Avicenna Journal of Medical Biochemistry
doi:10.15171/ajmb.2017.04 2017 June;5(1):22-28 http://ajmb.umsha.ac.ir

Research Article

# Effects of Different Doses of Metformin on Serum Fatty Acid Composition in Type 2 Diabetic Rats

**Nazi Aghaalikhani[1*], Mohammad Taghi Goodarzi[2], Zeinab Latifi[3], Azam Rezaei Farimani[2], Amir Fattahi[3]**

[1]Department of Nursing, Dezful Branch, Islamic Azad University, Dezful, Iran
[2]Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Department of Biochemistry and Clinical Laboratories, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

***Corresponding Author:**
Nazi Aghaalikhani, Department of Nursing, Dezful Branch, Islamic Azad University, Dezful, Iran.
Email: Alikhani234@yahoo.com

Received: 4 December 2016
Accepted: 13 April 2017
ePublished: 29 April 2017

## Abstract

**Background:** Several studies have shown association of fatty acids with type 2 diabetes (T2D), as well as metformin effects on blood glucose concentrations through affecting lipid metabolism.

**Objectives:** Since the exact therapeutic mechanism of metformin is not clear, in this study we investigated effects of different doses of metformin on serum fatty acids in rats with T2D.

**Materials and Methods:** Twenty-five adult albino male Wistar rats were divided into the following groups: Healthy, untreated T2D, and T2D rats receiving metformin for 4 weeks with doses of 100, 150, and 200 mg/kg/d. Serum insulin and triglyceride (TG) were measured using commercial kits. Serum total lipids were extracted by the Bligh-Dyer method and then compositions of fatty acids were evaluated using gas chromatograph.

**Results:** Monounsaturated fatty acid (MUFA) levels in T2D rats were lower than those in healthy rats ($P < 0.05$). We also observed that diabetic rats treated with 100 or 150 mg/kg/d of metformin had higher levels of arachidonic acid and polyunsaturated fatty acids (PUFA) in comparison with the healthy group ($P < 0.05$). Moreover, the T2D+Met (150 mg/kg) group showed increased levels of MUFA compared with the T2D group. Such a difference was seen in levels of arachidonic acid between the T2D+Met 100 mg/kg group and untreated T2D group. In the group treated with high doses of metformin (200 mg/kg/d), levels of palmitic acid, palmitoleic acid, and saturated fatty acid (SFA) were higher and levels of oleic acid, linoleic acid, arachidonic acid, MUFA, PUFA, and also SFA/UFA ratio were lower compared with other metformin treated and untreated groups ($P < .05$). In untreated T2D group, there were positive correlations between glucose levels and linoleic acid and PUFA levels ($r = 0.707$, $P = .049$ and $r = 0.726$, $P = .041$ respectively). Arachidonic acid levels were positively correlated with glucose levels in T2D rats treated with 100 mg/kg/d of metformin ($r = 0.969$, $P = .031$).

**Conclusions:** Our study showed that different doses of metformin could have different effects on serum levels of saturated and unsaturated fatty acids, as 200 mg/kg/d of metformin could increase and decrease saturated and unsaturated fatty acids respectively, while lower doses increased unsaturated fatty acids, particularly arachidonic acid.

**Keywords:** Diabetes, Metformin, Fatty acids, Insulin resistance

## Background

Diabetes mellitus is the most common endocrine/metabolic disorder which is caused by defects in insulin production, or cells response to insulin (1). Its main characteristic is hyperglycemia and impaired glucose tolerance (2). Type2 diabetes (T2D) or non-insulin dependent diabetes mellitus (NIDDM) occurs in those aged over 30 years, and those with a family history or insulin resistance, and accounts for more than 90% of diabetes cases (3,4). Various factors such as genetics, aging, obesity, and physical inactivity could increase the risk of T2D. More than 80% of patients with T2D suffer from obesity and insulin resistance (5).

Several factors have been proposed for such a relationship between obesity and T2D, and free fatty acids (FFA) are one of the major candidates. Reaven and Chen (6) stated for the first time that high plasma FFA levels are involved in insulin resistance. Various studies have investigated the association between plasma fatty acid concentrations and blood glucose levels in diabetic patients, and the existence of direct association has been reported (6-8). It has been supposed that the accumulation of fatty acids and its derivatives in different tissues including adipose, liver, and muscle tissues is a significant cause of insulin resistance (9,10). Several studies have shown that high intake of polyunsaturated fatty acids (PUFAs) could improve complications of diabetes (11,12). It was revealed that PUFAs have

beneficial effects on diabetes through preventing glucose exit from the cells via glucose phosphorylation, inhibition of glycogen synthesis, and oxidation of carbohydrates (13,14).

Metformin is a Biguanide which increases insulin sensitivity of liver, muscle, adipose, and other tissues, and also inhibits glycogenolysis and glucose production by the liver (15). In addition, it was reported that metformin reduces low-density lipoprotein (LDL), triglyceride (TG), and FFA (16,17). It was documented that metformin could change plasma levels of FFA in the fasting state and have no impact on their metabolism and lipolysis (18,19). While some have reported that part of the metformin effect on insulin sensitivity is exerted by reducing plasma FFA levlel (20).

Therapeutic effects of metformin on fatty acid metabolism have been investigated in several studies. Some results have indicated that metformin causes an increase in fatty acid oxidation (21,22), while some have not obtained such result (23.24). Considering the role of fatty acids in pathogenesis of T2D, and also antidiabetic effects of metformin, it is likely that this oral hypoglycemic agent can improve insulin resistance via increasing fatty acid oxidation rate and decreasing TG accumulation in adipose tissues and peripheral blood.

Since the exact therapeutic mechanism of metformin is not clear and based on our knowledge there is no report about effects of different doses of metformin on fatty acid levels in serum, in this study we investigated effects of different doses of metformin on serum fatty acids in rats with T2D. The results of present study could clarify one of the possible metformin therapeutic mechanisms in T2D.

## Materials and Methods

Twenty-five 2-month old adult albino male Wistar rats (weighing approximately 150-200 g) were purchased from RAZI Institute, Iran. All rats were fed standard pellet and fresh water, and were housed under standard conditions of temperature (25 ± 2°C), and humidity (60%–70%) with 12 hours light/dark cycle. All animal handling and experimental procedures were performed according to the guidelines and Ethical Committee Approval was obtained (Code No. 9309255065). Induction of T2D was done in 20 rats by streptozotocin (STZ, Amazon, USA) and nicotinamide (NA, Sigma, Germany) briefly as follows: rats were intraperitoneally injected with BWT STZ (60 mg/kg) (dissolved in 0.1 M sodium citrate pH 4.5); and after 15 minutes, they were injected with BWT (120 mg/kg), then they were injected with NA to prevent beta-cytotoxic effect of STZ and induction of type 1 diabetes mellitus (T1D). Co-treatment of STZ with NA causes T2D induction. To confirm T2D in the rats, their fasting blood glucose (FBS) was measured with glucometer (MediSense Optimum TM Xceed, Abott Diabetes Care Inc., USA) 72 hours after injection of STZ/NA. Rats with FBS value above 170 mg/mL were considered diabetic. Five healthy rats that had been not subjected for T2D induction were considered as healthy rats (group 1) and the diabetic rats were randomly divided into 4 groups as follows: Group 2 consisted of 5 untreated diabetic rats as control. Groups 3, 4, and 5 consisted of 5 diabetic rats in each group receiving metformin treatment for 4 weeks with doses of 100, 150, and 200 mg/kg/d. The blood samples were taken from inferior vena cava and the serums were separated and stored at -20°C. The weight of animals was measured before and after induction of diabetes and treatment with metformin. Insulin was measured using ELISA kits (ALPCO, USA). Serum TG levels were assessed 4 weeks after treatment with metformin using commercial kits (Pars Azmoon kit, Iran) based on colorimetric method (spectrophotometry).

The Bligh-Dyer method was used to extract serum total lipids and then the lipids were esterified with methanol during catalysis with acetyl chloride. After extraction of fatty acid methyl esters, the composition of fatty acids was evaluated using previously described method. Briefly, fatty acid methyl ester derivatives were separated on a 60×0.25-mm Teknokroma TR CN100 column using a Buck Scientific model 610 gas chromatograph (SRI Instruments, Torrance, USA) which was equipped with a split injector and a flame ionization detector and helium as carrier gas. The oven temperature was increased from 170°C to 210°C at the rate of 1°C/min and then maintained stable for 45 minutes. We used tridecanoic acid (13:0) as the known internal standard which was injected to identify peak retention times. The levels of fatty acids were calculated as the percentage of each fatty acid from the total.

### Statistical Analysis

Given that the study was performed on animal models and all conditions were the same for animals (except desired intervention), after confirming normal distribution of data using Kolmogorov-Smirnov test, parametric statistical methods were used for data analysis. To compare quantitative data among different groups, one-way analysis of variance (ANOVA), following Tukey post hoc test was used. For comparing quantitative data before and after the intervention in the same group, paired *t* test was applied. Pearson correlation coefficient was used to evaluate possible associations between various factors. *P* values < .05 were considered significant. SPSS version 16.0 was used for the statistical analyses.

## Results

Our results showed that serum glucose levels of the T2D group were significantly higher than those of the healthy rats and diabetic rats receiving various doses of metformin ($P < .05$) (Table 1). Blood glucose levels of

**Table 1.** Blood Glucose, Insulin and Triglyceride Levels 1 Month After Treatment With Metformin

| Groups | Healthy | Untreated T2D | T2D + Met (100 mg/kg/d) | T2D + Met (150 mg/kg/d) | T2D + Met (200 mg/kg/d) |
|---|---|---|---|---|---|
| Glucose (mg/dL) | 150.4 ± 26.14 | 403.12 ± 124.06 [a] | 242.50 ± 36.04 [a, b] | 156.50 ± 14.16 [b] | 136 ± 73.18 [b] |
| Insulin (μU/mL) | 7.35 ± 1.64 | 8.07 ± 1 | 8.45 ± 1.89 | 9.76 ± 1.63 [a] | 11.07 ± 1.31 [a, b, c] |
| Triglyceride (mg/dL) | 132.17 ± 30.36 | 101 ± 21.3 | 106.60 ± 23.76 | 122.33 ± 42.39 | 58.75 ± 18.88 [a, b, c, d] |

Healthy: non-diabetic rats; T2D: rats with type 2 diabetes; T2D+Met 100/150/200: rats with type 2 diabetes treated with 100, 150 or 200 mg/kg/d of metformin. Significant difference ($P < .05$) in comparison with [a] healthy group, [b] T2D, [c] T2D+Met 100 mg/kg/d, [d] T2D+Met 150 mg/kg/d.

diabetic rats which received 150 and 200 mg/kg/d of metformin were also statistically lower than the blood glucose levels of diabetic rats that were treated with 100 mg/kg/d of metformin ($P < .05$). We found that 1-month metformin treatment with doses of 150 and 200 mg/kg/d could reduce blood glucose levels to the levels of healthy rats (Table 1). Insulin and TG levels in T2D+Met (200 mg/kg) group were respectively higher and lower in comparison with healthy, T2D, and T2D+Met (100 mg/kg) groups ($P < .05$).

Using gas-liquid chromatography, we could analyze serum levels of saturated (palmitic acid and stearic acid) and unsaturated (palmitoleic acid, oleic acid, linoleic acid, and arachidonic acid) fatty acids. However, the amounts of other possible types of serum fatty acids were very low and undetectable. As shown in Table 2, levels of various serum fatty acids between healthy and T2D groups were not statistically different and only monounsaturated fatty acid (MUFA) levels in T2D rats were lower than those in healthy rats ($P < .05$). We also observed that diabetic rats treated with 100 or 150 mg/kg/d of metformin had higher levels of arachidonic acid and PUFA in comparison with the healthy group ($P < .05$). Moreover, the T2D+Met (150 mg/kg) group showed increased levels of MUFA in comparison with the T2D group. Such a difference was seen in levels of arachidonic acid between the T2D+Met 100 mg/kg and untreated T2D groups. In the group treated with high doses of metformin (200 mg/kg/d), levels of palmitic acid, palmitoleic acid, and SFA were higher and levels of oleic acid, linoleic acid, arachidonic acid, MUFA, PUFA, and also SFA/UFA ratio were lower compared with other metformin treated and untreated groups ($P < .05$).

The results of correlation analysis between various fatty acid levels and serum levels of glucose, insulin and TG and also the rats' weight are demonstrated in Table 3. There were negative correlations between serum TG levels and palmitic acid, palmitoleic acid, and SFA, and also positive correlations between TG concentrations and oleic acid, linoleic acid, MUFA, and PUFA levels in the healthy rats. In untreated T2D group, there were positive correlations between glucose levels and linoleic acid and PUFA levels ($r = 0.707$, $P = .049$ and $r = 0.726$, $P = .041$ respectively). Arachidonic acid levels were positively correlated with glucose levels in T2D rats treated with 100 mg/kg/d of metformin ($r = 0.969$, $P = .031$). TG levels in T2D+Met (150 mg/kg/d) were correlated negatively with stearic acid and SFA and positively with oleic acid, linoleic acid, and MUFA levels. We found no correlation between various fatty acid levels and insulin levels in any of studied groups.

## Discussion

Reaven (25) for the first time evaluated association between lipids and insulin resistance and showed the role of increased plasma fatty acid in insulin resistance. Studies also have shown that there is a direct relationship between plasma levels of fatty acids and blood glucose levels in

**Table 2.** Fatty Acid Composition of Serum in Healthy and Type 2 Diabetic Rats Treated With Different Doses of Metformin

| % Of Total Fatty Acids | Healthy | Untreated T2D | T2D + Met (100 mg/kg/d) | T2D + Met (150 mg/kg/d) | T2D + Met (200 mg/kg/d) |
|---|---|---|---|---|---|
| 16:0 (Palmitic acid) | 30.08 ± 17.5 | 27.02 ± 8.77 | 16.24 ± 5.99 | 18.15 ± 11.32 | 56.01 ± 7.12 [a, b, c, d] |
| 18:0 (Stearic acid) | 9.97 ± 3.16 | 13.34 ± 2.33 | 14.04 ± 3.56 | 13.11 ± 2.08 | 9.54 ± 3.69 |
| 16:1 (Palmitoleic acid) | 2.17 ± 1.42 | 2 ± 0.76 | 1.16 ± 0.84 | 1.28 ± 1.2 | 4.69 ± 0.39 [a, b, c, d] |
| 18:1n-9 (Pleic acid) | 31.49 ± 10.7 | 21.87 ± 2.58 | 24.23 ± 6.42 | 30.09 ± 8.12 | 12.73 ± 2.44 [a, b, c, d] |
| 18:2n-6 (Linoleic acid) | 20.68 ± 5.82 | 25.97 ± 7.89 | 27.64 ± 7.73 | 24.79 ± 5.9 | 12.07 ± 1.05 [a, b, c, d] |
| 20:4n-6 (Arachidonic acid) | 5.57 ± 1.77 | 9.77 ± 3.05 | 14.66 ± 2.55 [a, b] | 12.55 ± 4.95 [a] | 4.95 ± 0.81 [b, c, d] |
| SFA | 40.06 ± 15.7 | 40.36 ± 7.53 | 32.29 ± 3.43 | 31.26 ± 12.8 | 65.55 ± 3.7 [a, b, c, d] |
| MUFA | 33.67 ± 9.32 | 22.88 ± 2.91 [a] | 25.39 ± 6.76 | 31.38 ± 7.06 [b] | 17.42 ± 2.25 [a, b, c, d] |
| PUFA | 26.26 ± 6.7 | 35.75 ± 9.12 | 42.3 ± 9.39 [a] | 37.35 ± 6.48 [a] | 17.03 ± 1.86 [a, b, c, d] |
| SFA/UFA | 0.789 ± 0.594 | 0.703 ± 0.535 | 0.48 ± 0.077 | 0.503 ± 0.315 | 1.926 ± 0.326 [a, b, c, d] |

Healthy, non-diabetic rats; T2D, rats with type 2 diabetes; T2D+Met 100/150/200, rats with type 2 diabetes treated with 100, 150 or 200 mg/kg/d of metformin; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; SFA/UFA, ratio of saturated to unsaturated fatty acids. Significant difference ($P < .05$) in comparison with [a] healthy group, [b] T2D, [c] T2D+Met 100 mg/kg/d, [d] T2D+Met 150 mg/kg/d.

**Table 3.** Correlations Between Various Serum Fatty Acid Levels and Levels of Glucose, Insulin and Triglyceride and Also the Rats' Weight in Studied Groups

| | Weight | | Glucose | | Insulin | | Triglyceride | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| **Healthy Group** | | | | | | | | |
| Palmitic acid | 0.822 | .088 | 0.722 | .168 | 0.549 | .338 | -0.915 | **.03** |
| Stearic acid | -0.724 | .166 | -0.554 | .333 | -0.852 | .067 | 0.292 | .633 |
| Palmitoleic acid | 0.859 | .063 | 0.614 | .271 | 0.480 | .414 | -0.912 | **.031** |
| Oleic acid | -071 | .179 | -0.590 | .295 | -0.326 | .592 | 0.982 | **.003** |
| Linoleic acid | -0.849 | .069 | -0.717 | .173 | -0.501 | .390 | 0.879 | **.048** |
| Arachidonic acid | -0.436 | .463 | -0.721 | .169 | -0.674 | .212 | 0.440 | .458 |
| SFA | 0.77 | .128 | 0.693 | .194 | 0.44 | .458 | -0.961 | **.009** |
| MUFA | -0.684 | .203 | -0.583 | .302 | -0.301 | .623 | 0.988 | **.002** |
| PUFA | -0.853 | .066 | -0.814 | .093 | -0.613 | .271 | 0.878 | **.049** |
| **T2D Group** | | | | | | | | |
| Palmitic acid | -0.694 | .056 | -0.613 | .106 | 0.12 | .777 | -0.220 | .600 |
| Stearic acid | 0.478 | .231 | 0.213 | .613 | 0.255 | .543 | -0.253 | .545 |
| Palmitoleic acid | -0.756 | .51 | -0.705 | .051 | -0.159 | .707 | -00.168 | .691 |
| Oleic acid | 0.142 | .737 | -0.463 | .248 | -0.107 | .800 | 0.485 | .223 |
| Linoleic acid | 0.422 | .298 | 0.707 | **.049** | -0.193 | .648 | 0.173 | .683 |
| Arachidonic acid | 0.608 | .110 | 0.343 | .406 | 0.090 | .832 | 0.01 | .981 |
| SFA | -0.66 | .75 | -0.648 | .082 | 0.218 | .603 | -0.335 | .418 |
| MUFA | -0.072 | .865 | -0.597 | .118 | -0.137 | .746 | -0.388 | .343 |
| PUFA | 0.568 | .142 | 0.726 | **.041** | -0.136 | .747 | 0.153 | .718 |
| **T2D+Met 100 Group** | | | | | | | | |
| Palmitic acid | 0.344 | .571 | -0.118 | .882 | -0.373 | .536 | 0.586 | .300 |
| Stearic acid | -0.137 | .826 | -0.357 | .643 | 0.435 | .464 | -0.664 | .222 |
| Palmitoleic acid | 0.36 | .552 | 0.04 | .960 | -0.337 | .579 | 0.664 | .222 |
| Oleic acid | -0.408 | .495 | -0.411 | .589 | -0.811 | .095 | 0.252 | .683 |
| Linoleic acid | 0.022 | .972 | 0.34 | .660 | 0.524 | .365 | -0.190 | .759 |
| Arachidonic acid | 0.226 | .715 | 0.969 | **.031** | 0.833 | .080 | -0.722 | .168 |
| SFA | 0.458 | .438 | -0.523 | .477 | -0.2 | .747 | 0.333 | .584 |
| MUFA | -0.343 | .572 | -0.389 | .611 | -0.812 | .095 | 0.321 | .598 |
| PUFA | 0.079 | .899 | 0.465 | .535 | 0.658 | .228 | -0.353 | .560 |
| **T2D+Met 150 Group** | | | | | | | | |
| Palmitic acid | 0.285 | .584 | -0.264 | .613 | 0.128 | .810 | -0.750 | .086 |
| Stearic acid | -0.162 | .759 | -0.387 | .449 | 0.441 | .381 | -0.943 | **.005** |
| Palmitoleic acid | 0.406 | .424 | -0.057 | .914 | 0.160 | .762 | -0.679 | .138 |
| Oleic acid | -0.117 | .826 | 0.392 | .442 | -0.354 | .491 | 0.896 | **.016** |
| Linoleic acid | 0.190 | .718 | 0.576 | .232 | -0.356 | .489 | 0.938 | **.006** |
| Arachidonic acid | -0.717 | .109 | -0.549 | .259 | 0.488 | .326 | -0.311 | .549 |
| SFA | 0.226 | .667 | -0.297 | .568 | 0.185 | .726 | -0.817 | **.047** |
| MUFA | -0.065 | .903 | 0.441 | .381 | -0.380 | .457 | 0.915 | **.011** |
| PUFA | -0.375 | .464 | 0.105 | .844 | 0.049 | .926 | 0.616 | .193 |
| **T2D+Met 200 Group** | | | | | | | | |
| Palmitic acid | 0.950 | .202 | -0.901 | .285 | -0.889 | .302 | -0.905 | .280 |
| Stearic acid | -0.830 | .377 | 0.985 | .111 | 0.732 | .477 | 0.756 | .455 |
| Palmitoleic acid | -0.011 | .993 | -0.686 | .519 | 0.167 | .893 | 0.132 | .915 |
| Oleic acid | -0.823 | .385 | 0.987 | .103 | 0.724 | .485 | 0.748 | .462 |
| Linoleic acid | -0.893 | .297 | 0.331 | .785 | 0.952 | .197 | 0.941 | .219 |
| Arachidonic acid | -0.899 | .289 | 0.344 | .777 | 0.956 | .189 | 0.946 | .211 |
| SFA | 0.999 | .29 | -0.751 | .459 | -0.98 | .128 | -0.986 | .106 |
| MUFA | -0.896 | .293 | 0.953 | .195 | 0.816 | .392 | 0.835 | .37 |
| PUFA | -0.896 | .294 | 0.337 | .781 | 0.954 | .194 | 0.943 | .216 |

Healthy, non-diabetic rats; T2D, rats with type 2 diabetes; T2D+Met 100/150/200, rats with type 2 diabetes treated with 100, 150 or 200 mg/kg/d of metformin; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; SFA/UFA, ratio of saturated to unsaturated fatty acids.

diabetic patients (6,7,26). Due to the lack of information about effects of T2D and also administration of metformin on serum levels of saturated and unsaturated fatty acids, in this study we examined the influence of T2D and also the effect of treatment with metformin at doses of 100, 150 and 200 mg/kg/d on serum fatty acids in rats.

Our results showed that treatment with metformin at doses of 150 and 200 mg/kg/d reduced blood glucose levels up to normal range in T2D rats, but 1-month treatment with dose of 100 mg/kg/d could not bring blood glucose levels back down to normal, although the glucose levels were lower than those in diabetic rats without treatment. It is likely that one of the metformin mechanisms in reducing blood glucose is increasing insulin production and secretion, as we have seen higher insulin levels in T2D rats treated with metformin at doses of 150 and 200 mg/kg/d compared with healthy and untreated T2D rats. However, some previous studies have demonstrated that metformin further increases the insulin sensitivity instead of insulin levels (27,28). Such an effect of metformin can probably be seen in our studied low-dose group (100 mg/kg/d) because in this group the levels of glucose were lower than those in the untreated T2D group, while insulin levels were not significantly different between 2 groups. It seems that metformin at low doses decreases blood glucose level via some ways other than inducing insulin secretion (maybe via increasing insulin sensitivity); but at high doses, it can also increase the insulin levels. Though, to arrive at such a conclusion, further studies with long-term metformin treatment are required.

On the other hand, we found that in T2D rats metformin at low doses (100 and 150 mg/kg/d) could not reduce serum TG levels; but at dose of 200 mg/kg/d, it significantly reduced amounts of TG, which could be another glucose-lowering mechanism of metformin. Reducing TG amounts in pancreatic beta cells with insulin resistance due to addition of metformin at a medium level has been reported which could confirm our results (29). Similar animal studies on rats with genetica T2D (ZDF rats) have demonstrated reduction of TG levels in Langerhans islets (30,31). Consistent results were also documented from human studies (32). Failure of low doses of metformin (100 and 150 mg/kg/d) in reducing TG levels in our study possibly was due to the short duration of treatment (1 month).

Our results showed that short-term treatment of T2D significantly decreases only serum amounts of MUFA in rats. In a study, it was found that the amounts of palmitic, stearic and oleic acids were higher and also levels of arachidonic and linolenic acids were lower in the liver and kidneys of diabetic mice compared with normal mice (33). In the present study, it was found that treatment with metformin at doses of 100 and 150 mg/kg/d increased arachidonic acid levels, and also metformin doses of 150 mg/kg/d could increase MUFA levels and thus metformin could compensate the effect of diabetes on serum fatty acids. On the other hand, high dose of metformin (200 mg/kg/d) increased SFA/UFA ratio by decreasing the amounts of PUFA and MUFA and increasing SFA. In a previous study on non-diabetic obese subjects with insulin resistance, no significant changes were seen in fatty acids of serum phospholipids and TG following short period metformin therapy (32). Although in a study on diabetic mice, it was reported that metformin treatment could reduce the amounts of palmitic, stearic and oleic acids and increase levels of arachidonic and linolenic acids in liver and kidney (33). Moreover, other studies have shown reduction in plasma FFA level in patients treated with metformin (18,34). Association of insulin action with fatty acid levels of phospholipids in serum and red blood cells has been documented (35). Considering our findings and previously reported results about existence of positive correlation between levels of saturated fatty acids, such as palmitic acid and insulin resistance (36), it could be concluded that metformin at high doses has a negative effect on insulin resistance via increasing saturated fatty acid levels. Moreover, the levels of insulin in the T2D rats receiving 200 mg/kg/d of metformin were higher than those in other groups which could be considered as compensatory response against increased insulin resistance. Because of the contradictory results obtained from previous studies, the results of present study are difficult to interpret. For example, in a study it was mentioned that even unsaturated fatty acids, especially palmitoleic, oleic and linoleic acids could induce insulin resistance and none of unsaturated fatty acids could improve insulin resistance (37). Therefore based on this report, it can be concluded that reduction of unsaturated fatty acid levels in our study following administration of 200 mg/kg/d of metformin could also improve insulin resistance. Furthermore, due to the increasing effects of 100 and 150 mg/kg/d of metformin on PUFA (arachidonic acid) which were seen in our study, maybe these doses were not enough to improve insulin resistance via fatty acid reduction. While in another study, it was indicated that oral administration of unsaturated fatty acids, especially omega-3 improves insulin resistance in diabetic rats (38). Finally it could be postulated that although short-term treatment of metformin at high doses decreases blood glucose and increases insulin levels but the levels of both serum saturated and unsaturated fatty acids change in favor of insulin resistance. On the contrary, short duration treatment with lower doses of metformin increases unsaturated fatty acids maybe in favor of insulin sensitivity. However, it has been documented that in therapeutic doses, metformin induces intracellular signaling via activating AMP dependent protein kinase (AMPK), which ultimately inhibits the synthesis and

stimulates the oxidation of fatty acids (21). This process in long term could induce fatty acid uptake from plasma (39), so it seems further study with long term treatment is needed to clarify effects of metformin on serum fatty acids.

The present study has several limitations which would be considered in future investigations; first, we did not evaluate effects of very low doses of metformin. Secondly, in the present study we examined serum total fatty acids and it would be better to investigate fatty acids of phospholipids as well. Thirdly, our studied animals did not have long history of T2D and also we just administrated metformin for short period (1 month).

In conclusion, our study showed that high dose of metformin is more effective in reducing blood glucose and insulin levels. It was also found that different doses of metformin could have different effects on serum levels of saturated and unsaturated fatty acids, as 200 mg/kg/d of metformin could increase and decrease saturated and unsaturated fatty acids respectively, while lower doses increased unsaturated fatty acids, particularly arachidonic acid.

## Authors' Contribution

Study designing and interpretation of data: NA and MTG; Laboratory analysis and experimental works: ZL, ARF and AF; Manuscript drafting: NA and ZL; Data analysis: AF; Manuscript critical revision: NA and MTG.

## Conflict of Interest Disclosures

All authors declare that there is no conflict of interests.

## Funding/Support

This work was financially supported by Islamic Azad University of Dezful.

## References

1. Aune D, Norat T, Leitzmann M, Tonstad S, Vatten LJ. Physical activity and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis. Eur J Epidemiol. 2015;30(7):529-542. doi:10.1007/s10654-015-0056-z.
2. Newsholme P, Krause M. Nutritional regulation of insulin secretion: implications for diabetes. Clin Biochem Rev. 2012;33(2):35-47.
3. Association AD. Classification and diagnosis of diabetes. Diabetes care. 2015;38(suppl 1):S8-S16.
4. Skyler JS, Bergenstal R, Bonow RO, Buse J, Deedwania P, Gale EA, et al. Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA diabetes trials: a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association. J Am Coll Cardiol. 2009;53(3):298-304.
5. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman Med J. 2012;27(4):269-273.
6. Reaven GM, Chen Y-DI. Role of abnormal free fatty acid metabolism in the development of non-insulin-dependent diabetes mellitus. Am J Med. 1988;85(5):106-112.
7. Boden G, Chen X. Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. J Clin Invest. 1995;96(3):1261.
8. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes. 1999;48(6):1270-1274.
9. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IκB-α. Diabetes. 2002;51(7):2005-2011.
10. Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. Curr Opin Lipidol. 2008;19(3):235-241. doi: 10.1097/01.mol.0000319118.44995.9a.
11. Sirtori CR, Crepaldi G, Manzato E, Mancini M, Rivellese A, Paoletti R, et al. One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: reduced triglyceridemia, total cholesterol and increased HDL-C without glycemic alterations. Atherosclerosis. 1998;137(2):419-427.
12. Kesavulu M, Kameswararao B, Apparao C, Kumar E, Harinarayan C. Effect of omega-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. Diabetes Metab. 2002;28(1):20-6..
13. Kahn BB. Glucose transport: pivotal step in insulin action. Diabetes. 1996;45(11):1644-1654.
14. Boden G, Chen X, Ruiz J, White J, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest. 1994;93(6):2438.
15. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. Ann Intern Med. 2000;133(1):73.
16. Perriello G, Misericordia P, Volpi E, Santucci A, Santucci C, Ferrannini E, et al. Acute antihyperglycemic mechanisms of metformin in NIDDM: evidence for suppression of lipid oxidation and hepatic glucose production. Diabetes. 1994;43(7):920-928.
17. Jeppesen J, Zhou M-Y, Chen YI, Reaven GM. Effect of metformin on postprandial lipemia in patients with fairly to poorly controlled NIDDM. Diabetes Care. 1994;17(10):1093-1099.
18. Castro Cabezas M, Van Wijk JP, Elte JWF, Klop B. Effects of metformin on the regulation of free Fatty acids in insulin resistance: A double-blind, placebo-controlled study. J Nutr Metab. 2012;2012:394623. doi:10.1155/2012/394623.
19. Magalhães F, Gouveia L, Torquato M, Paccola G, Piccinato C, Foss M. Metformin increases blood flow and forearm glucose uptake in a group of non-obese type 2 diabetes patients. Horm Metab Res. 2006;38(08):513-517. doi:10.1055/s-2006-949522.
20. Zhang T, He J, Xu C, Zu L, Jiang H, Pu S, et al. Mechanisms of metformin inhibiting lipolytic response to isoproterenol in primary rat adipocytes. J Mol Endocrinol. 2009;42(1):57-66. doi:10.1677/JME-08-0130.
21. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108(8):1167-1174.
22. Wang C, Liu F, Yuan Y, Wu J, Wang H, Zhang L, et al. Metformin suppresses lipid accumulation in skeletal muscle by promoting fatty acid oxidation. Clin Lab. 2013;60(6):887-896.
23. Fulgencio J-P, Kohl C, Girard J, Pégorier J-P. Effect of metformin on fatty acid and glucose metabolism in freshly isolated hepatocytes and on specific gene expression in cultured hepatocytes. Biochem Pharmacol. 2001;62(4):439-446.
24. Brant S, Cobert M, West L, Holmes C, Jessen M, Peltz M. Metformin reduces fatty acid oxidation in the isolated perfused rat heart. J Surg Res. 2014;186(2):494.
25. Reaven GM. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595-1607.

26. Boden G. Free fatty acids, insulin resistance, and type 2 diabetes mellitus. Proc Assoc Am Physicians. 1999;111(3):241-248.
27. Schäfer G. Biguanides. A review of history, pharmacodynamics and therapy. Diabete Metab. 1982;9(2):148-163.
28. Bailey CJ. Biguanides and NIDDM. Diabetes Care. 1992;15(6):755-772.
29. Patanè G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F. Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. Diabetes. 2000;49(5):735-740.
30. Sreenan S, Sturis J, Pugh W, Burant CF, Polonsky KS. Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. American Journal of Physiology-Endocrinology And Metabolism. 1996;271(4):E742-E747.
31. Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. Diabetes. 1995;44(8):863-870.
32. Rodríguez Y, Giri M, Feyen E, Christophe AB. Effect of metformin vs. placebo treatment on serum fatty acids in non-diabetic obese insulin resistant individuals. Prostaglandins Leukot Essent Fatty Acids. 2004;71(6):391-397. doi:10.1016/j.plefa.2004.08.004.
33. Kumar NA, Pari L. Combined N-benzoyl-d-phenylalanine and metformin treatment reverses changes in the fatty acid composition of streptozotocin diabetic rats. J Basic Clin Physiol Pharmacol. 2006;17(1):17-28.
34. Prato SD, Marchetto S, Pipitone A, Zanon M, De Kreutzenberg SV, Tiengo A. Metformin and free fatty acid metabolism. Diabetes Metab Rev. 1995;11(S1):S33-S41.
35. Clifton P, Nestel P. Relationship between plasma insulin and erythrocyte fatty acid composition. Prostaglandins Leukot Essent Fatty Acids. 1998;59(3):191-194.
36. Salmeron J, Hu FB, Manson JE, et al. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr. 2001;73(6):1019-1026.
37. Kusunoki M, Tsutsumi K, Nakayama M, Kurokawa T, Nakamura T, Ogawa H, et al. Relationship between serum concentrations of saturated fatty acids and unsaturated fatty acids and the homeostasis model insulin resistance index in Japanese patients with type 2 diabetes mellitus. J Med Invest. 2007;54(3,4):243-247.
38. Kusunoki M, Tsutsumi K, Hara T, Ogawa H, Nakamura T, Miyata T, et al. Ethyl icosapentate (omega-3 fatty acid) causes accumulation of lipids in skeletal muscle but suppresses insulin resistance in OLETF rats. Metabolism. 2003;52(1):30-34. doi:10.1053/meta.2003.50012.
39. Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J Clin Invest. 2010;120(7):2355-2369. doi:10.1172/JCI40671.