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Effect of combination of metformin with B12 and folic acid on lipid profile and levels of homocysteine in adult male rabbits

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Abstract

The present study aims to evaluate the effects of metformin in combination with vitamin B12 and folic acid on lipid profiles and serum homocysteine levels in male rabbits. The study included twenty-four male rabbits that weighed (1.5-2 kg), and were divided randomly into four groups (six rabbits/group). The control first group was administrated (5 ml) of distilled water; The second group was given metformin (125g/kg B.W) plus vitamin B12 (500 µg/kg B.W) daily, the third group was given metformin (125g/kg B.W) plus folic acid (5g/kg B.W) daily and the fourth group was given metformin (125g/kg B.W), folic acid (5g/kg B.W) and vitamin B12 (500 µg/kg B.W) daily, the rabbits were followed up for one month. The results revealed a decrease in the final body weight and body weight gain in all treated groups as compared to the control group. The homocysteine parameter decreases significantly in the second group which was given metformin and B12 and the third group was given metformin in combination with folic acid as compared with the control group and group four. It showed a significant decrease in the fourth group (metformin + folic acid + vitamin B12) compared with the second and third groups also the glucose level increased significantly in group three compared to group second, fourth, and control group. The results showed that serum cholesterol and TG have decreased in G2 and G3 while an increase in G4 as compared with the control group, and the HDL was decreased in G2 and G4 compared with control and G3 groups. While the results of LDL and VLDL were increased in G3 and G4 compared with G2 and the control group.

Key words: metformin, vitamin B12, folic acid, lipid.

Introduction

Vitamin B12 is a vital nutrient for health. It plays an important role in the functioning of the brain and nervous system, and the formation of red blood cells. In addition to anemia, vitamin B12 deficiency may increase the severity of peripheral neuropathy in patients with type II Diabetic Mellitus (T2DM) (1). Furthermore, because vitamin B12 participates in the most important pathway of homocysteine (Hcy) metabolism, a reduction in vitamin B12 would increase plasma concentrations of Hcy, which is strongly linked to cardiovascular disease in patients with T2DM (2) and PCOS (3). Folic acid, a water-soluble vitamin B, has recently gained considerable attention because of its great potential to prevent many disorders through supplementation for the general population, folic acid was repeatedly reported to improve endothelial dysfunction in various clinical conditions, although the mechanisms of this beneficial effect are not fully understood, it seems that folate (a main circulating metabolite of folic acid in plasma is 5MeTHF) acts through at least four mechanisms in atherosclerosis: (i) indirectly, to decrease homocysteine level and ensure optimal functioning of the methylation cycle, (ii) directly, produce antioxidant effects, (iii) interact with enzyme endothelial nitric oxide synthase (eNOS), and (iv)affect cofactor bioavailability of nitric oxide (4). For folic acid it has been found: (a) revert dysfunction of eNOS (5) (b) influence on postprandial endothelial dysfunction (6) (c) improve endothelial function in coronary artery disease by reduction of intracellular (7) (d) to inhibit intimal hyperplasia induced by a high-homocysteine diet in a rat carotid endarterectomy model (8) (e) to reverse endocardial endothelial dysfunction in homocysteinemia hypertensive rats (9) and (f) anti- arrhythmia effects after reperfusion injury in rat heart (10). The present study aims to examine the evaluation effects of metformin in combination with vitamin B12 and folic acid on the level of lipid profile and homocysteine in male rabbits.

Materials and Methods

Experimental animals: The present study was carried out at the College of Veterinary Medicine/ University of Basrah. Twenty-four adult male rabbits were used for this study. The animals were kept in the animal house for acclimatization fourteen days

before the beginning of the experiments. The animals were maintained under optimum conditions (25+2 ºC) and (12/12 hours light/dark) cycle throughout the study, with standard pellets and tap water.

Experimental design: The experimental animals were included in this study: Twenty-four adult male rabbits are randomly divided into four groups as the following:

Group1 (control): 6 adult male rabbits orally administered distilled water (15 ml/animal) by gavage daily for thirty days.

Group2 (M+FA): 6 adult male rabbits were orally administration metformin hydrochloride (125 mg/kg BW) and folic acid (5mg/kg BW) dissolved in 15ml distilled water by gavage daily for thirty days.

Group 3 (M+B12): 6 adult male rabbits were orally administration metformin hydrochloride (125mg/kg BW) dissolved in 15ml distilled water and vitamin B12 (500 µg/kg BW) by gavage daily for thirty days.

Group4 (M+FA+B12): 6 adult male rabbits were orally administration metformin hydrochloride (125mg/kg BW) dissolved in 15ml distilled water plus folic acid (5mg/kg BW) and vitamin B12(5ooµg/kg BW) by gavage daily for thirty days.

Collected blood samples from the heart by cardiac puncture before sacrificed animals using a sterile 5 cc syringe dropped into a tubeless anticoagulant plain and blood serum was isolated from the blood by centrifugation at 3000 rpm for 15 min. They are separated in Eppendorf tubes and stored at -20°C until a biochemical analysis is performed.

Studied parameters:

Serum homocysteine level: It measured by Albean laboratory by special kits.

Total cholesterol measurement: Serum total cholesterol was enzymatically measurement by using a linear chemical kit (BIO-ABO S.A/CHOD-PAP, France) (11).

Serum triglyceride measurement: The concentration of serum triglyceride was measured by using a special chemical kit (SYRBIO/GPO-PAP/ Syria) based on (12).

Determination of serum lipoprotein cholesterol: Three types of lipoprotein cholesterol measured by the following:

Serum High-Density Lipoprotein Cholesterol (HDL-C): The principal role of HDL in the lipid metabolism is the uptake

and transport of cholesterol from peripheral tissues to the liver through a process known as revers cholesterol transport (11).

Serum Low-Density Lipoprotein Cholesterol (LDL-C): Serum LDL concentration can be calculated by the following equation (13). LDL = TC – (HDL+TG/5)

Serum very Low-Density Lipoprotein (VLDL): Serum VLDL concentration was calculated by dividing serum TG/5 (14). VLDL= TG/5

Result:

1- Effect of their combination M, FA and B12 on body weights and body weight gain of male rabbits: There were no significant changes in initial BW between all groups. The results in table (1) revealed significant ($p< 0.05$) decrease in the final BW in all treated groups compared with control group (G1).

2- **Effect of combination M, FA and B12 on serum Homocysteine and glucose concentrations of male rabbits:** Table (2) showed significant ($p<0.05$) increase in the concentration of Hyc in G2, G3 and G4 compared with control group. While there is significant ($p<0.05$) decrease in G4 compared with G2 and G3. The results revealed significant ($p<0.05$) decrease in serum glucose concentrations in G2 and increased significantly ($p<0.05$) in G3 and G4 compared with control group.

3- Effect of M, FA, B12 and their combination on serum lipid profile in male rabbits: In table (3) the results of TC showed significant ($p<0.05$) increase in the G4 compared with control and other treated groups while there is significant ($p<0.05$) decrease in G2 and G3. The results of TG concentration revealed a significant ($p<0.05$) decrease in G2 and G3 compare to the control group. Serum HDL concentrations significantly ($p<0.05$) decreased in G2 and G4 compared to the control group and G3. While serum LDL concentrations increased significantly in G3, G4 more than other treated groups and compared to the control group. Also, table (3) revealed the significant ($p<0.05$) increased in serum VLDL in G4 compared to G2, G3 and control group.

Table (1) Effect of M, FA, B_{12} and their combination on body weights and body weight gain of male rabbits (M±SD):

Groups	Initial BW	Final BW	BW gain
G1 (control)	1.65 ± 0.23^{Aa}	1.82 ± 0.09^{Aa}	0.17 ± 0.14^{A}
G2 (M+FA)	1.67 ± 0.05^{Aa}	1.45 ± 0.17^{Ba}	-0.22 ± 0.12^{C}
G3 (M+B12)	1.60 ± 0.73^{Aa}	1.46 ± 0.18^{Ba}	-0.16 ± 0.55^{A}
G4 (M+FA+B12)	1.61 ± 0.12^{Aa}	1.17 ± 0.15^{Ca}	-0.44 ± 0.03^{B}
LSD	N.S	0.27	0.20

Values are expressed in capital letters mean significant differences at (p< 0.05).

Table (2) Effect of M, FA, B12 and their combination on serum Homocystine and glucose concentrations of male rabbits:

Groups	Hyc	glucose
G1 (control)	4.02 ± 0.38^{D}	85.95 ± 1.88^{C}
G2 (M+FA)	11.90 ± 0.34^{A}	78.16 ± 1.16^{D}
G3 (M+B12)	11.02 ± 0.40^{B}	$12.05\pm6.63^{A}1$
G4 (M+FA+B12)	7.05 ± 1.02^{C}	90.64 ± 2.55^{B}
LSD	0.78	4.27

Values are expressed in capital letters mean significant differences at (p< 0.05).

Table (3) Effect of combination of M, FA, B12 and on serum lipid profile in male rabbits:

Groups	TC	TG	HDL	LDL	VLDL
G1 (control)	109.60 ± 7.25^{B}	105.20 ± 6.05^{C}	27.71 ± 1.72^{A}	77.08 ± 4.74^{B}	26.67 ± 0.78^{A}
G2 (M+FA)	96.54 ± 7.19^{B}	95.91 ± 5.14^{B}	21.44 ± 1.03^{B}	80.83 ± 1.98^{B}	$2.64^{A}\pm26.83$
G3 (M+B12)	95.05 ± 1.95^{B}	96.17 ± 3.29^{B}	27.35 ± 1.35^{A}	89.59 ± 2.11^{A}	31.03 ± 1.34^{B}
G4(M+FA+B12)	123.01 ± 4.24^{A}	119.95 ± 7.55^{A}	21.20 ± 0.99^{B}	87.13 ± 5.08^{A}	31.70 ± 1.74^{B}
LSD	13.07	9.03	5.91	5.44	4.02

Values are expressed in capital letters mean significant differences at (p< 0.05) levels(M±SD).

Discussion:

Homocysteine is an essential amino acid required for the growth of cells and tissues in the human body. Vitamin B6, B12 and folate are essential factors in the processes of synthesis of homocysteine. In the human body, the Hcy is an essential amino acid required for the growth of cells and tissues (15). The results of BW and BW gain showed decreased as compared with control group. Despite the well-known glucose-lowering effects of metformin, more recent clinical interest lies in its potential as a weight-loss drug. Here, we discuss potential mechanisms by which metformin reduces appetite and opposes unfavorable lipid storage in peripheral tissues. Recent findings: Many individuals struggle to maintain clinically relevant weight loss from lifestyle and bariatric surgical interventions. Long-term follow-up from the Diabetes Prevention Program demonstrates that metformin leads to permanent weight loss and that reducing food intake with metformin is the primary mechanism for weight loss. Although the effect of metformin on appetite is likely to be multifactorial, changes in hypothalamic physiology, including sensitivity to leptin and insulin, have been documented (16). This study showed a significant decrease in Hcy levels seen in the B-group vitamin and folic acid plus metformin (G4). The only source of Hcy comes from the methionine in dietary proteins, which are mainly of animal origin. Homocysteine may undergo remethylation to methionine or trans-sulphuration to cystathionin and cysteine. Plasma homocysteine levels are influenced by several variables including smoking, coffee consumption, renal function, vitB12, folate status, and some drugs such as methotrexate, nitrous oxide, metformin, or azaribine, that reacts with folic acid, vitamin B12 or B6 respectively can cause hyperhomocysteinemia. Ten percent of the risk of coronary artery disease in the general population is attributable to an increase in Hcy levels which may be due to vascular damage and alteration in the coagulation process (15). This study showed a significant decrease in Hcy levels was seen in the B-group vitamin and the folic acid plus metformin (G4) the agreement of the results with Wulffele et al. (17). The current study showed a significant increase in serum Hcy in group vitamin B12 plus metformin (G2, G3, and G4). The mechanisms by which drugs alter the plasma Hcy level vary. Any drug, such as methotrexate, nitrous oxide, metformin, or azaribine that reacts with folic acid, vitamin B12, or B6 can cause hyperhomocysteinemia (18). Interference with vitamin absorption from the gut may lead to increased plasma Hcy levels (19). The results also showed that serum cholesterol and TG were decreased in G2 and G3 and increased in G4 as compared with the control group, and the HDL was increased in all groups except G4 compared with the control group. Also, the result showed that LDL was increased in G3 and G4 as compared with the control group. This effect on lipid profile may be a due imbalance between normal rabbits of fat metabolism secretion (20). Metformin monotherapy significantly improves dyslipidemia in statin-naive subjects with T2DM. Its lipid-modifying effect may be

attributed to insulin sensitivity, irreversible reduction of LDL-C glucose, and weight loss. In practice, people with dyslipidemia who are not eligible to receive lipid-lowering agents may benefit from treatment with metformin. Furthermore, previous studies suggest a synergistic effect between metformin and statins, which may further reduce cardiovascular events in high-risk individuals. Overall, metformin is a safe and effective approach to relieving dyslipidemia in people newly diagnosed with T2DM (21; 22).

Conclusion: Administration of B-group vitamins and folic acid effective in reducing elevated Hcy levels in male rabbits undergoing short-term metformin therapy.

conflict of Interest

The author(s) declared that there is no conflict of interest.

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دراسة تأثير الميتفورمين وفيتامين ب 12 وحامص الفوليك على مستوى الهموسيستين وصور الدهون في ذكور الارانب البالغة

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الخلاصة

الهدف من هذه الدراسة هو تقييم تأثير تركيبة الميتفورمين مع فيتامين ب 12 وحمض الفوليك على ملامح الدهون ومستوى الهوموسيستين في الدم في ذكور الأرانب. اشتملت الدراسة على أربعة وعشرين أرنباً ذكور بوزن (1.5-2 كجم)، مقسمة إلى أربع مجموعات (ستة أرانب / مجموعة). تم استخدام مجموعة التحكم أولاً (5 مل) من الماء المقطر. المجموعة الثانية أعطيت الميتفورمين (125 جم / كجم من وزن الجسم) بالإضافة إلى فيتامين ب 12 (500 ميكروجرام / كجم من وزن الجسم) يوميًا، المجموعة الثالثة أعطيت الميتفورمين (125 جم / كجم من وزن الجسم) زائد حمض الفوليك (5 جم / كجم من وزن الجسم) يوميًا والمجموعة الخامسة أعطيت المجموعة ميتفورمين (125 غم / كجم من وزن الجسم) وحمض الفوليك (5 جم / كجم من وزن الجسم) وفيتامين ب 12 (500 مليغرام / كجم من وزن الجسم) يوميًا، وتمت متابعة هذه الأرانب لمدة شهر. أظهرت النتائج انخفاضًا في وزن الجسم النهائي ووزن الجسم المكتسب في جميع المجموعات المعالجة مقارنة بمجموعة السيطرة. انخفض معامل الهوموسيستين بشكل كبير في المجموعة الثانية التي أعطيت الميتفورمين و12B والمجموعة الثالثة أعطيت تركيبة الميتفورمين مع حمض الفوليك مقارنة مع المجموعة السيطرة والمجموعة الرابعة. كما ظهر مستوى الجلوكوز انخفاضا معنويا في المجموعة الرابعة (M + B12 + FA) مقارنة بالمجموعتين الثانية والثالثة بينما أظهر مستوى الجلوكوز زيادة معنوية في المجموعة الثالثة مقارنة بالمجموعة الثانية والرابعة والمجموعة السيطرة. أوضحت النتائج أن الكوليسترول في الدم والكليسريد الثلاثي انخفض في G2 و3G بينما زاد في G4 مقارنة بمجموعة السيطرة، وانخفض مستوى الدهون الجيدة (HDL) في G2 و4G مقارنة مع مجموعة السيطرة و3G. في حين زادت مستوية الدهون الضارة LDL وVLDL في G3 و4G مقارنة مع G2 ومجموعة السيطرة.

الكلمات المفتاحية: ميتفورمين، فيتامين ب 12, حامض الفوليك، دهون.