The effect of metformin on cytokines in Iraqi Patients with type 2 Diabetes

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Abstract:

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and is characterised by disorders of insulin action and insulin secretion, and associated with increase problem of insulin resistant. High plasma levels of insulin and glucose due to insulin resistance often lead to metabolic syndrome, chronic inflammation associated with metabolic and immune system involves a network of cellular and systemic responses that integrates many complex signaling pathways infiltration of inflammation cells in adipose tissue , abnormal pro-inflammatory cytokines production (IL-8,TNF-α). The study is designed to measure glycosylated hemoglobin (HbA1c), IL-8 and TNF-α. These parameters and measures applied for thirty newly diagnosed patients with diabetes before and after treatment divided into 3 group. Three groups of (10) patients each newly diagnosed with T2DM; group(1) receives:500mg metformin for 3 months with (HbA1c=9.54±1.95, IL-8=13.85±6.15,TNF-α=260.1±123.7). Group(2)receives:1000mg metformin for 3 months with(HbA1c=9.16±1.55,IL-8=15.02±6.28,TNF-α=230.86 ±49.26). Group(3)receives:1500mg metformin for 3 months with (HbA1c=9.80±1.94, IL-8 = 14.41±4.61, TNF-α = 207.42±40.45). Blood samples were withdrawn from the patients at pretreatment, then after 3 months of treatment. After 3 months of treatment, patient who received metformin 1500mg had greater reduction in HbA1c, IL-8 and TNF-α (27,9%, 44.20% and 49.75% respectively), compared with patient who received metformin 1000mg (18.3% ,15.77% , 34.42% respectively)and to metformin 500mg(10.3%.12.56% and 4.72%).

Metformin monotherapy are effective as an initial treatment of newly diagnosed diabetic patients in The national diabetes center / University of Al-Mustansiriyah, Iraq. Metformin has significant reduction effect on these three group, had significant reduction in HbA1c and also significant reduction in cytokines in the three dose in different percent.

Keywords: metformin, cytokines, diabetes

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Introduction:

The term diabetes mellitus (DM) describes a metabolic disorder of multiple aetiologies characterised chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1] Factors that influence the development of chronic hyperglycemia include genetic abnormalities; environmental causes, such as nutritional excess and lack of activity; increased hepatic production of glucose; increases in visceral fat; atherogenic dyslipidemias; increased of oxidation and inflammation, natural processes involved in maintaining a physiologic state. Long-term complications associated with chronic hyperglycemia include microvascular disease, such as retinopathy, nephropathy, neuropathy and macrovascular diseases, including fatal and non-fatal myocardial infarction, peripheral vascular disease and stroke [2]. The goal of treatment is typically an HbA1C of less than 7% or a fasting glucose of less than 6.7 mmol/l (120 mg/dl) however these goals may be changed after professional clinical consultation, taking into account particular risks of hypoglycemia and life expectancy.[3] Type 1 DM is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to a deficiency of insulin, while Type 2 DM is characterized differently and is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases becomes absolute.[4] Chronic inflammation associated with the metabolic and immune systems involves a network of cellular and systemic responses that integrate many complex signaling pathways.[5] Mediators of these pathways include major stress hormones, noradrenaline and adrenaline and cortisol; angiotensin-II (ang-II); pro-inflammatory cytokines.[6] Environmental factors plus a genetic predisposition can increase adiposity, which is associated with both a localised and systemic chronic inflammation characterised by infiltration of inflammatory cells in adipose tissue, abnormal pro-inflammatory cytokine production. This phenomenon, referred to as meta-inflammation (metabolic inflammation). [7] Recently, it has been reported that metformin activates AMPK in macrophages and this

results in the inhibition of biosynthesis of phospholipids as well as neutral lipids and down-regulates the expression of LPS-induced pro-inflammatory cytokines.[8] metformin has suppressed IL-8 release from human adipose tissue *in vitro,* [9] Thus, metformin seems to exert its anti-inflammatory role by reducing pro-inflammatory cytokine secretion in specific cell types.[9]

Materials, Subjects and Methods:

The study was conducted between October 2012 up to March 2013; during this period Thirty Iraqi patients of newly diagnosed patient with type 2 diabetes were attended of the (The National Diabetes Center , University of Al-Mustansiriyah) Their age ranging (35-77) year's mean±SD (51.5±10.248) years. They had no history of smoking or alcohol drinking. The diagnosis of T2DM was made on the basis of the recommended criteria by WHO (1999).[1] The selected patient diabetic patients were treated with different doses of oral hypoglycemic agents (Glucophage ® (Metformin 500mg) tablets -Merck, France- 500, 1000, 1500 mg) according to the patient condition and the physician opinion and randomized into three groups:

1- First group: includes (10) patient diabetic patients were treated with oral hypoglycemic agent metformin in a dose (500mg/day) for (3)months.

2- Second group: includes (10) patient diabetic patients were treated with metformin in a dose (1000 mg/day) for (3)months.

3- Third group: included (10) patient diabetic patients were treated with metformin in a dose (1500mg/day) for (3) months.

Ten ml of venous blood were drawn using a plastic disposable syringe of 10ml capacity. All patients were fasting (12-14) hr calories free diet. Two ml of blood collected in EDTA containing tubes for measurement of HbA1c. The remaining blood was allowed to clot and separated by centrifuge at speed of 3000 rpm for 20 minutes. Serum samples were stored at (-20 ^{O}C) until the time of examination for the other testes.

Parameters used for analysis involved:

Estimation of glycosylated hemoglobin (HbA1c); Glycated hemoglobin was measured by using the Variant Hemoglobin A1C program developed by BIO-Rad.[10]

Serum levels of cytokines (IL-8 & TNF-α) were quantitatively determined in patients by means of sandwich ELISA test using commercially available kit.

Statistical Analysis:

Data were statistically evaluated using paired *t*-test to compare between pre- and post-treatment results. Another way analysis of variance (ANOVA) was utilized to compare between the results of studied parameters among different patients groups. Values with $P<0.05$ were considered significantly different.

Results:

HbA1c:

Effect of treatment with different doses of metformin on HbA1c level in newly diagnosed patient with type 2 DM:

Table – 1: showed that all T2DM patients treated with different doses of metformin (500, 1000 and 1500)mg showed significant decrease in HbA1c levels after 3 months ($P<0.05$) using Paired t-test compared with pre-treatment values. However, the percent reduction in HbA1c produced due to the use of different doses of metformin (500, 1000 and 1500)mg were (10.3%, 18.3% and 27.9%) respectively compared with baseline values; while the patient groups treated which was statically not Significant ($P>0.05$) according to ANOVA test as in (Table-1) and (Figure-1).

Tab 1: HbA1c distribution of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months.

		Metformin 500 mg	Metformin1000 mg	Metformin1500 mg
HbA1C %	Before	9.54±1.95 (7.3-13.0)	9.16±1.55 (7.7-12.1)	9.80±1.94 (6.5-12.3)
	After 3 months	8.55±1.89 (6.0-11.9)	7.47±1.08 (6.5-10.2)	7.06±1.23 (4.6-9.3)
P value compare After to Before		0.0001*	0.0001*	0.001*
Before P value compare to 500mg		-	0.635	0.768
P value compare to 1000mg		-	-	0.425
P value comparing All doses				0.734
After P value compare to 500mg		-	0.134	0.051
P value compare to 1000mg		-	-	0.411
P value comparing All doses				0.072

-Data were presented as Mean±SD (Range)
*Significant using Paired t-test for difference between two dependent means (paired observations) at 0.05 level
#Significant using Student t-test for difference between two independent means at 0.05 level
@Significant using ANOVA test for difference among three independent means at 0.05 level

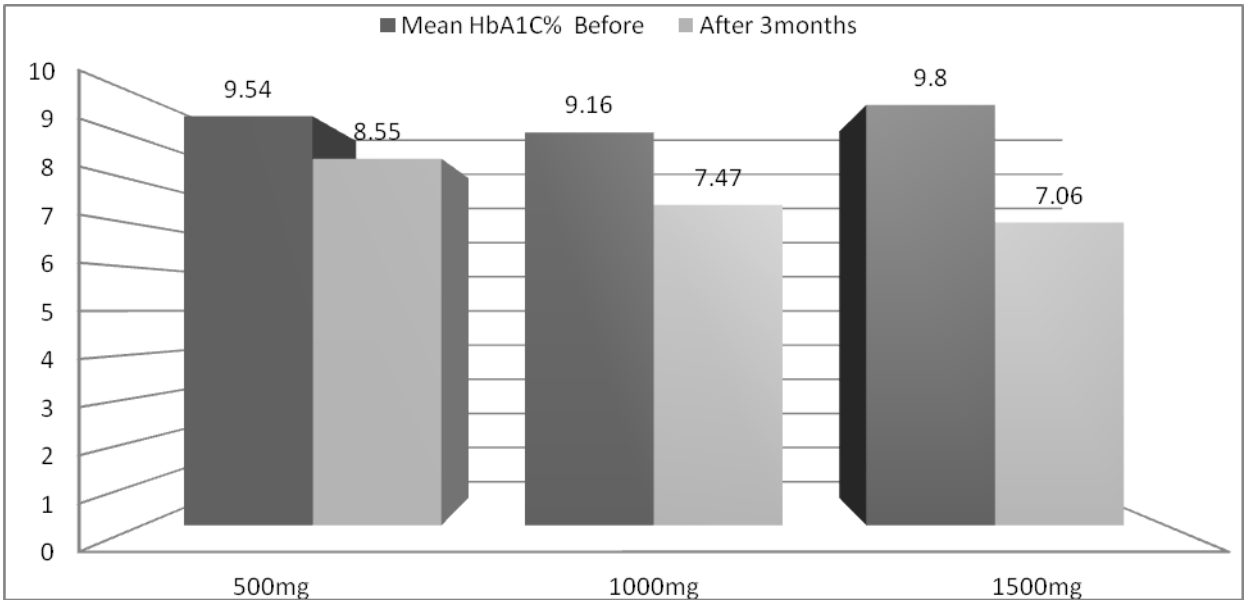

Fig 1: HbA1c distribution of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months.

Interleukin -8:

Effect of treatment with different doses of metformin on Interleukin 8 level in newly diagnosed patient with T2DM :

Tab -2; showed that there is a significant decrease in IL-8 in all groups of patients treated with different doses of metformin (500, 1000 and 1500)mg after 3 months (P<0.05) compared with baseline values according to Paired t-test . Moreover, (table -2) and (figure -2) showed that treatment with maximum dose of metformin (1500mg) produced a higher percent reduction in this parameter after 3 months (44.20 %) compared with base line values, and metformin treated group (1000mg), (500mg) produced (15.77% and 12.56% respectively). There is a significant difference among the three groups treated with metformin doses after 3 months (P<0.05), and also a significant according to ANOVA test (P<0.05).

Tab 2: IL-8 serum concentration of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months

	Metformin 500 mg	Metformin1000 mg	Metformin1500 mg
Interleukin 8 (pg/ml) Before	13.85±6.15	15.02±6.28	14.41±4.61
After 3 months	12.11±4.82	12.65±5.24	8.04±1.02
P value compare After to Before	0.018*	0.005*	0.004*
Before P value compare to 500mg	-	0.899	0.820
P value compare to 1000mg	-	-	0.807
P value comparing All doses			0.901
After P value compare to 500mg	-	0.048#	0.018#
P value compare to 1000mg	-	-	0.014#
P value comparing All doses			0.039@

-Data were presented as Mean±SD (Range)

*Significant using Paired t-test for difference between two dependent means (paired observations) at 0.05 level

#Significant using Student t-test for difference between two independent means at 0.05 level

@Significant using ANOVA test for difference among three independent means at 0.05 level

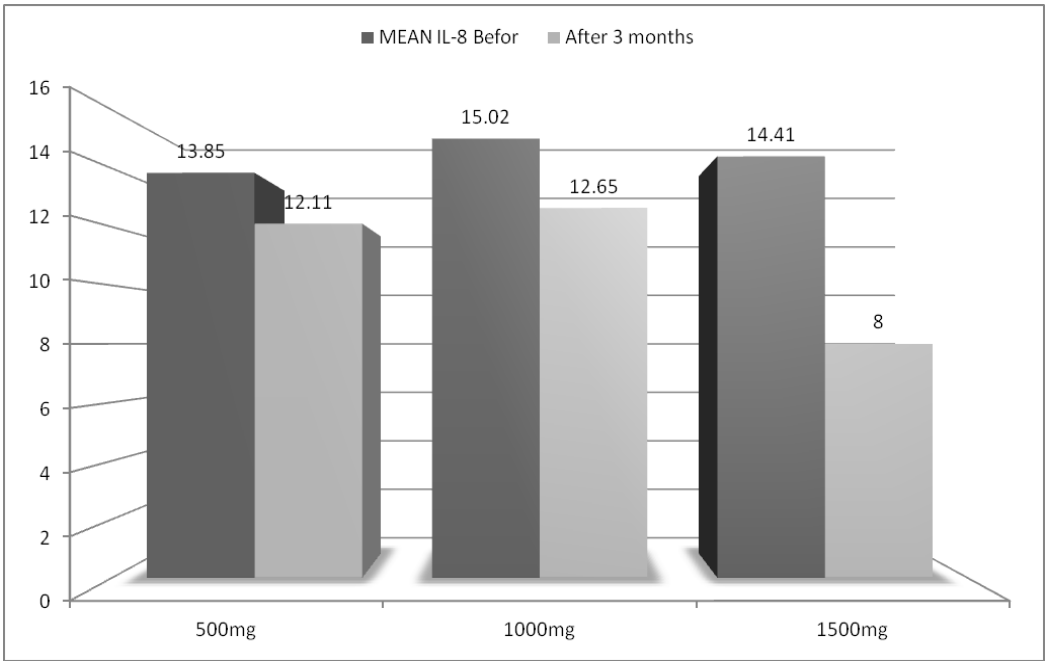

Fig 2: IL-8 serum concentration of newly diagnosed diabetic type 2 patient treated with different dose metformin before and after 3 months.

TNF-alpha:

Effect of treatment with different doses of metformin on TNF- α level in newly diagnosed patient with type 2 DM :

Tab -3: showed that there is a significant decrease in TNF- α in all groups of patients treated with different doses of metformin (500, 1000 and 1500)mg after 3 months (P<0.05) compared with baseline values according to Paired t-test . Moreover, table -3 and figure -3 showed that treatment with maximum dose of metformin (1500mg) produced a higher percent reduction in this parameter after 3 months (49.75 %) compared with base line values, and metformin treated group (1000mg),(500mg) produced (34.42 % and 4.72% respectively). There is a significant difference among the three groups treated with metformin doses after 3 months (P<0.05), and also a significant according to ANOVA test (P<0.05).

Tab 3: serum TNF- α concentration of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months

		Metformin 500 mg	**Metformin1000 mg**	**Metformin1500 mg**
TNF-alpha (pg/ml)	Before	260.1±123.7 (137.7-550)	230.86±49.26 (176.9-297.9)	207.42±40.45 (129.50-280.9)
	After 3 months	247.8±127.0 (122.2-546.0)	151.74±52.26 (110.2-284.2)	104.22±29.55 (62.8-141.5)
P value compare After to Before		0.001*	0.002*	0.0001*
Before P value compare to 500mg		-	0.496	0.217
P value compare to 1000mg		-	-	0.260
P value comparing All doses				0.354
After P value compare to 500mg		-	0.040#	0.003#
P value compare to 1000mg		-	-	0.025#
P value comparing All doses				0.002@

-Data were presented as Mean±SD (Range)

*Significant using Paired t-test for difference between two dependent means (paired observations) at 0.05 level

#Significant using Student t-test for difference between two independent means at 0.05 level

@Significant using ANOVA test for difference among three independent means at 0.05 level

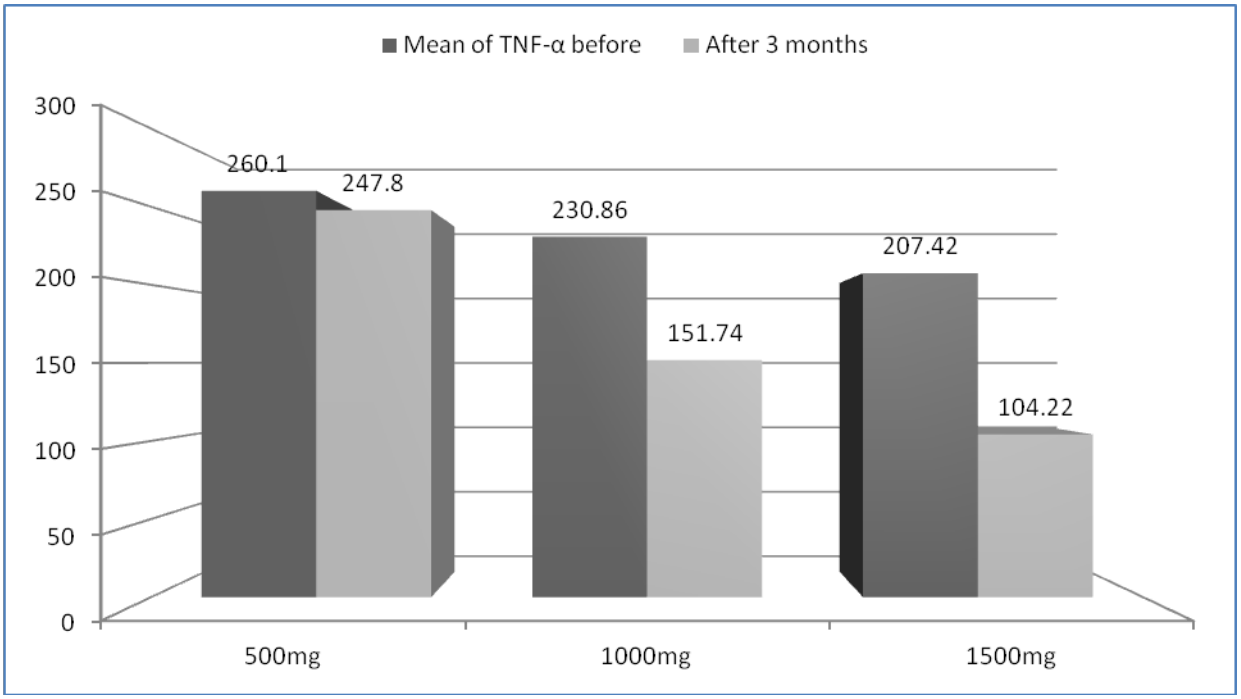

Fig 3: TNF-α serum concentration of newly diagnosed diabetic type 2 patient treated with different dose of metformin before and after 3 months.

Discussion:

Many specialist recommended the use of metformin as first drug of choice in newly diagnosed diabetic patients in The National Diabetes Center, University of Al-Mustansiriyah in Baghdad, strategy which was parallel to the strategy used in the treatment of diabetes mellitus in other countries.[11] Metformin lowers hyperglycemia by reducing hepatic gluconeogenesis in the present of insulin and increasing peripheral glucose uptake and utilization.[12,13,14] The results in the present study investigated the effects of different doses of metformin (500, 1000 and 1500)mg on HbA1c levels in newly diagnosed T2DM patients for periods of 3 months . The mean values of HbA1c in treated groups of diabetic patients are in agreement with other results.[15,16] However, the percent reduction in HbA1c produced following the use of different doses of metformin were (-10%,-18.3% and -28.2%) respectively compared with baseline values; This finding agrees with other previous studies [17,18,19] and the possible explanation for these results could be attributed to the effect of the short duration of treatment and also, the baseline values of HbA1c at zero time in different treated groups were lower than the fasting glucose values, which could probably reflect that the HbA1c values were not in steady state at the start of treatment.[17,20] Moreover, (table -1) and (figure-1) showed that treatment with maximum metformin (1500mg) produced the highest percent reduction in this parameter after 3 month rather than the other groups treated with 1000mg and 500mg doses compared with baseline values. These results are compatible with those of Garber *et al* (1997),[21] who

demonstrated that the hypoglycemic activity of metformin on glycemic control (%HbA1c) in diabetic patients is exhibited , generally, a dose-dependent manner.[21] High HbA1c regarded as predicator for the development of microangiopathy. This problem may be explained by the facts that HbA1c had high affinity for O_2 resulting in marked difficulty in unloading of O_2 to peripheral tissues resulting in tissue hypoxia and microangiopathy.[22]

It is increasingly recognized that markers of vascular inflammation play a role in the pathogenesis of T2DM, insulin resistance, and atherosclerosis,[23] the infiltration of macrophages into fat tissue and their subsequent release of pro-inflammatory cytokines into circulation occur at a greater rate in type 2 diabetics than non-diabetics [24,25,26] & pro-inflammatory cytokines clearly decrease insulin sensitivity.[27] The use of metformin during the first month of treatment of patient with coronary artery disease and T2DM led to the decrease of insulin resistance and reduced activity of systemic inflammation (significant decrease in the concentration of IL-8 and TNF-α).[28] The results in the present study investigated the effects of different doses of metformin (500, 1000 and 1500)mg on IL-8 & TNF-α levels in newly diagnosed T2DM patients for periods of 3 months . The mean values of IL-8 & TNF-α in treated groups of diabetic patients are in agreement with other results.[29,30,31] However, the percent of reduction in IL-8 & TNF-α produced following the use of different doses of metformin were (-12.56%,-15.77% and -44.20%) for IL-8 ,(-4.72 %,-34.42% and -49.75 %) for TNF-α respectively compared with baseline values; This finding agrees with other previous studies [32,33] and the possible explanation for these results could be attributed to the effect of the short duration of treatment and also, the baseline values of IL-8 & TNF-α in different treated groups were lower than that of newly diagnosed values, which could probably reflect that the IL-8 & TNF-α values were have significant decrease in the concentration compared with baseline values. [28] Metformin can exert a direct vascular anti-inflammatory effect by inhibiting NF-kappa B through blockade of the PI3K-Akt pathway. The novel anti-inflammatory actions of metformin may explain in part the apparent clinical reduction by metformin of cardiovascular events not fully attributable to its hypoglycemic action.[34]

Moreover, (table -2,3) and (figure -) showed that treatment with maximum dose of metformin (1500mg) produced the highest percent reduction in this parameter after 3 month rather than the other groups treated with 1000mg and 500mg doses compared with baseline values. These results are compatible with those of Yoshiyuki Hattori *et al* (2006)[33] who demonstrated that the effect of metformin on cytokine (IL-8 & TNF-α) in diabetic patients is exhibited, generally, a dose-dependent manner.[33] These results, therefore, should be discussed within this framework, and not necessarily to be comparable with other results. Furthermore, the exact mechanism action of metformin remains elusive.[35] On one hand, some experts consider metformin to be the drug of choice for newly diagnosed type 2 diabetics.[36] whereas the landmark Diabetes Prevention Program, on the other hand, concluded that metformin is efficacious in preventing the new onset of type 2 diabetes in middle-aged, obese persons with impaired glucose tolerance and fasting hyperglycemia but did not prevent diabetes in older, leaner prediabetics.[35] Finally, the present study did establish an improved efficacy of metformin use at different doses (500-1500mg/day); most of the changes were observed as early as 12 weeks of treatment. Furthermore, this study demonstrated an improved insulin sensitivity of

metformin & pro-inflammatory cytokines clearly decrease insulin sensitivity in the newly diagnosed type 2 diabetic patients. The current study was conducted on a small number of patients, and no power calculation was done, because of the paucity of data, on dose calculation of metformin in newly diagnosed diabetic patient. Therefore, the number of patients was too small to draw a strong conclusion from it, and a larger number of patients are essential for a better understanding of the present results.

Conclusion:

- The use of different doses of metformin as amonotherapy in all treated groups significantly improved the biochemical markers (HbA1c) in newly diagnosed patient with type 2 diabetes.
- Serum levels of the inflammatory marker(IL-8 and TNF-α) are decreased in all treated groups with different doses of metformin after 3 months in different percent of reduction; metformin has anti- inflammatory effect.

- We demonstrated that metformin inhibits the expression of pro-inflammatory resulting in suppression of cytokine-induced NF-κB activation. the present results suggest that metformin may serve for antiatherogenic drug for diabetic subjects.

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