TAURUNUM MEDICAL JOURNAL
VOL 40 No 1 2026

DOI: 10.63696/TMJ202601003

GLYCOLIPID AND INFLAMMATORY STATUS ANALYSIS IN METFORMIN-TREATED TYPE 2 DIABETICS

Nataša Đorđević[1], Sonja Zafirović[1], Jelena Radovanović[1], Julijana Stanimirović[1], Nikola Ninković[1], Zoran Gluvić[2,3], Aleksandra Klisić[4], Esma R. Isenović[1]

1. Department of Radiobiology and Molecular Genetics, VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia.
2. Department of Endocrinology and Diabetes, Clinic for Internal Medicine, Clinical Hospital Center Zemun, Belgrade, Serbia
3. University of Belgrade, Faculty of Medicine, Belgrade, Serbia
4. Primary Health Care Center, University of Montenegro- Faculty of Medicine, Podgorica, Montenegro

Corresponding to: Zoran Gluvić, 9th Vukova Street, Belgrade, Serbia
Phone: +381691109019, Email: zorangluvic@yahoo.com

ANALIZA GLIKOLIPIDNOG I INFLAMATORNOG STATUSA KOD PACIJENATA SA DIJABETESOM TIPA 2 NA TERAPIJI METFORMINOM

Nataša Đorđević[1], Sonja Zafirović[1], Jelena Radovanović[1], Julijana Stanimirović[1], Nikola Ninković[1], Zoran Gluvić[2,3], Aleksandra Klisić[4], Esma R. Isenović[1]

1. Laboratorija za radiobiologiju i molekularnu genetiku, Institut za nuklearne nauke VINČA - institut od nacionalnog značaja republike Srbije, Univerzitet u Beogradu, Beograd, Srbija.
2. Odeljenje za endokrinologiju, Klinika za internu medicinu, Kliničko bolnički centar Zemun, Beograd, Srbija.
3. Medicinski fakultet Univerziteta u Beogradu, Beograd, Srbija
4. Dom zdravlja u Podgorici, Univerzitet Crne Gore-Medicinski fakultet, 81000 Podgorica, Crna Gora.

Autor za korespondenciju: Zoran Gluvić, Vukova 9, Beograd, Srbija
Telefon: +381691109019, Email: zorangluvic@yahoo.com

ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is linked to increased vascular morbidity and mortality. Well-controlled diabetes is indicated by effective glycemic and HbA1c regulation and low-grade inflammation management. This study evaluates glycolipid control and inflammatory status in T2DM patients on metformin.

Methods: A pilot study involved 11 participants divided into two groups: a T2DM group on metformin therapy and a control, non-diabetic group. Demographic, clinical, anthropometric, glycolipid (glycemia, HbA1c, TC, LDL-C, HDL-C, TG), and inflammatory parameters (CRP, FFA, PL, UA, FLI) were evaluated.

Results: Significant intergroup differences were found in glycemia, HbA1c, TC, LDL-C, UA, FLI, and WC. Glycemia influenced PL ($p<0.05$), UA ($p<0.01$), and FLI ($p<0.01$), while HbA1c affected UA ($p<0.05$) and FLI ($p<0.01$). LDL-C and HDL-C impacted UA ($p<0.05$, $p<0.01$). Age affected FLI ($p<0.05$), gender influenced PL ($p<0.01$), and BW, BH, WC, and BMI significantly impacted UA and FLI ($p<0.01$).

Conclusion: T2DM patients showed signs of low-grade systemic inflammation, emphasizing the need for comprehensive diabetes management and early cardiovascular risk detection. Intensified interventions are necessary for patients with inadequate glycemic control on metformin.

Keywords: type 2 diabetes, metformin, systemic inflammation, glycemic regulation, lipid status

SAŽETAK

Uvod: Dijabetes melitus tipa 2 (T2DM) povezan je sa povećanim vaskularnim morbiditetom i mortalitetom. Dobro kontrolisan dijabetes je definisan efikasnom regulacijom glikemije i parametra glikoliziranog hemoglobina (HbA1c) odražavajući nizak stepen inflamacije. U okviru ove studije je praćena kontrola glikolipidnog i inflamatornog statusa kod pacijenata sa T2DM na terapiji metforminom.

Metode: Pilot studija je obuhvatila 11 učesnika podeljenih u dve grupe: grupu sa T2DM na terapiji metforminom i kontrolnu grupu bez dijabetesa. Praćeni su demografski, klinički, antropometrijski, glikolipidni (glikemija, HbA1c, TC, LDL-C, HDL-C, TG) i inflamatorni parametri (CRP, FFA, PL, UA, FLI).

Rezultati: Značajne razlike između grupa primećene su u glikemiji, kao i u parametrima HbA1c, TC, LDL-C, UA, FLI i WC. Glikemija je uticala na nivoe PL ($p<0,05$), UA ($p<0,01$) i FLI ($p<0,01$), dok je HbA1c uticao na UA ($p<0,05$) i FLI ($p<0,01$). LDL-C i HDL-C su uticali na nivo UA ($p<0,05$, $p<0,01$). Starost pacijenta uticala je na nivo FLI ($p<0,05$), pol je uticao na PL ($p<0,01$), dok su telesna težina, telesna visina, obim struka i BMI značajno uticali na nivoe UA i FLI ($p<0,01$).

Zaključak: Pacijenti sa T2DM pokazuju znakove sistemske inflamacije niskog stepena, što ističe potrebu za sveobuhvatnim pristupom lečenju i prevenciji dijabetesa i kardiovaskularnog rizika. Glikemijski i lipidni disbalans značajno utiču na inflamatorne parametre, naglašavajući važnost pažljivog praćenja ovih biomarkera kod pacijenata na metforminu. Individualizovane terapijske strategije i intenzivnije intervencije mogu poboljšati kontrolu glikemije i smanjiti dugoročne komplikacije.

Ključne reči: tip 2 dijabetesa, metformin, sistemska inflamacija, regulacija glikemije, lipidni status

INTRODUCTION

Diabetes mellitus (DM) represents a significant global healthcare issue [1]. Projections suggest that this metabolic disorder will affect over 700 million people by 2045 [2]. Type 2 DM (T2DM) constitutes approximately 90% of all DM cases globally [3]. Defining characteristics of DM include inadequate insulin action or insufficient secretion. Inflammatory cytokines produced in response to hyperglycemia and dyslipidemia impair pancreatic β-cell function and reduce the activation of signaling molecules involved in the molecular mechanisms of insulin activity [4, 5]. The development of DM-associated vascular complications is related to the increased synthesis of acute phase reactants, such as fibrinogen, sialic acid, C-reactive protein (CRP), and serum amyloid A and P, which are stimulated by proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [6-8].

The management of DM encompasses both non-pharmacological and pharmacological approaches [9]. Non-pharmacological interventions include enhancing dietary habits and promoting supervised physical activities and exercises. The pharmacological management comprises oral antihyperglycemic agents (OHAs) and insulin [10]. Based on their mechanism of action, OHAs are classified into two classes. The first class includes insulin secretagogues that enhance insulin secretion when pancreatic insulin stores are intact (e.g., sulphonylurea derivates, meglitinides, etc.). The second class comprises insulin sensitizers that improve insulin sensitivity (e.g., biguanides, thiazolidinediones, glucagon-like peptides, dipeptidyl peptidase-4 inhibitors, etc.). Diabetes mellitus can be treated pharmacologically via monotherapy or combination therapy, involving various medications, including insulin. The use of insulin analogs, which demonstrate improved glycemic control and safety compared to human insulins, represents a modern approach to DM management. Enhancements in glucometabolic, lipid, and inflammatory markers, resulting in reduced cardiovascular morbidity and mortality, signify effective DM management [11-13].

Metformin (1,1-dimethyl biguanide hydrochloride) is the most often used medicine for treating T2DM and belongs to the biguanide class [14]. It is derived from the Galega officinalis, commonly known as French lilac [15]. The Food and Drug Administration approved metformin in 1994, and by 2005, it was the most prospective treatment for T2DM [15]. Therapeutic doses of metformin typically range from 1000 to 2500 mg per day, resulting in plasma concentrations between 0.5 and 2.0 μg/mL [16]. Metformin primarily affects the liver, causing a reduction in glucose synthesis, improving insulin resistance, and increasing insulin sensitivity [17]. By influencing several signaling pathways, metformin promotes weight loss and cardiovascular homeostasis [18]. Since metformin optimizes gut microbiota and reduces insulin resistance in hypothalamic pro-opiomelanocortin neurons, it accelerates metabolism and regulates appetite [17-19]. Metformin mitigates the risk of cardiovascular disease (CVD) in people with T2DM by enhancing lipid profiles, reducing visceral and total body fat, and lowering low-grade systemic inflammation. This is mainly attributable to its effects on both AMPK-dependent and AMPK-independent pathways, as well as the mitochondrial electron transport chain complex [20-26]. Rarely, metformin causes modest adverse effects, mostly in the gastrointestinal system [27].

This research assesses the concentrations of glycolipids and inflammatory markers in

individuals with T2DM receiving metformin therapy. Glycolipid and inflammatory marker levels will be assessed regularly in a cohort of T2DM patients treated with metformin, further reinforcing the safety and efficacy of this treatment.

SUBJECTS AND METHOD

STUDY POPULATION

This cross-sectional pilot study included eleven participants, seven females and four males, aged between 42 and 73 years. The study population comprised five healthy individuals and six patients diagnosed with T2DM receiving metformin during the period of one to five years. Smoking, menstruation, thyroid diseases, hypertension, and the use of fibrates, statins, and salicylates were exclusion criteria. All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate institutional committee. Informed consent was obtained from all participants.

ANTHROPOMETRIC MEASUREMENTS

The patients' body weight (BW) and body height (BH) were measured using a medical scale (Metalija Subotica), while waist circumference (WC) (cm) was measured with a non-stretchable tape. The body mass index (BMI) (kg/m2) was calculated and represents the ratio of BW (kg) to the square of BH (m2).

CLINICAL MEASUREMENTS

After a five-minute rest, and immediately before the venipuncture, all subjects were measured for systolic (SBP) and diastolic (DBP) blood pressure (mmHg) with the sphygmomanometer, HS 201C1 Palm Type Sphygmomanometer, Wenzhou Hongshun Industries, and Trade Co.

MEASUREMENT OF SERUM PARAMETERS OF GLYCOLIPID STATUS

Determination of glycemia, glycated hemoglobin (HbA1c), lipids (total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TGs), as well as gamma-glutamyl transferase (GGT) and uric acid (UA), was carried out using a biochemical analyzer Roche Cobas c501 (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Low-density lipoprotein-cholesterol (LDL-C) levels were calculated using the Friedewald formula: LDL-C= TC – HDL-C – 0.45 * TG (mmol/L) [28].

MEASUREMENT OF SERUM-FREE FATTY ACID (FFA) CONCENTRATIONS

Free fatty acid (FFA) concentration in the patient's plasma was determined using a modified Duncombe colorimetric method, as previously described in our laboratory [29]. In brief, FFAs were measured by adding 225 µL of reagent into 45 µL of the patient's plasma and briefly shaking. The resulting solution was mixed with 1125 µL of chloroform and vigorously shaken for 20 minutes and then centrifuged for 10 minutes at 3000 rpm. After removing the upper blue-green protein layer, 45 µL of 0.2% diethyldithiocarbamate was

added to 450 µL of the remaining chloroform layer with extracted FFA. The samples were vigorously shaken and then incubated for 20 min at room temperature. A spectrophotometer SmartSpec™ 3000 (Bio-Rad, Hercules, CA, USA) was used to determine the absorbance at 436nm. The intensity of the compound's color was proportional to the concentration of FFA and expressed in mmol/L.

MEASUREMENT OF SERUM PHOSPHOLIPID (PL) CONCENTRATION

The concentration of phospholipids (PLs) in the serum of the patients was determined by the colorimetric method according to Stewart [30]. The procedure involved adding 600 µL of a 2:1 chloroform-methanol solution to 300 µL of the serum. The resulting solution was shaken for 20 seconds and then centrifuged at room temperature for 3 min at 7000 rpm. Before adding 500µL of the reaction reagent 1, 400µL of chloroform was added to the lower chloroform layer with extracted PL. This solution was shaken for 20 seconds and then centrifuged for 10 min at 1000 rpm. After the removal of the upper layer, the optical density of the sample and standard was measured using a SmartSpec™ 3000 spectrophotometer (Bio-Rad, Hercules, CA, USA). Phospholipid concentration was expressed in mg/mL.

CALCULATION OF FATTY LIVER INDEX (FLI)

Fatty liver index (FLI) was calculated using the following formula [31]:

$$FLI = \frac{e0.953 \times \log_e(TG) + 0.139 \times BMI + 0.718 \times \log_e(GGT) + 0.053 \times WC - 15.745}{1 + e(0.953 \times \log_e(TG) + 0.139 \times BMI + 0.718 \times \log_e(GGT) + 0.053 \times WC - 15.745} * 100$$

To calculate the FLI, the following variables were used: BMI, WC, as well as GGT levels, and TG. Calculated values for FLI range from 0 to 100. Fatty liver index values <30 indicate a low risk of developing fatty liver, while values ≥60 indicate a significant presentation of fatty liver. Fatty liver index scores between 30 and 60 (grey zone) require careful monitoring [31].

STATISTICAL ANALYSIS

Methods of descriptive statistics included relative numbers, measures of central tendency (arithmetic means and median), and measures of variability (standard deviation, interval, and coefficient of variation). Analytical statistics included tests for assessing the significance of correlations and differences. Spearman's rank correlation test was used to determine the correlation's significance. To evaluate the significance of the difference, the t-test for independent samples was used in the case of parametric data, and the chi-square test in the case of categorical, i.e., the Mann-Whitney test in cases of interval data that do not follow the normal distribution. The level of statistical significance is 0.05. The data obtained were analyzed using the statistical package SPSS for Windows 22.0.

RESULTS

COMPARATIVE ANALYSIS OF ANTHROPOMETRIC, CLINICAL, AND INFLAMMATORY PARAMETERS IN HEALTHY INDIVIDUALS AND THOSE WITH TYPE 2 DIABETES MELLITUS

The pilot study included 11 participants, with a mean age of 60±9 years, categorized into two groups: patients with T2DM undergoing metformin treatment (T2DM group) and healthy controls (C group). Table 1 presents the anthropometric, clinical and inflammatory parameters. There was no significant difference between the groups in age, gender, BW, BH, and BMI. The mean WC values exhibited a significant difference between the groups ($t = +3.653$, $p<0.01$). The systolic and diastolic arterial pressure values showed no significant differences among the groups. Furthermore, a statistically significant difference in the levels of glycemia and HbA1c was detected ($t_{glycemia} = +9.750$; $t_{HbA1c} = +3.646$, $p<0.01$). Regarding blood lipids, the levels of TC and LDL-C were significantly different ($t_{TC} =-2.472$, $p<0.05$; $t_{LDL-C}=-3.847$, $p<0.01$) in contrast to the levels of TG and HDL-C, which did not significantly differ among the groups. The concentrations of UA and FLI as inflammatory markers exhibited significant differences between groups ($T_{FA} = .000$, $FLI = 3.000$, $p<0.05$), unlike the values of other inflammatory markers (CRP, FFA, and PL). Statistical significance was assessed through t-tests, with p-values reflecting the likelihood of obtaining the results under the assumption that the null hypothesis is valid.

Variable	T2D group [n=6]	C group [n=5]	p
Age [X±SD [min-max]]	64±7 [56-73]	54±8 [42-63]	ns
Gender [, n [%]]	2 [33]	5 [100]	ns
BW [kg] [X±SD [min-max]]	95±8 [86-108]	72±6 [63-79]	ns
BH [m] [X±SD [min-max]]	1.72±0.1 [1.56-1.83]	1.64±0.1 [1.50-1.76]	ns
BMI [kg/m2] [X±SD [min-max]]	33±6 [26-42]	27±3 [25-32]	ns
WC [cm] [X±SD [min-max]]	110±10 [100-126]	90±7 [82-101]	**<0.01**
SBP [mmHg] [X±SD [min-max]]	143±17 [120-165]	120±21 [99-146]	ns
DBP [mmHg] [X±SD [min-max]]	83±17 [65-108]	80±17 [60-99]	ns
Gly [mmol/L] [X±SD [min-max]]	8.5±0.5 [8.0-9.0]	5.6±0.5 [5.1-6.0]	**<0.01**
HbA1c [%] [X±SD [min-max]]	7.3±1.0 [6.0-9.0]	5.5±0.5 [5.0-6.0]	**<0.01**
TC [mmol/L] [X±SD [min-max]]	5.3±0.5 [5.0-6.0]	6.9±1.5 [4.9-9.0]	**<0.05**
TG [mmol/L] [Med [min-max]]	1.0 [1.0-5.0]	2.0 [1.2-2.0]	ns
LDL-C [mmol/L] [X±SD [min-max]]	3.0±0.0 [3.0]	4.7±1.1 [3.0-6.0]	**<0.01**
HDL-C [mmol/L] [Med [min-max]]	1.0 [1.0-2.0]	1.64 [1.1-2.0]	ns
CRP [mg/L] [Med [min-max]	1.0 [0.0-7.0]	1.0 [0.8-2.0]	ns
FFA [mmol/L] [Med [min-max]]	0.72 [0.06-1.70]	1.06 [0.60-1.14]	ns
PL [mg/mL] [Med [min-max]]	6.54 [1.98-10.09]	3.13 [2.86-5.19]	ns
UA [µmol/L] [Med [min-max]]	334 [240-412]	198 [168-228]	**<0.05**
FLI [Med [min-max]]	66 [40-98]	32 [18-62]	**<0.05**

Table 1. Comparative analysis of anthropometric, clinical, and inflammatory parameters in healthy individuals and those with Type 2 Diabetes Mellitus

BH-body height; BMI-body mass index; BW-body weight; C-control, healthy participants; CRP-C-reactive protein; DBP-diastolic blood pressure; FFA-free fatty acid; FLI-fatty liver index; Glycemia; HbA1c-glycated hemoglobin; HDL-C-high-density lipoprotein cholesterol; LDL-C-low-density lipoprotein cholesterol; ns - non-significant difference; PL-phospholipids; SBP-systolic blood pressure; T2DM-subjects with Type 2 Diabetes Mellitus; TC-total cholesterol; TG-triglycerides; UA-uric acid; WC-waist circumference; * $p<0.05$; ** $p<0.01$.

CORRELATION BETWEEN GLYCOLIPID, DEMOGRAPHIC, ANTHROPOMETRIC, CLINICAL, AND INFLAMMATORY PARAMETERS

Table 2 presents the Spearman's ρ coefficient values between observed glycolipid parameters (glycemia, HbA1c, TC, TG, LDL-C, and HDL-C) and inflammatory parameters (CRP, FFA, PL, UA, and FLI). The results demonstrate statistically significant correlations between glycemia levels and PL ($p<0.05$), UA ($p<0.01$), and FLI ($p<0.01$). Additionally, HbA1c values correlate with UA ($p<0.05$) and FLI ($p<0.01$), while LDL-C and HDL-C values are associated with UA ($p<0.05$ and $p<0.01$, respectively). The correlations indicate a possible interaction between glycolipid and inflammatory parameters in T2DM. Fig. 1a, Fig. 2-a, b, c, d, Fig. 3-a and 3-b display the logarithmic regression lines corresponding to statistically significant correlations. Also, Spearman's ρ coefficient values between observed demographic (gender and age), anthropometric (BW, BH, BMI, WC), clinical (SBP and DBP), and inflammatory parameters (CRP, FFA, PL, UA, FLI) are shown in Table 2.

Statistically significant correlations were identified between FLI scores and age ($p<0.05$), as well as with BW ($p<0.01$), BMI ($p<0.01$), and WC ($p<0.01$). Correlations between BH and PL values ($p<0.01$), UA and BW values ($p<0.01$), and UA and WC values were statistically significant ($p<0.01$) as illustrated in Fig. 1-b, Fig. 2-e, f, and Fig. 3-c, d, e, and f. It is important to acknowledge that the limited sample size and the unique characteristics of the participants may restrict the generalizability of these findings.

Variable	CRP	FFA	PL	UA	FLI
Gly	+0.038	-0.426	**+0.613*** F1a	**+0.794**** F2a	**+0.823**** F3a
HbA1c	+0.324	-0.533	+0.446	**+0.776*** F2b	**+0.809**** F3b
TC	-0.146	-0.023	-0.345	-0.396	+0.028
TG	-0.007	-0.264	-0.108	+0.153	+0.273
LDL-C	-0.110	-0.075	-0.436	**-0.764*** F2c	-0.109
HDL-C	-0.212	+0.265	-0.172	**-0.873**** F2d	-0.521
Gender [man]	+0.544	+0.180	**-0.777****	+0.000	-0.270
Age	+0.147	-0.469	+0.482	+0.310	**+0.683*** F3c
BW	-0.120	-0.142	+0.446	**+0.766*** F2e	**+0.767**** F3d
BH	-0.557	+0.034	**+0.658**** F1b	+0.095	-0.224
BMI	+0.074	-0.241	+0.055	+0.643	**+0.934**** F3e
WC	+0.032	-0.350	+0.447	**+0.886**** F2f	**+0.888**** F3f
SBP	+0.133	-0.182	+0.264	+0.524	+0.255
DBP	+0.106	-0.160	-0.068	+0.216	+0.016

Table 2. Correlation between glycolipid, demographic, anthropometric, clinical and inflammatory parameters

BH-body height; BMI-body mass index; BW-body weight; CRP-C-reactive protein; DBP-diastolic blood pressure; FFA-free fatty acid; FLI-fatty liver index; Glycemia; HbA1c-glycated hemoglobin; HDL-C-high-density lipoprotein cholesterol; LDL-C-low-density lipoprotein cholesterol; PL-phospholipids; SBP-systolic blood pressure; TC-total cholesterol; TG-triglycerides; UA-uric acid; WC-waist circumference; Fig. 1-3 display results; * $p<0.05$; ** $p<0.01$.

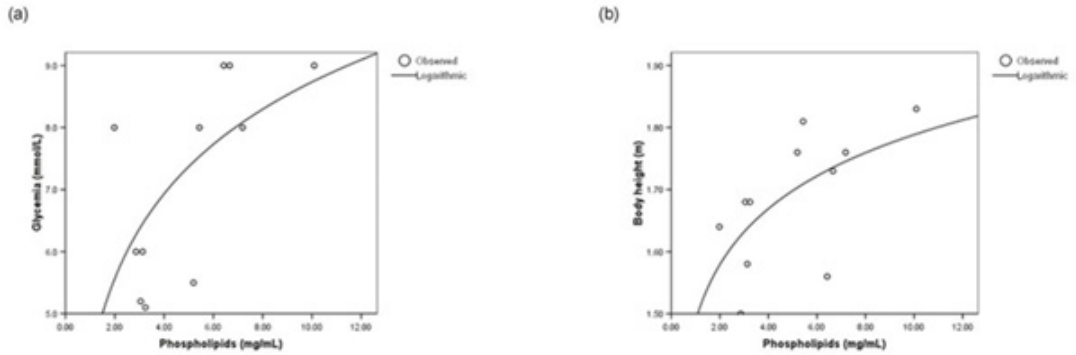

Fig 1. a-b. Logarithmic regression line illustrating: The relationship between glycemia and phospholipids (a). The association between body height and phospholipids (b)

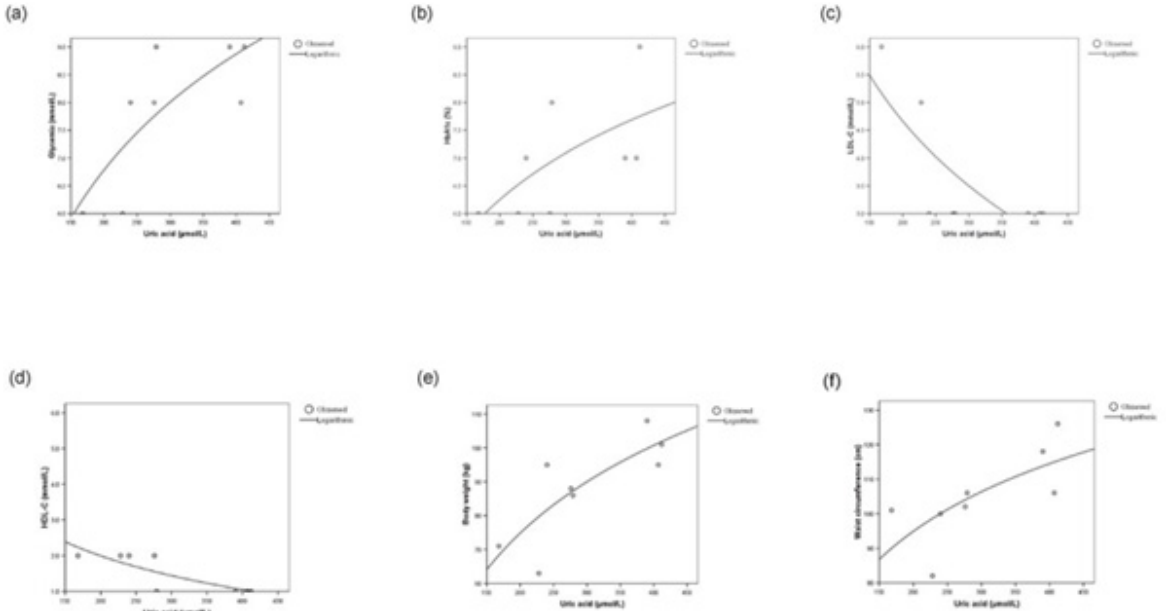

Fig 2. a-f. Logarithmic regression lines for: Glycemia vs. uric acid (a). HbA1c vs. uric acid (b). LDL-C vs. uric acid (c). HDL-C vs. uric acid (d). Body weight vs. uric acid (e). Waist circumference vs. uric acid (f)

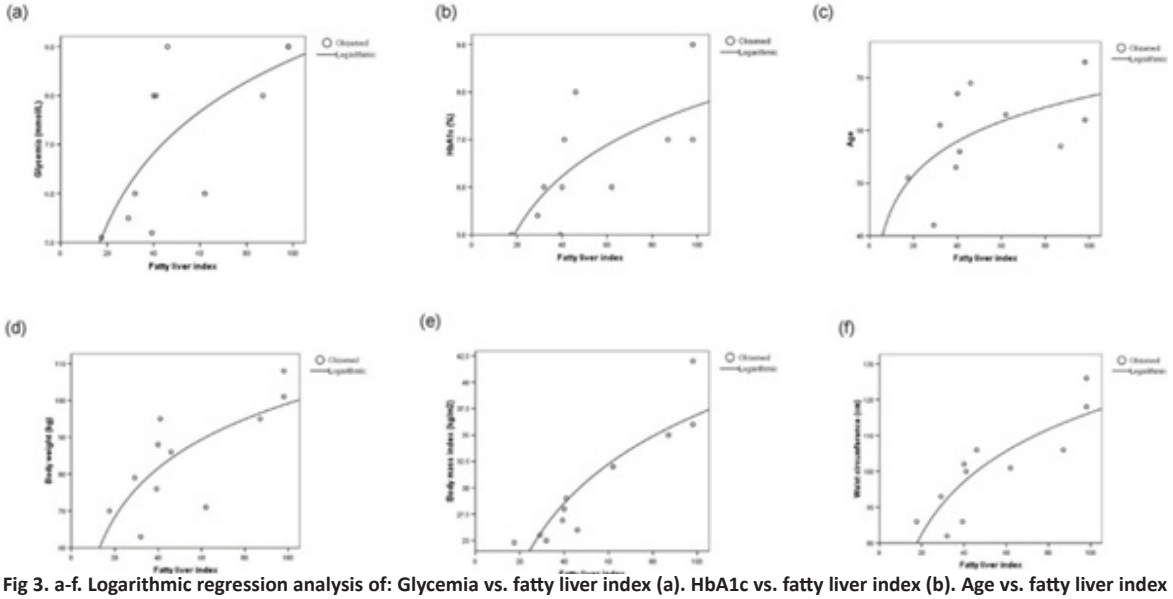

Fig 3. a-f. Logarithmic regression analysis of: Glycemia vs. fatty liver index (a). HbA1c vs. fatty liver index (b). Age vs. fatty liver index (c). Body weight vs. fatty liver index (d). Body mass index vs. fatty liver index (e). Waist circumference vs. fatty liver index (f)

DISCUSSION

Type 2 diabetes mellitus (T2DM) is a common chronic metabolic disorder that causes significant vascular complications, contributing to morbidity, disability, and mortality [32, 33]. Metformin is the preferred OHA for the initial treatment of T2DM [34]. This study examines the inflammatory and glycolipid status in T2DM patients receiving metformin treatment for one to five years, along with the influence of clinical, anthropometric, demographic, and glycolipid variables on inflammatory markers. Fasting glucose levels and insulin secretion are utilized for diagnostic purposes and treatment monitoring. Metformin reduces hepatic gluconeogenesis, improves insulin sensitivity, and promotes glucose uptake in the liver and skeletal muscle [17]. Additionally, metformin influences lipid metabolism following a single-dose administration in healthy individuals [35, 36]. Nonetheless, its impact on lipid metabolism in diabetes remains unclear. This pilot study indicates elevated glycemia and HbA1c levels in the T2DM cohort. Nonetheless, the reduced levels of TC and LDL-C were unexpectedly observed in the sera of diabetic patients in comparison to healthy subjects. A potential explanation for this phenomenon is the beneficial effect of metformin monotherapy on the specified lipid parameters, in contrast to inadequate management of T2DM [37]. The findings highlight the importance of both non-pharmacological strategies, such as dietary modifications, physical exercise, and dietary supplements with atheroprotective and hepatoprotective properties, and pharmacological interventions, including statins, fibrates, and salicylates for primary and secondary vascular protection [38-42]. Additionally, our study demonstrates that metformin decreases HbA1c and BW [43]. Multiple studies indicate that metformin alleviates cardiovascular problems [44-46]. Nevertheless, the findings of certain studies are inconsistent. Two meta-analyses have indicated that metformin does not possess a vasculoprotective effect [47, 48]; however, the SPREAD-DIMCAD study reported a 46% reduction in secondary cardiovascular events [43]. Inadequate adherence to both non-pharmacological and pharmacological interventions may have led to insufficient control of T2DM in certain participants in this trial. Additionally, the beneficial effects of metformin monotherapy in T2DM control are exhaustive, suggesting the need for intensification of both non-pharmacological and pharmacological therapy [49].

Recent research indicates a correlation between the systemic inflammatory response and insulin resistance in individuals with T2DM [50-52]. This pilot study also examined the inflammatory markers CRP, FFA, PL, UA, and FLI; only the levels of UA and FLI differed among the groups. Mitrovic et al. demonstrated that elevated FLI and UA levels serve as indicators of liver steatosis and increased atherogenic potential in patients with T2DM and metabolic-associated fatty liver disease (MAFLD) [37]. Metformin monotherapy maintains inflammation, which limits vascular effects and the advancement of liver steatosis to fibrosis and steatohepatitis. A higher risk of cardiovascular morbidity and mortality has been attributed to hyperuricemia [53-56]. Hyperuricemia and CVD are associated with the pro-inflammatory and pro-oxidative properties of UA, elevated levels of CRP and IL-6, and the production of vascular adhesion molecules, contributing to endothelial dysfunction [57-60]. Numerous studies have demonstrated a correlation between hyperuricemia, T2DM, and metabolic syndrome (MetS) [61-65]. Despite conflicting evidence, hyperuricemia is

linked to MAFLD [66-70]. Men experience fatty liver more frequently than women of the same age, and men also have higher reference values of UA. Consequently, some studies have raised concerns regarding this association in female patients. This is probably linked to the uricosuric effects of estrogen [71, 72]. Given the invasive nature of liver biopsy, which is the gold standard for diagnosing MAFLD, clinicians employ biochemical markers and non-invasive assessments for liver steatosis and fibrosis. From a clinical perspective, it is the most reliable strategy for selecting liver biopsy candidates [73]. The combination of biochemical markers, non-invasive scores, and radiological imaging findings, such as hepatic magnetic resonance imaging or transitional elastography, enhances the sensitivity, specificity, and accuracy of identifying the most prevalent chronic liver disease [74]. In addition to abdominal ultrasonography and the grading of hepatic steatosis severity, straightforward, quick, and economical scores such as FLI or Fibrosis-4 (FIB-4) may be utilized [75].

The risk of fatty liver diminishes with FLI scores under 30%. Ultrasound diagnostics detects fatty liver when the FLI score exceeds 60%, demonstrating a sensitivity of 87.3% and a specificity of 80.3% [31]. Furthermore, FLI values above 60% suggest the necessity of using methods to detect liver fibrosis early. The FIB-4 score is one of the most utilized non-invasive assessments for liver fibrosis in clinical settings, although its accuracy is greater for identifying advanced stages of the condition [76, 77]. Furthermore, to evaluate the risk and mitigate potential outcomes of MAFLD, such as steatohepatitis, cirrhosis, and hepatocellular carcinoma, patients identified as having an increased risk of liver fibrosis are referred for liver biopsy and pathohistological assessment of liver steatosis [75]. Elevated FLI scores are significantly associated with increased systemic inflammation, the presence of T2DM, and increased risk of atherosclerosis [78]. In our investigation, the control group showed a reduced FLI, yet it stayed above 30, indicating systemic inflammation that presents as clinically undetectable endothelial damage, a precursor to atherosclerosis in healthy individuals. However, type 1 DM individuals exhibit earlier onset of atherosclerosis [79].

The study's findings indicate a correlation between FLI score and BW, BMI, and WC, suggesting an elevated risk of liver cirrhosis and fibrosis, as well as atherosclerotic changes associated with increased cardiovascular morbidity and mortality. Atherosclerotic outcomes, including atherosclerotic CVD, in individuals with T2DM are predicted by the FLI score [80]. The FLI score can be evaluated by a general practitioner, enabling accurate expert referral for severe liver and cardiovascular late effects of MAFLD [78-81]. Glycemic values exhibit a positive association with FLI scores, PL, and UA levels; HbA1c levels show a positive correlation with UA and FLI scores; and LDL-C and HDL-C levels demonstrate a negative correlation with UA levels. Poorly controlled diabetes leads to systemic inflammation and hastens the progression of atherosclerosis, contributing to CVD [82]. The study's findings indicate that male T2DM patients demonstrated elevated blood levels of PL. In the group of T2DM patients, no evidence supports the reciprocal effect of inflammatory markers. The study's limitations, including the small sample size, the brief duration of T2DM, and the possible advantages of metformin therapy, may account for these trends. PLs, present in all cell membranes, are essential for various biological activities [83]. Fatty acids in PL act as substrates for eicosanoid synthesis, while their degradation products serve as intracellular signaling molecules. PLs regulate membrane permeability and fluidity. The fat-

ty acid composition of PL reflects the type of dietary lipids and serves as a biochemical biomarker for monitoring fatty acid intake [84]. Oxidatively modified phospholipid products in atherosclerotic lesions demonstrate their significance [85]. The primary source of FFAs is adipose tissue TGs, which break down, releasing FFAs into the bloodstream and serve as an important energy substrate [86]. Elevated blood levels of FFAs, resulting from hypertrophied adipose tissue, are associated with insulin resistance, DM, MetS, liver steatosis, hypertension, myocardial dysfunction, and the progression of atherosclerosis [86]. Additionally, increased levels of FFAs independently predict CVD mortality [87]. Free fatty acids impede the antilipolytic effects of insulin, leading to insulin resistance. The mechanisms of FFA-induced insulin resistance involve the intracellular accumulation of triacylglycerol and diacylglycerol in insulin target cells, a reduction in tyrosine phosphorylation, specifically the decreased activation of insulin receptor substrate (IRS) 1/2, and a subsequent decline in IRS/phosphatidylinositol 3-kinase signaling pathways. Furthermore, the activation of nicotinamide adenine dinucleotide phosphate oxidase by protein kinase C results in elevated production of reactive oxygen species due to FFAs, causing oxidative damage to endothelial cells, hepatocytes, immune cells, and other cell types [86]. FFA activates nuclear factor kappa B in endothelial cells, leading to an increased production of inflammatory markers, including monocyte chemoattractant protein, TNF-α, IL-6, and interleukin 1β, which play a role in the progression of atherosclerosis [88]. Additionally, FFAs enhance the production of vascular adhesion molecule-1 and intracellular adhesion molecule-1, which are critical markers in the development of atherosclerosis [89].

CONCLUSION

The increasing prevalence of T2DM and its related components of metabolic syndrome—obesity, dyslipidemia, and fatty liver disease—necessitates greater attention from healthcare professionals. This study, despite its limited sample size, supports the notion that both healthy individuals and T2DM patients on metformin exhibit low-grade systemic inflammation, highlighting the necessity for proactive and innovative disease management strategies. The findings highlight the necessity of implementing proactive preventive measures to address diabetes, obesity, and hypertension. The insufficiency of existing glycemic control in T2DM patients highlights the need for intensifying or adjusting therapeutic strategies according to established guidelines. The presence of low-grade systemic inflammation in the control group indicates a critical necessity for proactive preventive measures to address diabetes, obesity, and hypertension prior to the onset of irreversible complications. Early detection is fundamental to effective intervention. Addressing additional cardiovascular risk factors through non-invasive screening methods, including the FLI and liver fibrosis scores alongside ultrasound, is essential for refining risk assessment and identifying patients who may need more advanced diagnostic procedures, such as liver biopsy. Reducing systemic inflammation in patients with T2DM is essential. Targeted interventions can significantly enhance patient quality of life by mitigating atherosclerotic changes in major arteries, thereby reducing or delaying the onset of severe cardiovascular morbidity. An enhanced and proactive strategy for disease prevention and management is crucial for mitigating the long-term effects of T2DM.

FUNDING

This work is part of the collaboration between the Department of Radiobiology and Molecular Genetics, "VINČA" Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia, Clinic for Internal Medicine, Department of Endocrinology and Diabetes, Zemun Clinical Hospital, School of Medicine, University of Belgrade, Belgrade, Serbia, and Primary Health Care Center, University of Montenegro- Faculty of Medicine, Podgorica, Montenegro. This work was funded by the Ministry of Science and Technological Development and Innovation of the Republic of Serbia (Contract No#451-03-33/2026-03/200017) under the Research Theme "Hormonal regulation of expression and activity of nitric oxide synthase and sodium-potassium pump in experimental models of insulin resistance, diabetes, and cardiovascular disHorders" (No.0802601 to ERI).

ETHICS STATEMENT

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The official Ethical Committee of Primary Health Care Center, Podgorica, Montenegro approved experimental protocols (No. 05/01-E.K.-4692/1).

DECLARATION OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

1. Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Sci Rep. 2024;7(3):e2004. https://doi.org/10.1002/hsr2.2004

2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract. 2019;157:107843. https://doi.org/10.1016/j.diabres.2019.107843

3. Lushchak O, Zayachkivska A, Vaiserman A. Metallic Nanoantioxidants as Potential Therapeutics for Type 2 Diabetes: A Hypothetical Background and Translational Perspectives. Oxid Med Cell Longev. 2018;2018:3407375. https://doi.org/10.1155/2018/3407375

4. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet. 2018;391(10138):2449-62. https://doi.org/10.1016/S0140-6736[18]31320-5

5. Quinn L. Mechanisms in the development of type 2 diabetes mellitus. J Cardiovasc Nurs. 2002;16(2):1-16. https://doi.org/10.1097/00005082-200201000-00002

6. Gluvic Z, Zaric B, Resanovic I, Obradovic M, Mitrovic A, Radak D, et al. Link between Metabolic Syndrome and Insulin Resistance. Curr Vasc Pharmacol. 2017;15(1):30-

9. https://doi.org/10.2174/1570161114666161007164510

7. Hunter SJ, Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. Am J Med. 1998;105(4):331-45. https://doi.org/10.1016/s0002-9343[98]00300-3

8. Boden G. Free fatty acids, insulin resistance, and type 2 diabetes mellitus. Proc Assoc Am Physicians. 1999;111(3):241-8. https://doi.org/10.1046/j.1525-1381.1999.99220.x

9. Wang X, Kang J, Liu Q, Tong T, Quan H. Fighting Diabetes Mellitus: Pharmacological and Non-pharmacological Approaches. Curr Pharm Des. 2020;26(39):4992-5001. https://doi.org/10.2174/1381612826666200728144200

10. Majety P, Lozada Orquera FA, Edem D, Hamdy O. Pharmacological approaches to the prevention of type 2 diabetes mellitus. Front Endocrinol (Lausanne). 2023;14:1118848. https://doi.org/10.3389/fendo.2023.1118848

11. Gu S, Hu X, Shi L, Zhen X, Sun X, Huang M, et al. Choice of Glucose-Lowering Drugs as Initial Monotherapy for Type 2 Diabetes Patients with Contraindications or Intolerance to Metformin: A Systematic Review and Meta-Analysis. J Clin Med. 2022;11(23). https://doi.org/10.3390/jcm11237094

12. Kim JY, Kim NH. Initial Combination Therapy in Type 2 Diabetes. Endocrinol Metab (Seoul). 2024;39(1):23-32. https://doi.org/10.3803/EnM.2023.1816

13. Bu Y, Peng M, Tang X, Xu X, Wu Y, Chen AF, et al. Protective effects of metformin in various cardiovascular diseases: Clinical evidence and AMPK-dependent mechanisms. J Cell Mol Med. 2022;26(19):4886-903. https://doi.org/10.1111/jcmm.17519

14. Gonzalez-Lopez C, Wojeck BS. Role of metformin in the management of type 2 diabetes: recent advances. Pol Arch Intern Med. 2023;133(6). https://doi.org/10.20452/pamw.16511

15. Bailey CJ. Metformin: historical overview. Diabetologia. 2017;60(9):1566-76. https://doi.org/10.1007/s00125-017-4318-z

16. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. Am J Med. 1997;103(6):491-7. https://doi.org/10.1016/s0002-9343(97)00254-4

17. LaMoia TE, Shulman GI. Cellular and Molecular Mechanisms of Metformin Action. Endocrine reviews. 2021;42(1):77-96. https://doi.org/10.1210/endrev/bnaa023

18. Pillai AA, Melo L, Frishman WH, Aronow WS. The Effects of Metformin on Weight Loss, Cardiovascular Health, and Longevity. Cardiol Rev. 2024. https://doi.org/10.1097/CRD.0000000000000832

19. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med. 2017;23(7):850-8. https://doi.org/10.1038/nm.4345

20. Koffert JP, Mikkola K, Virtanen KA, Andersson AD, Faxius L, Hällsten K, et al. Metformin treatment significantly enhances intestinal glucose uptake in patients with type 2 diabetes: Results from a randomized clinical trial. Diabetes Res Clin Pract. 2017;131:208-16. https://doi.org/10.1016/j.diabres.2017.07.015

21. Mohamed HA, Mohamed NA, Macasa SS, Basha HK, Adan AM, Crovella S,

et al. Metformin-loaded nanoparticles reduce hyperglycemia-associated oxidative stress and induce eNOS phosphorylation in vascular endothelial cells. Sci Rep. 2024;14(1):30870. https://doi.org/10.1038/s41598-024-81427-6

22. Padhy DS, Aggarwal P, Velayutham R, Banerjee S. Aerobic exercise and metformin attenuate the cognitive impairment in an experimental model of type 2 diabetes mellitus: focus on neuroinflammation and adult hippocampal neurogenesis. Metab Brain Dis. 2025;40(1):92. https://doi.org/10.1007/s11011-024-01489-3

23. Gillani SW, Ghayedi N, Roosta P, Seddigh P, Nasiri O. Effect of Metformin on Lipid Profiles of Type 2 Diabetes Mellitus: A Meta-analysis of Randomized Controlled Trials. J Pharm Bioallied Sci. 2021;13(1):76-82. https://doi.org/10.4103/jpbs.JPBS_370_20

24. Bailey CJ. Metformin: Therapeutic profile in the treatment of type 2 diabetes. Diabetes Obes Metab. 2024;26 Suppl 3:3-19. https://doi.org/10.1111/dom.15663

25. Kim JW, Kim SM, Park JS, Hwang SH, Choi J, Jung KA, et al. Metformin improves salivary gland inflammation and hypofunction in murine Sjögren's syndrome. Arthritis Res Ther. 2019;21(1):136. https://doi.org/10.1186/s13075-019-1904-0

26. Lin H, Ao H, Guo G, Liu M. The Role and Mechanism of Metformin in Inflammatory Diseases. J Inflamm Res. 2023;16:5545-64. https://doi.org/10.2147/JIR.S436147

27. Aroda VR, Knowler WC, Crandall JP, Perreault L, Edelstein SL, Jeffries SL, et al. Metformin for diabetes prevention: insights gained from the Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study. Diabetologia. 2017;60(9):1601-11. https://doi.org/10.1007/s00125-017-4361-9

28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18(6):499-502. https://doi.org/10.1093/clinchem/18.6.499

29. Duncombe WG. THE COLORIMETRIC MICRO-DETERMINATION OF NON-ESTERIFIED FATTY ACIDS IN PLASMA. Clin Chim Acta. 1964;9:122-5. https://doi.org/10.1016/0009-8981(64)90004-x

30. Stewart JCM. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. Analytical biochemistry. 1980;104(1):10-4. https://doi.org/10.1016/0003-2697(80)90269-9

31. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:33. https://doi.org/10.1186/1471-230X-6-33

32. Heintjes E, Houben E, Beekman-Hendriks W, Lighaam E, Cremers S, Penning-van Beest F, et al. Trends in mortality, cardiovascular complications, and risk factors in type 2 diabetes. Neth J Med. 2019;77(9):317-29.

33. Constantino MI, Molyneaux L, Limacher-Gisler F, Al-Saeed A, Luo C, Wu T, et al. Long-term complications and mortality in young-onset diabetes: type 2 diabetes is more hazardous and lethal than type 1 diabetes. Diabetes care. 2013;36(12):3863-9. https://doi.org/10.2337/dc12-2455

34. Bosi E. Metformin--the gold standard in type 2 diabetes: what does the evidence tell us? Diabetes, obesity & metabolism. 2009;11 Suppl 2:3-8. https://doi.org/10.1111/j.1463-1326.2008.01031.x

35. Dahabiyeh LA, Mujammami M, Arafat T, Benabdelkamel H, Alfadda AA,

Abdel Rahman AM. A metabolic pattern in healthy subjects given a single dose of metformin: a metabolomics approach. Frontiers in Pharmacology. 2021;12:705932. https://doi.org/10.3389/fphar.2021.705932

36. Dahabiyeh LA, Mujammami M, AlMalki RH, Arafat T, Benabdelkamel H, Alfadda AA, et al. Lipids alterations associated with metformin in healthy subjects: an investigation using mass spectrometry shotgun approach. International Journal of Molecular Sciences. 2022;23(19):11478. https://doi.org/10.3390/ijms231911478

37. Mitrovic B, Gluvic Z, Macut D, Obradovic M, Sudar-Milovanovic E, Soskic S, et al. Effects of Metformin-Single Therapy on the Level of Inflammatory Markers in Serum of Non-Obese T2DM Patients with NAFLD. Endocr Metab Immune Disord Drug Targets. 2022;22(1):117-24. https://doi.org/10.2174/1871530321666210225110140

38. Mutie PM, Pomares-Millan H, Atabaki-Pasdar N, Jordan N, Adams R, Daly NL, et al. An investigation of causal relationships between prediabetes and vascular complications. Nat Commun. 2020;11(1):4592. https://doi.org/10.1038/s41467-020-18386-9

39. Schallmoser S, Zueger T, Kraus M, Saar-Tsechansky M, Stettler C, Feuerriegel S. Machine Learning for Predicting Micro- and Macrovascular Complications in Individuals With Prediabetes or Diabetes: Retrospective Cohort Study. J Med Internet Res. 2023;25:e42181. https://doi.org/10.2196/42181

40. Yu ES, Hong K, Chun BC. Incidence and risk factors of vascular complications in people with impaired fasting glucose: a national cohort study in Korea. Scientific reports. 2020;10(1):19504. https://doi.org/10.1038/s41598-020-76661-7

41. Barkas F, Bathrellou E, Nomikos T, Panagiotakos D, Liberopoulos E, Kontogianni MD. Plant Sterols and Plant Stanols in Cholesterol Management and Cardiovascular Prevention. Nutrients. 2023;15(13). https://doi.org/10.3390/nu15132845

42. Rizzo M, Colletti A, Penson PE, Katsiki N, Mikhailidis DP, Toth PP, et al. Nutraceutical approaches to non-alcoholic fatty liver disease (NAFLD): A position paper from the International Lipid Expert Panel (ILEP). Pharmacol Res. 2023;189:106679. https://doi.org/10.1016/j.phrs.2023.106679

43. Hong J, Zhang Y, Lai S, Lv A, Su Q, Dong Y, et al. Effects of metformin versus glipizide on cardiovascular outcomes in patients with type 2 diabetes and coronary artery disease. Diabetes Care. 2013;36(5):1304-11. https://doi.org/10.2337/dc12-0719

44. Ala M, Ala M. Metformin for Cardiovascular Protection, Inflammatory Bowel Disease, Osteoporosis, Periodontitis, Polycystic Ovarian Syndrome, Neurodegeneration, Cancer, Inflammation and Senescence: What Is Next? ACS Pharmacol Transl Sci. 2021;4(6):1747-70. https://doi.org/10.1021/acsptsci.1c00167

45. Ding Y, Zhou Y, Ling P, Feng X, Luo S, Zheng X, et al. Metformin in cardiovascular diabetology: a focused review of its impact on endothelial function. Theranostics. 2021;11(19):9376-96. https://doi.org/10.7150/thno.64706

46. Schernthaner G, Brand K, Bailey CJ. Metformin and the heart: Update on mechanisms of cardiovascular protection with special reference to comorbid type 2 diabetes and heart failure. Metabolism. 2022;130:155160. https://doi.org/10.1016/j.metabol.2022.155160

47. Boussageon R, Supper I, Bejan-Angoulvant T, Kellou N, Cucherat M, Boissel JP, et al. Reappraisal of metformin efficacy in the treatment of type 2 diabetes: a me-

ta-analysis of randomised controlled trials. PLoS Med. 2012;9(4):e1001204. https://doi.org/10.1371/journal.pmed.1001204

48. Lamanna C, Monami M, Marchionni N, Mannucci E. Effect of metformin on cardiovascular events and mortality: a meta-analysis of randomized clinical trials. Diabetes, obesity & metabolism. 2011;13(3):221-8. https://doi.org/10.1111/j.1463-1326.2010.01349.x

49. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Care in Diabetes-2023. Diabetes Care. 2023;46(Suppl 1):S140-s57. https://doi.org/10.2337/dc23-S009

50. Llop D, Rehues P, Paredes S, Guardiola M, Girona J, Rosales R, et al. Triglyceride-independent associations between circulating levels of apolipoprotein C-III and biomarkers of inflammation. Cardiovasc Diabetol. 2025;24(1):9. https://doi.org/10.1186/s12933-024-02553z

51. Fajkić A, Lepara O, Jahić R, Ejubović M, Kurtović A, Džidić-Krivić A, et al. The role of resistin and adiponectin ratios with uric acid in assessing metabolic syndrome in type 2 diabetes. Mol Cell Biochem. 2025. https://doi.org/10.1007/s11010-024-05200-z

52. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity. 2022;55(1):31-55. https://doi.org/10.1016/j.immuni.2021.12.013

53. Viazzi F, Leoncini G, Ratto E, Pontremoli R. Serum uric acid as a risk factor for cardiovascular and renal disease: an old controversy revived. J Clin Hypertens (Greenwich). 2006;8(7):510-8. https://doi.org/10.1111/j.1524-6175.2006.04755.x

54. Milionis HJ, Kalantzi KJ, Goudevenos JA, Seferiadis K, Mikhailidis DP, Elisaf MS. Serum uric acid levels and risk for acute ischaemic non-embolic stroke in elderly subjects. J Intern Med. 2005;258(5):435-41. https://doi.org/10.1111/j.1365-2796.2005.01565.x

55. Niskanen LK, Laaksonen DE, Nyyssönen K, Alfthan G, Lakka HM, Lakka TA, et al. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. Arch Intern Med. 2004;164(14):1546-51. https://doi.org/10.1001/archinte.164.14.1546

56. Timsans J, Kauppi JE, Kerola AM, Lehto TM, Kautiainen H, Kauppi MJ. Hyperuricaemia: prevalence and association with mortality in an elderly Finnish population. BMJ Open. 2023;13(5):e072110. https://doi.org/10.1136/bmjopen-2023-072110

57. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension. 2003;41(6):1183-90. https://doi.org/10.1161/01.HYP.0000069700.62727.C5

58. Tsouli SG, Liberopoulos EN, Mikhailidis DP, Athyros VG, Elisaf MS. Elevated serum uric acid levels in metabolic syndrome: an active component or an innocent bystander? Metabolism. 2006;55(10):1293-301. https://doi.org/10.1016/j.metabol.2006.05.013

59. Bo S, Gambino R, Durazzo M, Ghione F, Musso G, Gentile L, et al. Associations between serum uric acid and adipokines, markers of inflammation, and endothelial dysfunction. J Endocrinol Invest. 2008;31(6):499-504. https://doi.org/10.1007/BF03346397

60. Ghazizadeh H, Rezaei M, Avan A, Fazilati M, Pasdar A, Tavallaie S, et al. Association between serum cell adhesion molecules with hs-CRP, uric acid and VEGF genetic polymorphisms in subjects with metabolic syndrome. Mol Biol Rep. 2020;47(2):867-75.

https://doi.org/10.1007/s11033-019-05081-2

61. Yoo TW, Sung KC, Shin HS, Kim BJ, Kim BS, Kang JH, et al. Relationship between serum uric acid concentration and insulin resistance and metabolic syndrome. Circ J. 2005;69(8):928-33. https://doi.org/10.1253/circj.69.928

62. Tu YC, Liu YH, Chen SC, Su HM. Metabolic Syndrome and Its Components Are Associated with New-Onset Hyperuricemia in a Large Taiwanese Population Follow-Up Study. Nutrients. 2023;15(5). https://doi.org/10.3390/nu15051083

63. Fayazi HS, Mortazavi Khatibani SS, Motamed B, Yaseri M. Evaluation of levels of uric acid and lipid profile in hospitalized patients with diabetes. BMC Res Notes. 2023;16(1):154. https://doi.org/10.1186/s13104-023-06429-5

64. Bhole V, Choi JW, Kim SW, de Vera M, Choi H. Serum uric acid levels and the risk of type 2 diabetes: a prospective study. Am J Med. 2010;123(10):957-61. https://doi.org/10.1016/j.amjmed.2010.03.027

65. Lv Q, Meng XF, He FF, Chen S, Su H, Xiong J, et al. High serum uric acid and increased risk of type 2 diabetes: a systemic review and meta-analysis of prospective cohort studies. PLoS One. 2013;8(2):e56864. https://doi.org/10.1371/journal.pone.0056864

66. Lee YJ, Lee HR, Lee JH, Shin YH, Shim JY. Association between serum uric acid and non-alcoholic fatty liver disease in Korean adults. Clin Chem Lab Med. 2010;48(2):175-80. https://doi.org/10.1515/CCLM.2010.037

67. Sirota JC, McFann K, Targher G, Johnson RJ, Chonchol M, Jalal DI. Elevated serum uric acid levels are associated with non-alcoholic fatty liver disease independently of metabolic syndrome features in the United States: Liver ultrasound data from the National Health and Nutrition Examination Survey. Metabolism. 2013;62(3):392-9. https://doi.org/10.1016/j.metabol.2012.08.013

68. Liu Z, Wang Q, Huang H, Wang X, Xu C. Association between serum uric acid levels and long-term mortality of metabolic dysfunction-associated fatty liver disease: a nationwide cohort study. Diabetol Metab Syndr. 2023;15(1):27. https://doi.org/10.1186/s13098-023-00997-z

69. Xu C, Yu C, Xu L, Miao M, Li Y. High serum uric acid increases the risk for nonalcoholic Fatty liver disease: a prospective observational study. PLoS One. 2010;5(7):e11578. https://doi.org/10.1371/journal.pone.0011578

70. Wei F, Li J, Chen C, Zhang K, Cao L, Wang X, et al. Higher Serum Uric Acid Level Predicts Non-alcoholic Fatty Liver Disease: A 4-Year Prospective Cohort Study. Front Endocrinol (Lausanne). 2020;11:179. https://doi.org/10.3389/fendo.2020.00179

71. Lee K, Sung JA, Kim JS, Park TJ. The roles of obesity and gender on the relationship between metabolic risk factors and non-alcoholic fatty liver disease in Koreans. Diabetes Metab Res Rev. 2009;25(2):150-5. https://doi.org/10.1002/dmrr.924

72. Yahyaoui R, Esteva I, Haro-Mora JJ, Almaraz MC, Morcillo S, Rojo-Martínez G, et al. Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. J Clin Endocrinol Metab. 2008;93(6):2230-3. https://doi.org/10.1210/jc.2007-2467

73. Milic S, Mikolasevic I, Krznaric-Zrnic I, Stanic M, Poropat G, Stimac D, et al. Nonalcoholic steatohepatitis: emerging targeted therapies to optimize treatment options. Drug Des Devel Ther. 2015;9:4835-45. https://doi.org/10.2147/DDDT.S64877

74. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol. 2019;70(3):531-44. https://doi.org/10.1016/j.jhep.2018.10.033

75. Gluvic Z, Tomasevic R, Bojovic K, Obradovic M, Isenovic ER. Non-alcoholic fatty liver disease: a multidisciplinary clinical practice approach—the institutional adaptation to existing Clinical Practice Guidelines. Emergency and Critical Care Medicine. 2022;2(1):12-22. https://doi.org/10.1097/EC9.0000000000000016

76. Kahl S, Straßburger K, Nowotny B, Livingstone R, Klüppelholz B, Keßel K, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One. 2014;9(4):e94059. https://doi.org/10.1371/journal.pone.0094059

77. Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology. 2005;42(3):641-9. https://doi.org/10.1002/hep.20842

78. Mitrovic B, Gluvic ZM, Obradovic M, Radunovic M, Rizzo M, Banach M, et al. Non-alcoholic fatty liver disease, metabolic syndrome, and type 2 diabetes mellitus: where do we stand today? Arch Med Sci. 2023;19(4):884-94. https://doi.org/10.5114/aoms/150639

79. Resanović I, Gluvić Z, Zarić B, Sudar-Milovanović E, Vučić V, Arsić A, et al. Effect of Hyperbaric Oxygen Therapy on Fatty Acid Composition and Insulin-like Growth Factor Binding Protein 1 in Adult Type 1 Diabetes Mellitus Patients: A Pilot Study. Can J Diabetes. 2020;44(1):22-9. https://doi.org/10.1016/j.jcjd.2019.04.018

80. Gastaldelli A, Kozakova M, Højlund K, Flyvbjerg A, Favuzzi A, Mitrakou A, et al. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. Hepatology. 2009;49(5):1537-44. https://doi.org/10.1002/hep.22845

81. Mitrovic B, Gluvic Z, Klisic A, Obradovic M, Macut D, Tomasevic R, et al. A non-invasive method for estimating the severity of liver steatosis and the risk of fibrosis in non-obese type 2 diabetes patients with NAFLD. Acta Endocrinol (Buchar). 2022;18(4):480-7. https://doi.org/10.4183/aeb.2022.480

82. Resanović I, Zarić B, Radovanović J, Sudar-Milovanović E, Gluvić Z, Jevremović D, et al. Hyperbaric Oxygen Therapy and Vascular Complications in Diabetes Mellitus. Angiology. 2020;71(10):876-85. https://doi.org/10.1177/0003319720936925

83. Sakuragi T, Nagata S. Regulation of phospholipid distribution in the lipid bilayer by flippases and scramblases. Nat Rev Mol Cell Biol. 2023;24(8):576–96. https://doi.org/10.1038/s41580-023-00604-z

84. Nikkari T, Luukkainen P, Pietinen P, Puska P. Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. Ann Med. 1995;27(4):491-8. https://doi.org/10.3109/07853899709002458

85. Lee S, Birukov KG, Romanoski CE, Springstead JR, Lusis AJ, Berliner JA. Role of phospholipid oxidation products in atherosclerosis. Circ Res. 2012;111(6):778-99. https://doi.org/10.1161/CIRCRESAHA.111.256859

86. Ghosh A, Gao L, Thakur A, Siu PM, Lai CWK. Role of free fatty acids in endothelial dysfunction. J Biomed Sci. 2017;24(1):50. https://doi.org/10.1186/s12929-017-0357-5

87. Pilz S, Scharnagl H, Tiran B, Seelhorst U, Wellnitz B, Boehm BO, et al. Free

fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease. J Clin Endocrinol Metab. 2006;91(7):2542-7. https://doi.org/10.1210/jc.2006-0195

88. Virdis A. Endothelial Dysfunction in Obesity: Role of Inflammation. High Blood Press Cardiovasc Prev. 2016;23(2):83-5. https://doi.org/10.1007/s40292-016-0133-8

89. Pilz S, März W. Free fatty acids as a cardiovascular risk factor. Clin Chem Lab Med. 2008;46(4):429-34. https://doi.org/10.1515/CCLM.2008.118