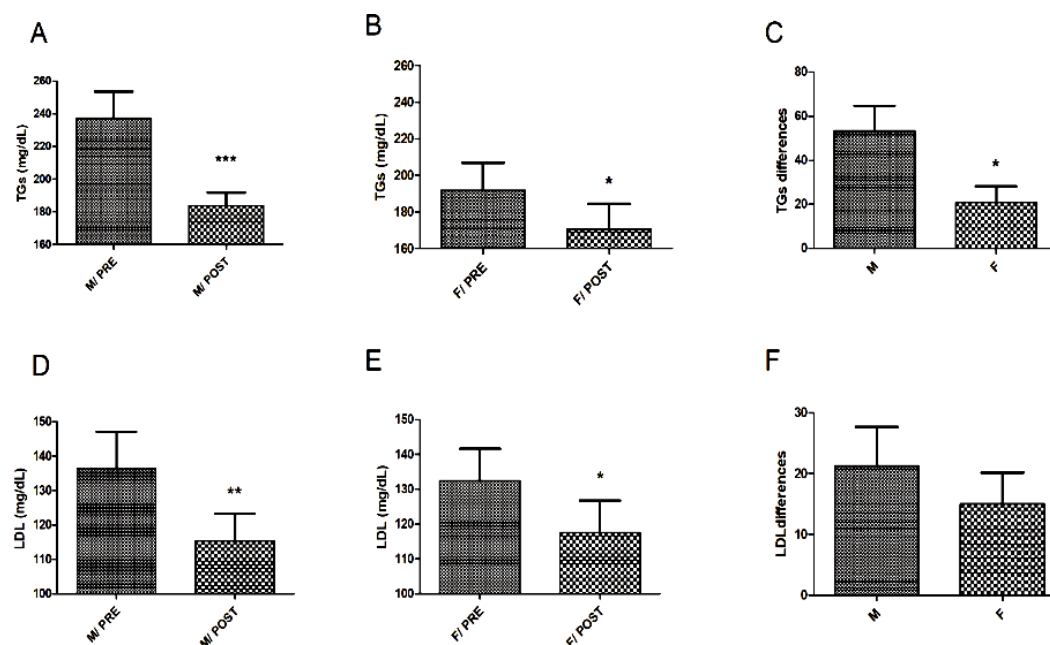


Figure 1: Effects of dapagliflozin on TG and LDL levels. Values are shown as means $\pm$ standard deviations (SD). Males before treatment (M/PRE), males after treatment (M/POST), females before treatment (F/PRE), and females after treatment (F/POST). * $P < 0.05$, ** $p < 0.01$ *** $p < 0.001$ vs before treatment

Effect of dapagliflozin on MDA and TAS levels

Treatment with dapagliflozin following 6 months of metformin administration resulted in significantly lower MDA levels in males (A) and females (B; $p < 0.05$), with no significant change in MDA difference (pre and post) between male and female (C; $p > 0.05$). Total antioxidant status (TAS) was significantly higher in females (E) compared to males (D; $p < 0.05$). Unpaired t-test showed a significant difference in TAS (pre and post) between males and females (F; $p < 0.05$; Figure 2).

Effect of dapagliflozin on VCAM-1 and ICAM-1 levels

Treatment with dapagliflozin following 6 months of metformin administration resulted in no significant change in serum VCAM-1 and ICAM-1 in males (A, D) and females (B, E). There was no significant variation in mean difference between pre and post-values in males and females (C, F; Figure 3).

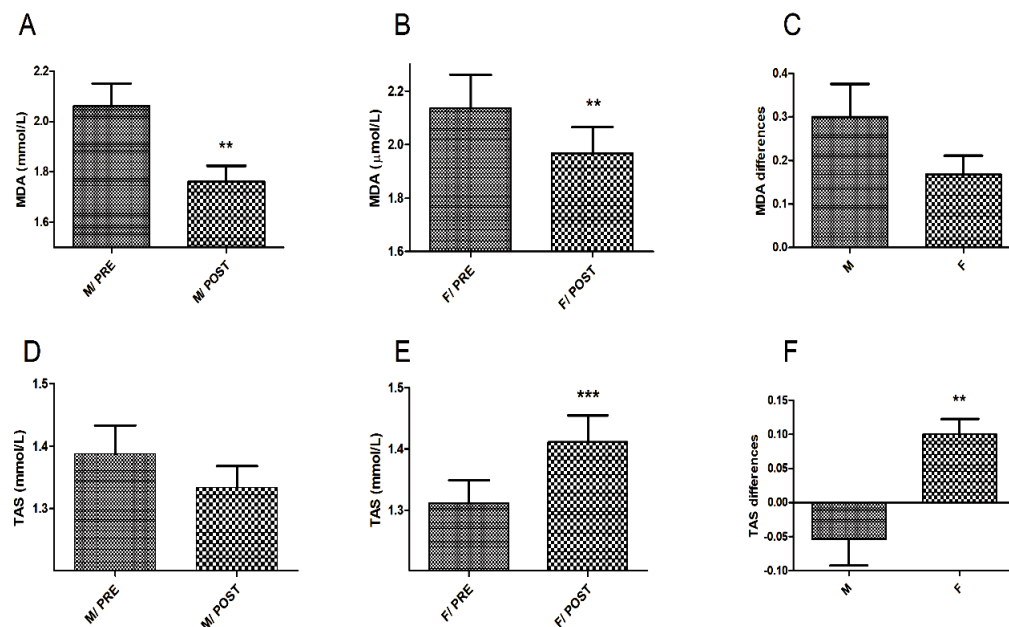


Figure 2: Effects of dapagliflozin on MDA and TAS levels in males and females. Values are presented as means $\pm$ standard deviations (SD). Males before treatment (M/PRE), males after treatment (M/POST), females before treatment (F/PRE), and females after treatment (F/POST). * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs before treatment

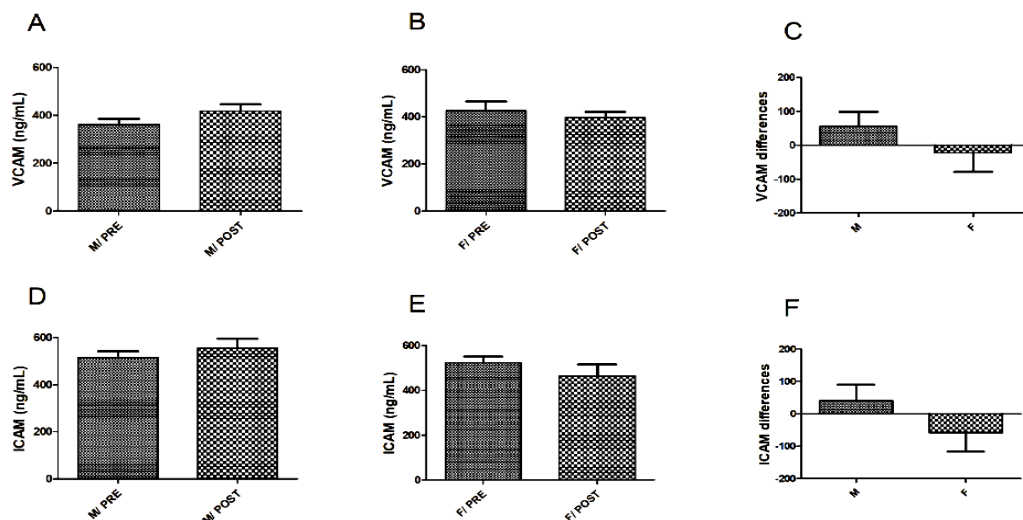


Figure 3: Effects of dapagliflozin on VCAM-1 and ICAM-1 levels. Results are expressed as mean $\pm$ standard deviation (SD). Males before treatment (M/PRE), males after treatment (M/POST), females before treatment (F/PRE), and females after treatment (F/POST)

Table 2: Effect of dapagliflozin on HbA1c and AI (mean $\pm$ SD)

Parameter	Gender	Metformin	Metformin+ dapagliflozin	P-value
Hb1C (%)	Female	7.76 $\pm$ 0.94	6.34 $\pm$ 0.80	< 0.0001***
Hb1C (%)	Male	8.02 $\pm$ 0.91	6.33 $\pm$ 0.74	< 0.0001***
AI (%)	Female	5.79 $\pm$ 1.63	5.16 $\pm$ 1.76	0.0264*
AI (%)	Male	6.16 $\pm$ 1.28	4.96 $\pm$ 1.12	0.0041**

Effect of dapagliflozin on HbA1c and AI

Treatment with dapagliflozin following 6 months of metformin administration resulted in significantly lower HbA1c compared to metformin alone ($p < 0.0001$). Furthermore, AI was significantly lower in males compared to females ($p < 0.01$; Table 2).

DISCUSSION

The present data clearly showed that dapagliflozin and metformin combination therapy significantly reduced glycated haemoglobin (HbA1c), low-density lipoprotein, total cholesterol and triglycerides. However, based on gender, the lipid profile significantly improved in male patients. By contrast, MDA was decreased similarly in both male and female patients, while total antioxidant status significantly improved only in female patients. There was a mild reduction in endothelial inflammatory markers (VCAM-1 and ICAM-1) in female patients. These results indicated that dapagliflozin favourably affected glucose and lipid metabolism, MDA and TAS with variable effects in both male and female. Dapagliflozin influenced metabolism by enhancing glucose excretion, increasing fatty acid oxidation, decreasing dependence on glucose metabolism and increasing β -oxidation and ketogenesis in the liver.

Studies have shown that enhanced ketone body generation, especially β -hydroxybutyrate, resulted in improved mitochondrial potential with a reduction in reactive oxygen species generation [13]. Increased lipolysis enhances triglyceride concentration due to higher fatty acid utilization in the liver. Dapagliflozin inhibits SGLT2 in the kidney proximal tubules, leading to significant urinary glucose excretion (typically 60-80 g/day), creating a perceived energy deficit. To compensate for this deficit, the body increases the breakdown of fat stores (lipolysis), releasing more free fatty acids into the bloodstream. This result reflects a sustained caloric deficit that promotes weight loss, particularly visceral and ectopic fat.

Reduction in adipose tissue mass improves lipid metabolism and insulin sensitivity, leading to favorable changes in lipid profiles, including

decreased LDL-C and increased HDL-C [14]. On the other hand, metformin induces a mild increase in ketone bodies by partial reversible inhibition of complex I of liver mitochondria, which results in inhibition of hepatic ATP-sensitive gluconeogenesis with consequent AMPK activation. Inhibition of mitochondrial complex I lowers mitochondrial membrane potential and promotes fatty acid entry into the mitochondria for oxidation. Similarly, decreased mitochondrial potential activates fatty acid translocation into muscles, and metformin may result in mild ketone body generation within physiological levels. Inhibition of liver mitochondrial complex I causes reduction in triglyceride levels. Switching substrate from glucose to lipid improved insulin resistance and reduced lipid accumulation [15]. Addition of dapagliflozin to metformin enhances mitochondrial function by increasing β -hydroxybutyrate formation, thus improving the cellular redox environment without significant pathological ketoacidosis. This result reflects sex-related differences in redox regulation and mitochondrial function [16].

The significant increase in TAS among females compared to males may underlie differences in hormonal regulation of antioxidant mechanisms. Estrogen increases the synthesis and potency of antioxidant defense mechanisms like catalase and superoxide dismutase, with additional improvement in mitochondrial electron transport chain. However, females generally have enhanced adiponectin levels and more subcutaneous adipose tissue than visceral adipose tissue, which improves insulin sensitivity and creates a favourable oxidative environment. By contrast, males have higher visceral adipose tissue accompanied by more oxidative stress, which may ultimately lessen the changes observed in TAS [17]. Visceral fat participates in metabolism and produces higher levels of interleukin-6 and TNF- α , which in turn activate mitochondrial ROS generation. The lower TAS gain in males may be explained by the persistent oxidative burden, which impaired antioxidant response to medication [18].

The SGLT2 inhibitors, like dapagliflozin, have direct antioxidant action (in addition to being euglycaemic) due to inhibition of ROS generation and inflammatory biomarkers in the tubular,

cardiac and endothelial cells. On the other hand, elevated glucose levels activate pro-oxidant pathways, boost formation of ROS in the mitochondria (via the electron transport chain), and weaken antioxidant defenses. Furthermore, dapagliflozin reduced blood glucose levels by promoting urine glucose excretion through SGLT2 inhibition in the proximal tubule. As a result, there is less substrate available for detrimental oxidative pathways mediated by glucose.

These effects play an important role in the anti-inflammatory protective effect of SGLT2 inhibitors in addition to their glycaemic and lipid control. This study suggested that dapagliflozin has metabolic effects by different mechanisms in males and females instead of a single universal mechanism. In females, the protective effect of dapagliflozin is through enhancement of TAS activity, which complements the protective effect induced by the female sex hormone.

Although this study did not examine the exact role of oestrogen, such an antioxidant-associated role may be important for endothelial and overall cardiovascular health. In male patients, the treatment showed significant reduction in total triglycerides, LDL, total cholesterol and overall atherogenic index, suggesting that the drug reduced cardiovascular events in men probably through improved adipose tissue metabolism. The effect in both sexes suggests a similar downstream effect on mitochondrial respiration with overall reduction in oxidative status. The results of the current study agree with previous trials which demonstrated the protective effect of SGLT2 inhibitors in both males and females [19].

Limitations of the study

The primary limitation of this study is the relatively small sample size of patients and the short duration of follow-up. The study also did not include ketone body estimation, which made the results inconclusive.

CONCLUSION

Treatment with dapagliflozin following 6 months of metformin administration significantly reduced HbA1c, total cholesterol, LDL, triglyceride levels and atherogenic index, with significant improvement in HDL level, especially in male patients. Furthermore, dapagliflozin significantly reduced MDA and increased TAS, particularly in females, suggesting sex-related metabolic action, with no significant change in endothelial factors (VCAM-1 and ICAM-1) between males and females.

DECLARATIONS

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Ethical approval

It was obtained from the Medical Research and Ethics Committee of the College of Pharmacy at the University of Mosul, Iraq (CCMRE phA-25-13).

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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