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Effects of Metformin alone, Metformin with Flaxseeds Oil on Serum 1,5 anhydroglucitol, Adiponectin and Insulin Resistance in Patients with Type 2 Diabetes Mellitus

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Abstract

Background Adiponectin is an amino acid collagen-like protein that is secreted by adipocytes to acts as a hormone with anti-inflammatory and insulin-sensitizing properties. 1,5 anhydroglucitol, is 1-deoxy form of glucose, is a validated marker of short-term glycemic control. Flaxseed oil is a colorless to yellowish oil obtained from the dried, ripened seeds of the flax plant (*Linumusitatissimum*, L).

Objectives To investigate the effects of metformin alone, metformin with flaxseed oil on fasting serum 1,5 anhydroglucitol, adiponectin and insulin resistance.

Methods Newly diagnosed (≤ 1year) male and female patients with type 2 diabetes mellitus aged 25 to 70 years were enrolled and divided into two groups; group 1 consisted of 32 patients, treated by oral metformim alone over a period of 12 weeks an group 2 consisted of 30 patients treated by oral metformim with flaxseed oil. Fasting serum 1,5 anhydroglucitol, adiponectin, fasting plasma glucose and fasting serum insulin were estimated. All parameters were measured initially, before any intervention, and later on at two steps, the 6th and the 12th week of the study.

Results After 12 weeks of treatment with metformin alone, metformin with flaxseed oil there was significant improvement in both 1,5 anhydroglucitol (p = 0.010 and 0.013, respectively) and adiponectin (p = 0.041 and 0.037, respectively). For insulin resistance, p value with metformin, metformin with flaxeed oil was 0.105 and 0.110, respectively. Both results showed an apparent improvement only which was statistically insignificant.

Conclusion Metformin, metformin with flaxseed oil associated with statistically significant improvement in 1,5 anhydroglucitol and adiponectin. Insulin resistance in both treatment groups showed only insignificant apparent improvement. Metformin with flaxseed oil group was more effective in insulin resistance improvement and elevation of adiponectin hormone.

Key words Adiponectin, 1,5 anhydroglucitol, Homeostasis model assessment for insulin resistance.

List of abbreviation: T2DM = type 2 diabetes mellitus, FSO = flaxseed oil, 1,5AG = 1,5 anhydroglucitol, ADP = adiponectin, HOMA-IR = Homeostasis model assessment of insulin resistance.

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency [(1)]. T2DM accounts for ~90-95% of cases of diabetes [(2)]. Metformin has been the most recommended monotherapy of T2DM [(3,4)].

Maida et al [(5)] and Zhou et al [(6)] reported that the activation of adenosine monophosphate - activated protein kinase by metformin in the liver, and probably in other tissue provides a unified explanation for the pleiotropic beneficial effects of this drug.

Flaxseed oil (FSO), also known as linseed oil, is a colorless to yellowish oil obtained from the

dried, ripened seeds of the flax plant (*Linumusitatissimum*, L) [7]. Many of flaxseed's health effects are attributed to its alpha-linolenic acid lignan and fiber components [8]. The anti-diabetogenic property of flaxseed is the active fraction: LU6.

Obesity is a major public health problem and it increases insulin resistance, reactive oxygen species (ROS) generation and nuclear factor NF-κBactivation [9]. The increase in NF-κB activation leads to low grade inflammation and contributes to the development of diabetes [10].

1,5 anhydroglucitol (1,5AG) was first discovered in the plant family *Polygala senega*in 1888. The presence of the compound in human blood [11] and cerebrospinal fluid was established in 1972 and 1973, respectively. 1,5-AG, is 1-deoxy form of glucose, is a major metabolically inert circulating polyol arising primarily from ingestion and excreted competitively with glucose [12]. Research studies have shown that 1,5AG originates mostly from foods with a mean daily intake of ~4.4 mg/day. The rate of intake is matched by the rate of daily excretion with a bodily pool of about 500–1000 mg of 1,5AG is constantly maintained [13].

1,5AG is a validated marker of short-term glycemic control and its levels in blood respond within 24 hour as a result of glucose's competitive inhibition of 1,5AG reabsorption in the kidney tubule [14].

Adiponectin (ADP) is a 244–amino acid collagen-like protein that is solely secreted by adipocytes to acts as a hormone with anti-inflammatory and insulin-sensitizing properties [15] and to be involved in cardiovascular tone. Obesity caused down-regulation of adiponectin which is the mechanism whereby obesity could cause insulin resistance and diabetes [15]. The high molecular weight oligomer of ADP has been implicated as the major active form responsible for the insulin-sensitizing effects of ADP in the liver and peripherally than total ADP levels [16].

ADP may decrease the risk of T2DM, including suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and stimulation of insulin secretion [15,17].

ADP receptors have been cloned in the skeletal muscle (AdipoR1) and liver (AdipoR2), AdipoR1 and AdipoR2 have been shown to mediate adenosinmonophosphate-activated protein kinase, peroxisome proliferator-Activated receptor-alpha (PPAR-α) ligand activities, glucose uptake and fatty-acid oxidation by adiponectin [18] .

T2DM is caused by a combination of progressive β-cell dysfunction, relative insulin deficiency, and variable degrees of insulin resistance that lead to dysregulation of glucose homeostasis [19]. Homeostasis model assess-ment of insulin resistance (HOMA-IR) is a mathematical model which can estimate an individual's degree of insulin sensitivity (HOMA % S) and level of beta cell function (HOMA % B) from simultaneous measurements of fasting plasma glucose and insulin or C-peptide concentrations.

IR is a condition in which normal amounts of insulin are inadequate to produce normal responses from fat, muscle (promote glucose uptake) and liver (inhibit glucose output) cells [20]. It is a major hallmark in the development of T2DM [21]. In non-diabetic individuals the best HOMA-IR cut-off levels ranged from 1.85 in men to 2.07 in women. A lower cut-off value for diabetic than non-diabetic individuals ranged from 1.60 in men to 2.05 in women probably because in the diabetic population there is an increased prevalence of hypertension, obesity, and dyslipidemia [22].

The objective of the study is to investigate the effects of metformin alone, metformin with FSO on fasting serum 1,5AG, adiponectin and HOMA-IR.

Methods

This study is a randomized, single blinded interventional, dose escalation study of 12 weeks treatment duration, comparative and prospective study. The study was conducted on

adult patients with T2DM attending the Diabetic and Endocrine Diseases Clinic in Mosul city, Iraq over the period from March 3, 2014 through February 15, 2015.

The study concept and design were approved by the Institute Review Board of the College of Medicine, Al-Nahrain University. The study included newly or recently diagnosed (≤ 1year) male and female patients (N = 62) with T2DM whose ages ranged between 25 and 70 years.

The study excluded patients who were known to have hepatobiliary diseases, hypo-thyroidism, chronic kidney diseases or nephrotic syndrome, cigarette smoking, the use of any glucose altering medications, such as oral contraceptive pills, diuretics, steroids and neuroleptics during the last month. In addition, pregnant or lactating women, patients with hematological abnormalities such as hemolytic anemia.

A 30, apparently healthy, volunteers whose age matched enrolled patients were involved. All the enrolled participants were informed about the aim of the study and an written consent was obtained from each of them. Thereafter, the patients were divided into two groups as follows:

Group 1: consisted of 32 patients, treated by oral metformim alone (merk-Germany), 500 mg b.d initially and the dose was adjusted according to the glycosylated hemoglobin and fasting plasma glucose readings over a period of 12 weeks, which is the period of the study.

Group 2: consisted of 30 patients treated by metformin plus FSO in a form of soft gel capsules obtained from a local source in Mosul city (Al-Emad Factory). Each flaxseed capsule contains 500 mg of FSO. The dose of flaxseed was two capsules given in a single dose (1 g) after lunch.

Thereafter, each patient instructed to have fasting serum 1,5AG, ADP, fasting plasma glucose and fasting plasma insulin. Standard kits were used to measure biochemical profiles suggested in this study using double-sandwich ELISA technique for both 1,5AG and ADP. Both fasting plasma glucose and fasting plasma insulin that required in HOMA-IR equation (HOMA-IR = fasting plasma glucose (mg/dl) x fasting plasma insulin (µIU/L)/405) was measured by an enzymatic immunoassay technique. An initial physical examination was conducted and all the above parameters were assessed initially, before any intervention, and later on at two steps, the sixth and the twelfth week of the study time. The data were recorded in specially preformed case record.

Statistical Analysis

The statistical analysis was carried out using Statistical Package for the Social Science (SPSS); version 21. Descriptive statistic; mean ± standard deviation (±SD), was used to describe numerical values [23]. The differences between the means were considered significant at the 5% confidence level and the level of significance was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$ as significant, highly significant and very highly significant respectively.

The inferential statistics; one way analysis of variance (ANOVA) followed by T test comparison *t*-test for one sample was used to compare between parameters within treatment groups and to compare the same parameter with its analogue in other treatment groups. Independent *t*-test was used to compare between the results of studied parameters obtained at the 12th week from treatment for each group with their corresponding at the controls.

Results

The patients were 62; 33 males and 29 females. Their ages ranged between 33 and 70 years with a mean age ±SD (49.5 ± 7.93) for females and (46.5 ± 11.57) for males. The BMI on initial visit in general was (33.51 ± 5.85).

After 12 weeks of treatment with metformin alone, there was significant improvement in1,5 AG with mean±SD at base line level and at week 12 was (7.4 ± 1.5 → 10.5 ± 0.7). A significant rise in ADP with mean±SD before and after treatment was (9.0 ± 3.6 → 18.3 ± 4.3). HOMA-IR improved, mean±SD was (14.7 ±

8.4 → 3.9 ± 1.8) however, and these alterations were statistically insignificant (Table 1).

Table1. Effect of treatment by metformin on 1,5 anhydroglucitol, adiponectin, and HOMA-IR from baseline and at the 6th and 12th week of treatment

Duration	HOMA	1-5AG(µg/ml)	ADP (ng/ml)
Base line	14.7 ± 8.4	7.4 ± 1.5	9.0 ± 3.6
Week 6	8.1 ± 4.3	9.2 ± 1.5	12.1 ± 3.9
Week 12	3.9 ± 1.8	10.5 ± 0.7	18.3 ± 4.3
p value	0.105	0.010	0.041

1-5AG = 1-5 anhydroglucitol, ADP = adiponectin

On comparing the results of metformin at the 12th week with the control group, the parameters didn't approach the control group values (Table 2).

Table 2. Comparison between metformin group and control group in regard to HOMA-IR, 1-5 anhydroglucitol, adiponectin at the 12th week of treatment

Group	HOMA	1-5AG (µg/ml)	ADP (ng/ml)
Treated	3.9 ± 1.8	10.5 ± 0.7	18.3 ± 4.3
Control	3.7 ± 1.5	25.9 ± 8.6	23.8 ± 4.3
p value	0.000	0.000	0.000

1-5AG = 1-5 anhydroglucitol, ADP = adiponectin

After 12 weeks of treatment by metformin with flaxseed, there was significant improvement in 1,5AG with a mean ±SD before and after treatment was (6.9 ± 1.5 → 10.4 ± 0.8). A significant rise in ADP (9.4 ± 4.1 → 18.4 ± 4.9) However, HOMA-IR (mean±SD) was (20.2 ± 10.2 → 5.1 ± 3.2), showed an apparent improvement only which was statistically insignificant (Table 3).

Table 3.Effect of treatment by metformin with flax seed oil on HOMA, 1-5 anhydroglucitol, adiponectin from baseline and at 6th and 12th week of treatment

Duration	HOMA	1-5AG (µg/ml)	ADP (ng/ml)
Base line	20.2 ± 10.2	6.9 ± 1.5	9.4 ± 4.1
Week 6	11.0 ± 5.1	9.0 ± 1.4	12.3 ± 4.2
Week 12	5.1 ± 3.2	10.4 ± 0.8	18.4 ± 4.9
p value	0.110	0.013	0.037

1-5AG = 1-5 anhydroglucitol, ADP = adiponectin

On comparing effect of therapy by metformin with FSO after 12 weeks of treatment with the control group values, it was shown that the parameters didn't approach the control group values (Table 4).

The effects of metformin alone, metformin with flaxseed on1,5 AG showed statistically significant improvement (Table 5).

Table 4. Comparison between metformin and flax seed oil group with control group in regard to HOMA, 1-5 anhydroglucitol, adiponectin at the 12th week of treatment

Group	HOMA	1-5AG (µg/ml)	ADP (ng/ml)
Treated	5.1 ± 3.2	10.4 ± 0.8	18.4 ± 4.9
Control	3.7 ± 1.5	25.9 ± 8.6	23.8 ± 4.3
p value	0.036	0.000	0.000

1-5AG = 1-5 anhydroglucitol, ADP = adiponectin

Table 5. Effect of treatment by metformin, metformin with flaxseed oil on 1-5 anhydroglucitol from baseline and at the 6th and 12th week of treatment

Duration	Metformin	Metformin + flaxseed oil	*p* value (t-test)
Base line	7.4 ± 1.5	6.9 ± 1.5	0.000
Week 6	9.2 ± 1.5	9.0 ± 1.4	0.000
Week 12	10.5 ± 0.7	10.4 ± 0.8	0.001
p value (t-test)	0.010	0.013	(F test) 0.000

HOMA-IR among both groups did not show significant improvement although there were marked reduction by using metformin alone, metformin with flaxseed (14.7 ± 8.4 → 3.9 ± 1.8), and (20.2 ± 10.2 → 5.1 ± 3.2) respectively. However, this apparent improvement was most marked in metformin with flaxseed group (improvement of HOMA-IR by 15.2) and least in metformin alone group (improvement of HOMA-IR by 10.8) as shown in table 6.

Table 6. Effect of treatment by metformin, metformin with flaxseed oil on HOMA from baseline and at the 6th and 12th week of treatment

Duration	Metformin	Metformin + flax seed oil	*p* value (t-test)
Base line	14.7 ± 8.4	20.2 ± 10.2	0.009
Week 6	8.1 ± 4.3	11.0 ± 5.1	0.011
Week 12	3.9 ± 1.8	5.1 ± 3.2	0.010
p value (t-test)	0.105	0.110	(F test) 0.001

Metformin and metformin with flaxseed significantly increased ADP level (9.0 ± 3.6 → 18.3 ± 4.3) and (9.4 ± 4.1 → 18.4 ± 4.9) respectively (Table 7).

Table 7. Effect of treatment by metformin, metformin with flaxseed oil on ADP from baseline and at the 6th and 12th week of treatment

Duration	Metformin	Metformin + flax seed oil	*p* value (t-test)
Base line	9.0 ± 3.6	9.4 ± 4.1	0.001
Week 6	12.1 ± 3.9	12.3 ± 4.2	0.007
Week 12	18.3 ± 4.3	18.4 ± 4.9	0.003
p value (t-test)	0.041	0.037	(F test) 0.001

ADP = adiponectin

Discussion

After 12 weeks of treatment with metformin alone, there was significant elevation in 1,5AG. To the best of our knowledge, no comparable data exist on the effects of metformin on 1,5AG. In this ADP was increased after treatment with metformin for 12 weeks. Adamia *et al* [24], reported an increment in the ADP after 6 months of metformin therapy together with significant reduction in HOMA-IR and the magnitude of the change in ADP levels positively correlated with the magnitude of IR reduction.

On the contrary, Fujita *et al* [25] found that after 4 weeks of treatment with metformin, the serum ADP levels were not significantly elevated in metformin-treated patients, which might indicate that the 4 weeks period might not be enough for metformin to exert a change in ADP serum level.

Moreover, Cannon *et al* [26] observed significant lowering of ADP levels following 4 months treatment with metformin.

Metformin-mediated improvements in insulin sensitivity may be associated with several mechanisms, including increased insulin receptor tyrosine kinase activity, enhanced glycogen synthesis, and an increase in the recruitment and activity of GLUT4 glucose transporters [27].

In our study, HOMA-IR was apparently reduced; however, this attenuation was statistically insignificant. In accordance to our study, Moghetti *et al* [28], Ponssen *et al* [29] and Freemark and Bursey [30] demonstrated that metformin induces significant reduction in fasting plasma insulin and increased insulin sensitivity; and hence a significant reduction in HOMA-IR. In contrast, Pau *et al* [31] where metformin did not improve insulin sensitivity but just improve glucose effectiveness. Furthermore, Shaker et *al* [32], after three months treatment with metformin, showed a significant reduction in serum concentrations of insulin and HOMA-IR.

After 12 weeks of treatment by metformin with FSO, there was significant improvement in 1,5 AG. To the best of our knowledge, no comparable data about the effect of flaxseed oil on fasting serum 1,5 AG exists.

In the current study, HOMA-IR was insignificantly increased. In congruence with our results, Barre *et al* [33] showed that flaxseed oil addition (10g/day) had no impact on insulin levels. Likewise, Taylor *et al* [34] studied the effect of 12 weeks treatment with metformin and FSO and found that fasting plasma insulin was unchanged. In agreement with our study, Viguiliouk *et al* [35] found that flaxseed oil diet produced no significant effect for fasting insulin and HOMA-IR.

Nelson *et al* [36], studied the effect of metformin with FSO for 8 weeks; they disclosed no significant changes in fasting insulin, or quantitative insulin sensitivity check index values as a result of this intervention.

On the contrary, Rhee and Brunt [8] determined the antioxidant activity of flaxseed and its role in inflammation and insulin resistance in obese glucose intolerant people. They used a randomized crossover design, with 12 weeks treatment with FSO and metformin. HOMA-IR decreased and plasma insulin concentration significantly reduced compared to baseline data.

After 12 weeks of treatment by metformin with flaxseed, there were significant rise in ADP level. In agreement with our result, Sekine *et al* [37] suggested that α-linolenic acid-rich FSO intake might exhibit beneficial effects through an increase of the ADP level. The experimental period with metformin and FSO was 4 weeks. Pan *et al* [38] in their study using flaxseed found that ADP level was associated with significant increases as well. However, Paschos *et al* [39] using flaxseed oil rich in α-linolenic acid (8.1 g/day) for 12 weeks found the ADP plasma levels did not changed after the increase in dietary intake of α-linolenic acid in the flaxseed oil supplementation group. On contrary, Nelson *et al* [36] who studied the effect of metformin and FSO for 8 weeks disclosed significant decreases in ADP after the intervention.

As a conclusion, metformin, metformin with flaxseed oil associated with statistically significant improvement in 1,5 AG and ADP. HOMA-IR in both treatment groups showed only insignificant apparent improvement. Metformin with flaxseed oil group was more effective in HOMA-IR improvement and elevation of ADP hormone.

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Author contribution
All authors contribute equally in the literature review and drafting this paper.

Conflict of Interest
We declare no conflict of Interest

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