Iranian Journal of Pharmaceutical Sciences
2023: 19 (2): 176- 187
https://journals.sbmu.ac.ir/IJPS

Original Article

SGLT2 Inhibitor-Induced Ketonemia and Its Related Metabolic and Anti-Inflammatory Impacts in Adult Patients with Type 2 Diabetes

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Abstract

Ketosis and attenuation of low-grade inflammation have been reported after consumption of sodium-glucose cotransporter-2 (SGLT2) inhibitors, but their mechanism and metabolic consequences have not been clearly defined. This clinical trial study aimed to assess SGLT2 inhibitor-induced ketonemia and its association with metabolic profiles and interleukin 6 (IL-6) levels in patients with type 2 diabetes mellitus (T2D). Biochemical variables, including fasting blood sugar (FBS), beta-hydroxybutyrate (BOBH), uric acid (UA), phosphate, insulin, glucagon, adrenocorticotropic hormone (ACTH), cortisol, and IL-6, were measured in 77 patients with T2D before and one month after treatment with empagliflozin (38 patients) and compared with a matched control group (39 patients). The patients in the intervention group received metformin and 10 mg empagliflozin, while those in the control group received metformin alone. The results revealed a significant decrease in IL-6, UA, FBS, glycated hemoglobin (HbA_1C), body mass index (BMI), blood pressure, and the homeostasis model assessment–estimated insulin resistance (HOMA-IR) index after one month in the intervention group. However, the BOHB concentrations were significantly higher only in the empagliflozin recipients (p=0.040), with no symptoms or signs of ketoacidosis. Despite the increase in the concentration of BOHB, no significant difference was observed regarding insulin, glucagon, ACTH, and cortisol levels before and after taking empagliflozin. Besides, both groups observed a positive correlation between BOHB and UA levels (r=0.680, p=0.0001 in the empagliflozin group and r=0.646, p=0.002 in controls). Nevertheless, HOMA-IR and UA concentrations decreased significantly only in the intervention group (p=0.020 and p=0.011, respectively). At the end of the study, IL-6 levels showed a significant reduction within the groups and more in the intervention group compared to the controls. IL-6 levels were positively correlated to HOMA-IR (r=0.401, p=0.013) in the intervention group, but no relationship was detected between IL-6 and BMI, BOHB, UA, and insulin concentrations in the two groups. Based on the results, SGLT2 inhibitor-associated ketonemia was independent of changes in the insulin, glucagon, or cortisol levels. However, low-grade ketosis was associated with improved insulin sensitivity. The results also suggest that SGLT2 inhibitors possess anti-inflammatory activity, possibly mediated by their ability to reduce insulin resistance.

Keywords: Beta-hydroxybutyrate; Empagliflozin; Homeostasis model assessment–estimated insulin resistance index; Interleukin 6; Type 2 diabetes; Uric acid.

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Cite this article as: Karimi F, Arya A, SGLT2 Inhibitor-Induced Ketonemia and Its Related Metabolic and Anti-Inflammatory Impacts in Adult Patients with Type 2 Diabetes, Iran. J. Pharm. Sci., 2023, 19 (2): 176- 187.
DOI: https://doi.org/10.22037/ijps.v19i2.42072

1. Introduction

Type 2 Diabetes (T2D) is one of the most common metabolic disorders in the world, and its treatment with particular attention to the pathophysiological aspects of the disease is of

particular importance. Pharmacological inhibition of the sodium-glucose cotransporter-2 (SGLT2) is an attractive and novel approach to lowering plasma glucose by inhibiting renal glucose reabsorption in the proximal tubule [1]. Despite the evidence on the clinical benefits of SGLT2 inhibitors in diabetic and nondiabetic patients, the mechanisms underlying their effects are still debated [2, 3]. Nevertheless, euglycemic diabetic ketoacidosis (EDKA) has been mentioned as one of their side effects unrelated to the duration of exposure [4]. The proposed mechanism of SGLT2 inhibitors-induced EDKA implicates decreased insulin secretion and plasma glucose concentration secondary to glucosuria, leading to increased glucagon release and upregulation of lipolysis [5].

The ketogenic properties of SGLT2 inhibitors have raised new questions about their effects on insulin, glucagon, and the hypothalamic-pituitary-adrenal axis. Previous studies have yielded conflicting results regarding the impact of SGLT2 inhibitors on glucagon or insulin secretion [6-9]. Moreover, few studies have been performed regarding the effects of gliflozins on the adrenal axis [10, 11]. On the other hand, an increase in ketone bodies has been shown to improve insulin resistance (IR) [12]. In addition, low-grade inflammation (LGI), as an underlying mechanism of the whole spectrum of vascular complications of diabetes, has been suggested to be attenuated by SGLT2 inhibitors, mostly in animal models [13, 14]. Some studies are available on humans in this regard, and all of them have compared the patients treated with SGLT2 inhibitors to those who have received sulphonylureas, but research about comparison with other drugs is scant [15, 16]. Another important outcome of SGLT2 inhibitors is their uricosuric effect [17, 18]. UA is independently related to IR in nondiabetic and diabetic people [19]. Therefore, UA reduction by SGLT2 inhibitors may positively affect cardiovascular and renal functions [20, 21]. Still, there are unanswered questions about the mechanisms of action of SGLT2 inhibitors. The present study aimed to provide more information about various metabolic effects of SGLT2 inhibitors. Especially the impact of SGLT2 inhibitors on interleukin 6 (IL-6) levels as a marker of LGI compared to metformin. We hypothesized that (1) SGLT2 inhibitors-induced ketonemia would be associated with hormonal changes similar to other cases of diabetic ketoacidosis (DKA) and (2) their proposed anti-inflammatory effects would be dependent on their effects on insulin levels.

2. Materials and Methods

2.1. Subjects

The study participants were selected from the patients with T2D who attended Shahid Motahari Clinic affiliated with Shiraz University of Medical Sciences in 2020-2021. The inclusion criteria were 30-75 years, uncontrolled blood sugar (HbA1c ≥7%) on metformin for at least three months, and not taking SGLT2 inhibitor. The exclusion criteria were suffering from type 1 diabetes mellitus or using insulin, lactation, pregnancy, alcohol consumption, glomerular filtration rate (GFR) ≤30 mL/min, liver enzymes more than three times the normal level, malignancy, hospitalization history in the past six months, hypersensitivity to empagliflozin, experiencing diabetic ketoacidosis, and limb amputation due to diabetes.

The study was conducted on 77 participants (38 in the intervention group and 39 age-, sex-, and BMI-matched controls). The participants were 33-74 years old, with a mean age of 54.34±9.73 years. The mean duration of diabetes was 4.03±3.31 years, ranging from 1-10 years. The participants were also matched for the duration of diabetes and HbA1c. The patients who took metformin were randomly assigned to the intervention or the control group to compare the empagliflozin users and non-users for the clinical and laboratory data. In the intervention group, 10 mg of empagliflozin (Abidi, Iran, Batch number: 21012) was added to metformin for four weeks, while those in the control group received metformin alone. The participants in the two groups were matched based on different doses of metformin, which ranged from 1000 to 2000 milligrams daily. Also, 15 persons in the control group and 21 in the empagliflozin group received statins, 13 in the control group, and 16 in the intervention group received antihypertensive medications.

The review board and local Ethics Committee of Shiraz University of Medical Sciences approved the study protocol. The study was also registered in the Iranian Registry of Clinical Trials (IRCT code: IRCT20210730052022N1, date:15/11/2021). After explaining the research objectives, written informed consent was obtained from the participants.

2.2. Anthropometric Measurements and Laboratory Data

The patient's height, weight, age, blood pressure, hip and waist circumferences, drug history, diabetes duration, history of comorbidities, and previous admissions were recorded. In addition, BMI was calculated as body weight in kilograms divided by height in meters squared. Waist circumference was measured while standing upright with light clothing, using tape halfway between the lowest rib and the top of the iliac crest. Blood samples were obtained early, between 7 a.m. and 9 a.m., after an overnight fast at baseline and the end of the clinical trial. The collected samples were processed immediately and frozen at −70°C until assayed. All the tests were performed at the Endocrinology and Metabolism Research Center of Shiraz University of Medical Sciences. Therein, the serum levels of glucose, phosphorus, UA, creatinine, and beta-hydroxybutyrate (BOHB) (Randox kit) were measured by enzymatic colorimetric assay, using a Dirui autoanalyzer (Dirui, CS-T240, china). Besides, the serum concentration of insulin was measured via immunoradiometric assay (RK-400CT, Hungary), serum cortisol level by radioimmunoassay (RK240-CT, Hungary), and glucagon by enzyme-linked immunosorbent assay (Bioassay technology, E1023Hu, China). Moreover, the adrenocorticotropic hormone (ACTH) plasma concentration was determined by automated fluorescent enzyme-linked immunoassay (Immulite- 2000 Siemens Healthineers, Los Angeles, CA). The serum level of IL-6 was measured by enzyme-linked immunosorbent assay (Demeditec Diagnostics, 24145 Kiel, Germany). IR was also evaluated using the homeostasis model assessment–estimated insulin resistance (HOMA-IR) index according to the following formula: HOMA-IR = [fasting plasma glucose (mg/dL) × fasting plasma insulin (μU/mL] / 405.

2.3. Statistical Analysis

Student's t-test was used to compare the mean anthropometric, biochemical, and hormonal levels with normal distribution. Mann-Whitney test was used if the desired variable did not follow a normal distribution. In addition, significant differences between the baseline and post-treatment levels of the studied parameters in each group were assessed using paired t-test and Wilcoxon signed-rank test in case of variables with and without normal distribution, respectively. The chi-square test was used to compare the categorical variables. Pearson's and Spearman's correlation tests were also used to analyze correlations between the variables with and without normal distribution. All data analyses were conducted using the SPSS 23 software (Chicago. IL, USA), and $p<0.05$ was considered statistically significant.

The local Ethics Committee of Shiraz University of Medical Sciences has approved this study involving humans. The Ethics approval code is IR.SUMS.MED.REC.1400.256. All procedures have been performed per the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. (IRCT code: IRCT20210730052022N1, date:15/11/2021).

3. Results and Discussion

SGLT2 inhibitors are a new class of antidiabetic agents that target the ignored significant role of kidneys in glucose homeostasis. These drugs reduce hyperglycemia by inhibiting the renal reabsorption of glucose [1]. These inhibitors' mode of glucose-lowering is unique since they are independent of beta-cell function and IR. Nonetheless, the observation that they reduce cardiovascular and renal events in diabetic and nondiabetic patients suggests that their benefits cannot be explained only by glucose-lowering effects [22, 23]. The baseline characteristics of the participants regarding the studied parameters have been presented in **Table 1**.

The current study results revealed improved blood pressure, blood sugar, insulin sensitivity, reduced total cholesterol, low-density lipoprotein cholesterol (LDL-C), and weight reduction in the patients with T2D after a one-month treatment with empagliflozin (**Table 2**).

Table 1: Baseline characteristics of the participants in the two groups.

	Empagliflozin (n=38)	**Control (n=39)**	**P value**
Sex	Female: 25 Male: 13	Female: 24 Male: 15	1.000
Age (year)	54.710±9.855	51.600±10.358	0.266
Duration of diabetes (year)	4.026±3.316	3.450±3.103	0.392
Anti-lipid medication	Yes: 21, No: 17	Yes: 15, No: 24	0.828
Antihypertensive edication	Yes:16, No: 22	Yes: 13, No: 26	1.000
Weight (kg)	69.934±11.683	70.650±12.520	0.980
WCF (cm)	93.540±10.367	88.833±8.240	0.175
BMI (kg/m^2)	27.987±3.850	27.361±3.508	0.547
Sys BP (mmHg)	132.500±12.343	129.000±17.517	0.275
Dias BP (mmHg)	82.500±8.601	81.250±7.411	0.631
FBS (mg/dL)	166.684±41.124	161.300±37.055	0.626

HbA_1C (%)	7.702±0.806	7.593±1.145	0.696
BOHB (mmol/L)	0.802±0.416	0.872±0.591	0.993
IL-6 (pg/mL)	6.518±4.053	6.930±3.785	0.617
Creatinine (mg/dL)	0.911±0.205	0.936±0.183	0.532
Phosphate (mg/dL)	4.042±0.591	3.965±0.585	0.638
Uric acid (mg/dL)	5.144±1.459	5.090±1.605	0.896
Insulin (µIU/mL)	9.134±4.608	9.310±4.673	0.676
Glucagon (ng/L)	45.264±37.851	45.307±33.300	0.804
I/G	0.320±0.234	0.336±0.297	0.948
HOMA-IR	3.623±1.725	3.704±1.940	0.871
ACTH (pg/mL)	20.603±11.298	19.720±12.895	0.719
Cortisol (µg/dL)	14.794±4.504	13.790±4.288	0.291
TG (mg/dL)	144.459±50.739	145.412±36.164	0.945
Cholesterol (mg/dL)	161.135±34.766	180.470±43.748	0.086
LDL-C (mg/dL)	90.128±29.651	99.312±38.619	0.353
HDL-C (mg/dL)	41.628±9.239	44.866±9.575	0.267

Data are given as mean ± SD. WCF waist circumference, BMI body mass index, Sys BP systolic blood pressure, Dias BP diastolic blood pressure, FBS fasting blood sugar, HbA1c glycated hemoglobin, BOBH beta-hydroxybutyrate, IL-6 interleukin 6, I/G insulin to glucagon ratio, ACTH adrenocorticotropic hormone, HOMA-IR homeostasis model assessment–estimated insulin resistance (HOMA-IR) index, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol.

Table 2: Comparison of clinical and biochemical parameters at baseline and after one month of empagliflozin treatment.

	Before empagliflozin	**After empagliflozin**	**P value**
Weight (kg)	69.934±11.683	68.723±11.598	0.006
WCF (cm)	93.540±10.367	91.784±9.678	0.008
BMI (kg/m^2)	27.987±3.850	27.512±3.998	0.006
Sys BP (mmHg)	132.500±12.343	125.921±12.673	0.002
Dias BP (mmHg)	82.500±8.601	78.947±8.555	0.013
FBS (mg/dL)	166.684±41.124	152.079±35.667	0.007
HbA_1C (%)	7.702±0.806	7.092±0.936	0.000
BOHB (mmol/L)	0.802±0.416	0.981±0.529	0.040
IL-6 (pg/mL)	6.518±4.053	4.258±2.576	0.000
Creatinine (mg/dL)	0.911±0.205	0.939±0.224	0.197
Phosphate (mg/dL)	4.042±0.591	4.142±0.600	0.423
Uric acid (mg/dL)	5.144±1.459	4.639±1.274	0.011
Insulin (µIU/mL)	9.134±4.608	8.363±4.532	0.258
Glucagon (ng/L)	45.264±37.851	41.657±36.699	0.155
I/G	0.320±0.234	0.307±0.211	0.890
HOMA-IR	3.623±1.725	3.111±2.063	0.020
ACTH (pg/mL)	20.603±11.298	20.73±14.63	0.706
Cortisol (µg/dL)	14.794±4.504	15.07±5.59	0.783
TG (mg/dL)	144.459±50.739	140.805±50.567	0.557
Cholesterol (mg/dL)	161.135±34.766	143.946±30.283	0.001
LDL-C (mg/dL)	89.777±29.811	71.479±26.619	0.001
HDL-C (mg/dL)	41.628±9.239	43.206±8.875	0.228

Data are given as mean ± SD. WCF waist circumference, BMI body mass index, Sys BP systolic blood pressure, Dias BP diastolic blood pressure, FBS fasting blood sugar, HbA1c glycated hemoglobin, BOBH beta-hydroxybutyrate, IL-6 interleukin 6, I/G insulin to glucagon ratio, ACTH adrenocorticotropic hormone, HOMA-IR homeostasis model assessment–estimated insulin resistance (HOMA-IR) index, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density. lipoprotein cholesterol

3.1. Ketogenic Effect

The results also indicated significantly increased BOHB one month after the administration of empagliflozin in the treatment group (p=0.040). Clinical studies have demonstrated that lipolysis and ketogenesis occur following short- and long-term gliflozins consumption [4, 6]. A novel hypothesis has linked low-grade ketonemia to cardio-renal benefits of SGLT2 inhibitors. It has been suggested that exposure to SGLT2 inhibitors leads to a fuel shift from glucose to ketone bodies in cardiomyocytes and renal tubular cells, which produce more energy per oxygen consumption [6, 24]. In line with this hypothesis, Mudaliar et al. recently reported that SGLT2 inhibitor–induced low-grade ketonemia ameliorated retinal hypoxia in persons with diabetes [25]. The previous studies on gliflozins-associated ketoacidosis have not provided a unifying mechanism. Some studies performed on patients with T2D have shown an increase in plasma glucagon following SGLT2 inhibitors administration [27]. However, the resulting increase in plasma glucagon represents a possible concerning side effect and may counteract their efficacy in patients already affected by hyperglucagonemia.

Consequently, the stimulatory impacts of SGLT2 inhibitors on glucagon secretion might result from their glucose-lowering effect [9, 28]. Nevertheless, the current study results disclosed that the mean level of glucagon did not change significantly after the one-month treatment with empagliflozin despite the increased BOHB concentrations and improved glucose control in the intervention group (**Table 2**). In agreement with our results, Capozzi et al. also demonstrated that the effects of SGLT2 inhibition ketoacidosis induction were independent of hyperglucagonemia [29]. In the present study, no relevant complications or clinical symptoms were reported by the participants taking empagliflozin despite the increased levels of BOHB.

Our study also revealed a reduction in the insulin level in the intervention group, but the difference was not statistically significant (**Table 2**). Some reports in the literature indicate a reduction in insulin levels after taking SGLT2 inhibitors [12, 30]. Nevertheless, more recent investigations have concluded that the decreased insulin level results from improved glycemic control [31].

A recent animal study demonstrated that glucose availability, but not the related changes in hormone levels (i.e., insulin to glucagon (I/G) ratio), promoted ketogenesis in response to fasting [32]. However, in the present study, despite the significant reduction of blood glucose, changes in the I/G ratio were not statistically significant (**Table 2**).

3.2. Hypothalamic-Pituitary-Adrenal Axis

In another research on rats, Perry et al. identified the combination of insulinopenia and dehydration as a potential mechanism for ketoacidosis associated with SGLT2 inhibitors. They found that the volume loss secondary to SGLT2 inhibition was associated with increased hypothalamic-pituitary-adrenal axis activity, as reflected by increased ACTH and corticosterone concentrations in healthy and diabetic rats. Supporting this hypothesis, they showed that saline administration could

neutralize the effect of SGLT2 inhibitors on increasing gluconeogenesis without affecting the glucagon level [33]. Nevertheless, scant research has been done in this regard among patients with T2D. The results of the present study revealed no significant changes in the cortisol and ACTH levels after taking empagliflozin (Table 2). These findings agreed with those obtained by Herring [10], but not with those obtained in the study by Higashikawa, which indicated that SGLT2 inhibition reduced ACTH and cortisol concentrations [11]. Thus, further studies in this area must confirm or reject this hypothesis.

3.3. Uricosuric Effect

The study results revealed a significant positive correlation between BOHB and UA levels in both groups. (r=0.680, p=0.0001 in cases and r=0.646, p=0.002 in controls). However, at the end of the study, UA levels decreased only in the empagliflozin-treated group (p=0.011). Additionally, serum creatinine levels remained unchanged (**Table 2**), and there was no correlation between creatinine concentration and UA and BOHB levels.

Recent studies have demonstrated that an increase in the level of ketone bodies led to urate retention and increased UA levels. It has been suggested that ketone bodies compete as a weak acid with urate excretory transporters [34]. The questionable point is the reason for the contradictory findings obtained in the current research, i.e., reduced UA levels in the presence of increased BOHB concentrations. UA is independently related to IR in nondiabetic and diabetic people [35]. Moreover, the current study results also revealed that changes in UA levels were directly correlated with ΔHOM-IR (r=0.460, p=0.004) and Δinsulin (r=0.496, p=0.002) in the empagliflozin recipients but not in controls. Therefore, the reduction in urate concentration might partly be due to the improvement in IR.

Additionally, insulin increases UA and sodium reabsorption in the proximal tubule, but the serum UA concentration decreases in case of glycosuria development. Hence, the UA-lowering effect of SGLT2 inhibitors can be due to the increased urinary excretion of UA secondary to glycosuria [17]. Experimental studies have suggested that luminal glucose possibly increases renal UA excretion via GLUT9 isoform 2 or other transporters [17, 18].

3.4. Insulin Sensitivity

The current study results revealed a significant reduction in HOMA-IR in the empagliflozin group (**Table 2**) but not in the controls (**Table 3**). It may result from improved insulin sensitivity being related to the reduced demand for glucose disposal due to renal glycosuria. Recent studies have investigated the other potential mechanisms for IR improvement by SGLT2 inhibitors in patients with T2D. In this regard, Huttl et al. reported increased adiponectin levels and improved muscular insulin sensitivity following SGLT2 inhibitor administration [35]. In addition, Kullmann et al. conducted a study on prediabetes subjects and concluded that an eight-week treatment with empagliflozin could restore hypothalamic insulin sensitivity. Thus, they introduced SGLT2 inhibitors as the first pharmacological approach to improve brain insulin resistance [36].

Table 3: Comparison of clinical and biochemical parameters at baseline and after one month of the control group.

	At the beginning	After one month	P value
Weight (kg)	70.650±12.520	71.325±12.927	0.322
WCF (cm)	88.833±8.240	89.800±8.787	0.698
BMI (kg/m^2)	27.361±3.508	27.651±3.876	0.272
Sys BP (mmHg)	129.000±17.517	125.000±14.779	0.136
Dias BP (mmHg)	81.250±7.411	79.500±9.017	0.283
FBS (mg/dL)	161.300±37.055	158.000±32.810	0.465
HbA1c (%)	7.593±1.145	7.176±0.959	0.822
Phosphor (mg/dL)	3.965±0.585	4.195±0.625	0.207
Uric acid (mg/dL)	5.090±1.605	5.255±1.513	0.440
Creatinine (mg/dL)	0.936±0.183	0.925±0.181	0.688
BHOB (mmol/L)	0.782±0.591	1.041±0.709	0.059
IL6 (pg/mL)	6.930±3.785	5.845±3.258	0.0001
Insulin (µIU/mL)	9.310±4.673	8.998±4.176	0.475
HOMA-IR	3.704±1.940	3.447±1.534	0.230
Glucagon (ng/L)	45.307±33.300	37.403±30.078	0.363
I/G	0.336±0.297	0.368±0.232	0.972
ACTH (pg/mL)	19.720±12.895	21.978±10.459	0.222
Cortisol (µg/dL)	13.790±4.288	12.658±4.793	0.313
TG (mg/dL)	147.971±44.404	146.613±43.845	0.794
Cholesterol (mg/dL)	170.885±40.136	154.054±30.406	0.004
LDL-C (mg/dL)	99.206±38.203	82.189±28.126	0.009
HDL-C (mg/dL)	43.093±11.425	42.795±8.199	0.488

Data are given as mean ± SD. WCF waist circumference, BMI body mass index, Sys BP systolic blood pressure, Dias BP diastolic blood pressure, FBS fasting blood sugar, HbA1c glycated hemoglobin, BOBH beta-hydroxybutyrate, IL-6 interleukin 6, I/G insulin to glucagon ratio, ACTH adrenocorticotropic hormone, HOMA-IR homeostasis model assessment–estimated insulin resistance (HOMA-IR) index, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol.

3.5. Anti-Inflammatory Effect

We showed that patients who received a one-month treatment with empagliflozin had lower levels of IL-6 (p=0.047), a prototypical marker of LGI, compared to the controls (**Table 4**). While we did not find a relationship between IL-6 and BOHB, UA, or insulin levels in the two groups, we observed a direct correlation between IL-6 and HOMA-IR in the empagliflozin group (r=0.401, p=0.013), supporting a potential pro-inflammatory role for insulin resistance. In this context, pro-inflammatory effects have been attributed to insulin and UA. Besides, ketone bodies have been identified as potent anti-inflammatory and anti-oxidative molecules [13]. Previous studies indicate that inflammation

plays a causal role in the development of IR [37]. Hence, SGLT2 inhibitors might attenuate LGI through their ability to reduce IR, UA, and insulin levels and increase ketone body concentrations in the circulation.

Consequently, the cardiovascular and renal benefits of SGLT2 inhibitors have been attributed to their anti-inflammatory effects [13, 26]. In line with our results, a recent study in patients treated with sulphonylurea demonstrated a link between the increase in BOHB and decrease in insulin levels and attenuation of LGI after initiation of SGLT2 inhibitor [16]. Our results extend these data to metformin-treated patients.

Table 4: Comparison of clinical and biochemical parameters of the two groups after one month.

	Empagliflozin	**Control**	**P value**
Weight (kg)	68.723±11.598	71.325±12.927	0.502
WCF (cm)	91.784±9.678	89.800±8.787	0.449
BMI (kg/m^2)	27.512±3.998	27.651±3.876	0.900
Sys BP (mmHg)	125.921±12.673	125.000±14.779	0.777
Dias BP (mmHg)	78.947±8.555	79.500±9.017	0.863
FBS (mg/dL)	152.079±35.667	158.000±32.810	0.423
HbA1c (%)	7.092±0.936	7.176±0.959	0.623
Phosphor (mg/dL)	4.142±0.600	4.195±0.625	0.754
Uric acid (mg/dL)	4.258±2.576	5.255±1.513	0.128
Creatinine (mg/dL)	0.939±0.224	0.925±0.181	0.902
BHOB (mmol/	0.981±0.529	1.041±0.709	0.762
IL6 (pg/mL)	4.639±1.274	5.845±3.258	0.047
Insulin (µIU/mL)	8.363±4.532	8.998±4.176	0.466
HOMA-IR	41.657±36.699	3.447±1.534	0.150
Glucagon (ng/L)	0.307±0.211	37.403±30.078	0.792
I/G	3.111±2.063	0.368±0.232	0.336
ACTH (pg/mL)	20.73±14.63	21.978±10.459	0.252
Cortisol (µg/dL)	15.07±5.59	12.658±4.793	0.108
TG (mg/dL)	140.805±50.567	146.613±43.845	0.584
Cholesterol (mg/dL)	143.946±30.283	154.054±30.406	0.156
LDL-C (mg/dL)	71.479±26.619	82.189±28.126	0.108
HDL-C (mg/dL)	43.206±8.875	42.795±8.199	0.833

Data are given as mean ± SD. WCF waist circumference, BMI body mass index, Sys BP systolic blood pressure, Dias BP diastolic blood pressure, FBS fasting blood sugar, HbA1c glycated hemoglobin, BOBH beta-hydroxybutyrate, IL-6 interleukin 6, I/G insulin to glucagon ratio, ACTH adrenocorticotropic hormone, HOMA-IR homeostasis model assessment–estimated insulin resistance (HOMA-IR) index, TG triglyceride, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol.

3.6. Serum Phosphate Change

Finally, in contrast to the previous reports about the increase in serum phosphate levels after SGLT2 inhibitor administration, the current study results did not show any significant changes in phosphate levels after four weeks of treatment with empagliflozin (**Table 2**). in this context and in agreement, Rau et al. disclosed that empagliflozin treatment of patients with T2D transiently increased the serum phosphate, parathyroid hormone, and fibroblast growth factor-23 and decreased 1,25-dihydroxy vitamin D after three days, while no significant difference was recorded in these parameters after three months [38]. Therefore, the reported increase in phosphate level in the previous studies might reflect a temporal increase in sodium-driven phosphate reabsorption in the proximal tubule caused by SGLT2 inhibition. However, further investigations are required to determine the interaction between SGLT2 inhibitors and phosphate homeostasis.

4. Conclusion

The current study findings revealed increased BOHB concentrations, reduced UA levels, and improved IR in addition to glucose, blood pressure, and weight reduction after a four-week empagliflozin administration. The results showed no significant differences between the two groups regarding glucagon, insulin, ACTH, cortisol, and phosphate levels after one-month consumption of empagliflozin. Moreover, a direct correlation was observed among the BOHB and UA concentrations. We also showed that SGLT2 inhibitor treatment attenuated LGI, as shown by lower levels of IL-6, an effect possibly mediated by decreased IR. In addition, a direct relationship was detected between IL-6 and HOMA-IR. However, if the activity of SGLT2 inhibitors against LGI is confirmed, this class of drugs might be used for a more extensive range of disorders. Furthermore, the interaction among the ketogenic, uricosuric, insulin-sensitizing, and anti-inflammatory properties of SGLT2 inhibitors could be an attractive issue for further research regarding their metabolic benefits and long-term efficacy in ameliorating the chronic complications of diabetes. Yet, the endocrine and metabolic implications of SGLT2 inhibitors require further investigations with more significant numbers to explore correlations among the variables adequately.

Acknowledgments

The authors thank the research Vice Chancellor of Shiraz University of Medical Sciences for financial support of this work (grant number 22641). The authors also wish to thank Ms. A. Keivanshekouh at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for her assistance in editing this manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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