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Review Article
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Mechanism by which Metformin Influence Metabolic Changes in Human: A Systematic-Review

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Abstract

Metformin is the most widely used oral antihyperglycemic agent for the improvement of type 2 diabetes mellitus (T2DM). In patients with T2DM, metformin increases insulin sensitivity and reduces glucose production. In this systematic review, we identified and compared metabolic changes induced by metformin administration.

We searched articles published within the past 5 years on Pubmed.gov by using the keywords "metformin and metabolomics." No restrictions were placed on the languages, species, or gender during the selection of articles. From the 29 identified articles, we excluded non-human *in vivo* or *in vitro* studies, studies in cancer patients, and studies in which the focus was not on variation in the levels of metabolites after administration of medication such as pharmacogenetic studies. Finally, we selected 8 articles and compared their results.

In the selected study results, a treatment with metformin and a combination of metformin and other anti-diabetic agents decreased the levels of phenylalanine, tyrosine, lysine, and glutamate to 5.58 ± 2.23%, 11.50 ± 2.12%, 25.28 ± 27.90%, and 35.49 ± 26.18% (mean ± SD), respectively, but increased the levels of alanine to 16.00% in 3 months-5 years of follow-up after metformin treatment compared to the control. The levels of total cholesterol and low-density lipoprotein (LDL) cholesterol (5.28%) decreased, whereas those of high-density lipoprotein (HDL) cholesterol increased by 34.65% in 18–30 months of follow-up after administration of metformin.

Metformin administration in human alters the blood concentration of phenylalanine, tyrosine, glutamate and lysine. Further confirmatory studies are required to understand how these metabolites related to the drug mechanism and also drug response, and to develop the biomarkers for predicting metformin response in humans.

Keywords: Metformin; Metabolite; Metabolomics

Introduction

Type 2 diabetes mellitus (T2DM) which counts approximately 90% of diabetes is a complex endocrine disorder [1] that affects many metabolic homeostatic processes such as energy and lipid metabolism, insulin resistance, and hemostasis stress [2,3]. The incidence of T2DM is increasing worldwide [4] and the WHO has predicted that the incidence of T2DM will be set to rise exponentially 366 million by 2030 [4]. The treatment of choice for patients with T2DM is the oral anti-diabetic drug metformin [5]. Metformin increases glucose utilization in target organs [5] decreases hepatic glucose production [6] and subsequently, decreases the blood glucose concentration [7]. In addition, metformin decreases insulin resistance and improves insulin sensitivity [5] Therefore, metformin is used in other metabolic disorders (e.g., hyperinsulinemic androgen excess [HIAE] [8] and polycystic ovary syndrome [PCOS] [9] apart from diabetes, such as insulin resistance-related diseases.

Despite the beneficial effects of metformin, 38% of metformin administered to T2DM patients is not in the successful therapeutic range, and thus, does not exert beneficial effects (fasting blood glucose [FBG]<6.7 mmol/l) [10-13]. A previous study indicated that among obese patients treated with metformin for 9 years, only 13% achieved glycosylated hemoglobin (HbA1c) levels within the target therapeutic range (HbA1c<7%) [14]. Although metformin exerts its effect in patients with T2DM by inhibiting gluconeogenesis in the liver, predicting non-responders to metformin is difficult. Thus, the pathophysiological characteristics of patients with complicated metabolic diseases such as T2DM and the mechanism of metformin's action have not been completely understood thus far.

A previous study showed that T2DM can be induced because of an imbalance in the ATP ratio caused by an adenosine monophosphate (AMP)-activated protein kinase (AMPK) disorder [10]. AMPK activation plays a significant role in glucose and lipid metabolism [11]. Thus, metformin can exert its effect in T2DM by activating AMPK through phosphorylation [15].

Metabolomics is a novel technology that has been increasingly used recently. This technology enables the measurement of various metabolites present in all organisms and provides new risk markers of T2DM and clinical efficacy by indicating the overall drug response index [16-18]. Identification of small molecular metabolites in bodily fluids will enable determination of drug effectiveness and prediction of treatment according to the phenotype of patients. Furthermore, this technology can identify mortality and morbidity associated with the disease and prognostic potential biomarkers of diseases and drug response [19-21].

The purpose of the current review is to investigate metabolic changes induced by metformin administration and compare the levels

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of metabolites and identify representative metabolites after metformin treatment that provide further clues for progress of T2DM, response mechanism to metformin and also the drug mechanism.

Methods

Search strategy

A comprehensive literature search was conducted using the PubMed electronic database. Search keywords included "metformin and metabolomics," and we selected articles that were published in the past five years. The last search was performed on March 5, 2016. There were no restrictions, except date of publication, on languages, gender, or biological fluids (e.g., blood or urine) during the selection of articles.

Study selection

From the initially identified 29 articles, we excluded non-human *in vivo* or *in vitro* studies, studies in cancer patients, and studies in which the focus was not on variation in the levels of metabolites after administration of medication such as pharmacogenetic studies. Thus, we selected 8 articles, and compared the levels of metabolites after metformin monotherapy or combination therapy. The search-term strategy has been described in detail in Figure 1. Then we identified metabolites pathway from searching through Metabolomics pathway analysis (Met PA) and Kyoto encyclopedia of genes and genomes (KEGG) databases.

Results

Literature information

We identified 30 articles from PubMed; from these, 10 studies published in English were reviewed. We excluded 2 studies that did not focus on human biological fluids but were animal experiments. Finally, we selected 8 articles for our systematic review and compared their results.

We identified many metabolites according to (1) various disease types (e.g., healthy [22] T2DM [9,22-25] coronary disease [25] hyperinsulinemic androgen excess [HAE] [8] polycystic ovary syndrome [PCOS] [26] (2) diverse number of patients (n=9 to 151); (3) different doses of metformin and its treatment period in the included trials (the shortest treatment period, 12 h and the longest follow-up period, 7 years); and (4) various analytical techniques used to identify metabolites from biological fluids (e.g., gas chromatography mass spectrometry [GC/MS], liquid chromatography mass spectrometry [LC/MS] and nuclear magnetic resonance [NMR]). We evaluated further details in the full-text article and included literature on the "metformin medication" regardless of above all conditions. The levels of several metabolites overlapped with each other in some articles (Table 1).

The most common metabolites were those found in plasma analysis, except one article in which the metabolites were obtained in urinary analysis [22].

The levels of urinary metabolites, including cortisol, hydroxycortisol (at boundary line statistically tolerance p=0.0575), betaine, and glucose decreased 12 h after metformin administration, while those of retinyl β-glucuronide and cholic acid glucuronide increased [22]. The studies by Henk den Ouden et al. [23] and Irving et al. [24] examining treatment with metformin alone for 5 years and in combination with pioglitazone for 3 months showed a decrease in the plasma glucose concentration. The results of studies by Henk den Ouden et al. [23] Irving et al. [24] and Vinaixa et al. [26] showed a significant decrease in blood glutamate levels compared to those before treatment. Treatment with metformin 850 mg/d, pioglitazone 7.5 mg/d, and flutamide 62.5 mg/d in PCOS showed an approximately 54.0% decrease in glutamate levels [26] and another study indicated a 17.0% decrease [24] in these levels at 3 months follow-up after combination therapy. Moreover, the results of the study by Vinaixa et al. showed that the serum levels of lysine, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol varied considerably; the levels of lysine and LDL cholesterol decreased by approximately 45.0% and 5.28%, respectively; however, the HDL cholesterol levels increased by 34.0%. Henk den Ouden et al. [22] showed that after 5 years of follow up, metformin decreased the total plasma cholesterol. Samino et al. [8] and Xu Tao et al. [27] showed that the serum HDL cholesterol increased and LDL cholesterol decreased, respectively. Irving et al. [24] showed that the lysine levels decreased by 5.55% after treatment with metformin plus pioglitazone in 12 T2DM patients in less than 3 months. The plasma levels of phenylalanine and tyrosine decreased by 7.76% and 13.0%, respectively [24]. In addition, the results reported by Henk den Ouden et al. showed a decrease in the plasma levels of l-phenylalanine [23]. Preiss et al. [25] showed that metformin monotherapy in 86 patients with coronary disease for 18 months induced a decrease in the levels of aromatic amino acid metabolites such as tyrosine by 10% and phenylalanine by 4% and an increase in the levels of alanine by approximately 16%. Henk den Ouden et al. [26] showed that the levels of HbA1c as an indicator of T2DM decreased after metformin administration at 5 years follow up in 132 patients with T2DM and 82 controls. Samino et al. [8] and Irving et al. [27] showed a decrease in insulin levels in the clinical blood specimen.

Lipid metabolite characteristics

Zhang et al. [9] included 23 patients with T2DM receiving metformin 1500 mg/d alone for the 3-year follow-up, and compared the changes in the lipid metabolite levels at 3 years and those at baseline. Their results showed that the levels of phosphatidylcholine (PC) (36:6, 38:4, 40.5, o-36:5) and sphingomyelin (SM) (d18:1-14:0, d18:1-16:1, d18:1-20:1) decreased by about 40.0%, 28.22%, 33.33%,

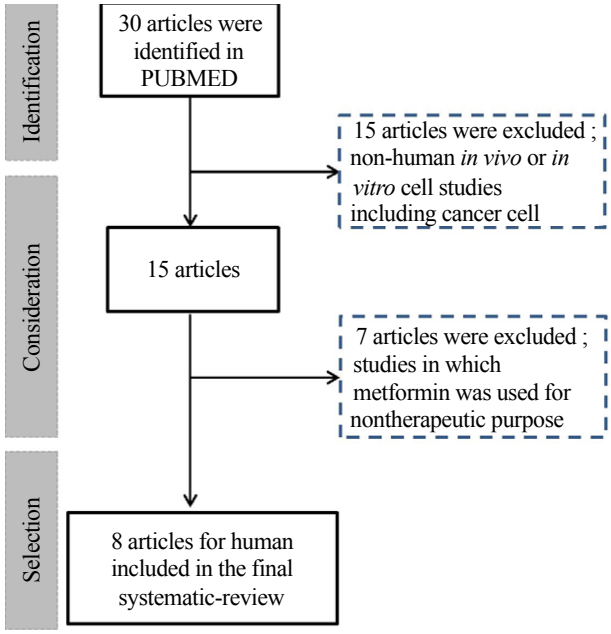

Figure 1: Flowchart of the search for appropriate studies.

Disease	Number of patients		Medication	Period	Specimen	Metabolites	Variation	Approach	Reference
	Case	Control							
Healthy subjects	14	14 baseline	Met 1000 mg	12 hours	Urine	Glucose	↓	LC/Q-TOF/MS	22
T2DM	132	82 no medication	Met	5 years	Plasma	Glucose Glutamate L-phenylalanine LCB 18:1–17:0 Cholesterol HbA1c	↓ ↓ ↓ ↓ ↓ ↓	GC-MS	23
T2DM	12	13 placebo	Met 1000 mg twice per day + Pio 45 mg/d	3 months	Plasma	Phenylalanine Tyrosine Lysine Insulin Glucose Glutamate	↓ ↓ ↓ ↓ ↓ ↓	UPLC-MS/MS	24
Coronary disease	86	87 placebo	Met	18 months	Plasma	Tyrosine Phenylalanine Alanine	↓ ↓ ↑	NMR	25
HIAE	12	12 baseline	Met 850 mg/d+Pio 7.5 mg/d+ Flu 62.5 mg/d	18 months	Serum	Insulin HDL chol	↓ ↑	LC/Q-TOF/MS	8
T2DM	23	23 baseline	Met 1500 mg/d	3 years	Serum	PC(36:6) PC(38:4) PC(40:5) PC(o-34:2) PC(o-36:5) SM(d18:1-14:0) SM(d18:1-16:1) SM(d18:1-20:1)	↓ ↓ ↓ ↑ ↓ ↓ ↓ ↓	LC/Q-TOF/MS	9
PCOS	12	12 baseline	Met 850 mg/d + Pio 7.5 mg/d + Flu 62.5 mg/d	30 months	Serum	Lysine HDL chol Glutamate LDL chol	↓ ↑ ↓ ↓	NMR	26
T2DM	151	NR	Met	7 years	Serum	PC ae C36:4 PC ae C38:5 PC ae C38:6 LDL chol	↓ ↓ ↓ ↓	UPLC/Q-TOF/MS	27

T2DM: Type 2 Diabetes Mellitus; HIAE: Hyperinsulinemic Androgen Excess; PCOS: Polycystic Ovary Syndrome; Met: Metformin; Sul: Sulfonylurea; Pio: Pioglitazone; Flu: Flutamide; SM: Sphingomyelin; Chol: Cholesterol; PC: Phosphatidylcholine; Ae: Acyl-Alkyl; ↑: Increase; ↓: Decrease; NR: No Reported

Table 1: Significant difference in the levels of metabolites or characteristics of included journals of human blood or urine.

23.81%, 41.25%, 73.89%, and 17.03%, respectively. The levels of one lipid PC (o-34:2) increased by approximately 66.67%. Henk den Ouden et al. [28] reported that LCB 18:1-17:0 SM decreased after metformin monotherapy.

Discussion

Pharmacometabolomics could provide certain advantages compared to other "omics" fields because metabolites that intermediates and end products in metabolic pathway necessary to reflect better physiological dysfunctions, drug response and enable to diagnosis using biological fluids [25,29-38]. A previous study reported the integration of metabolite levels of bio fluids and other biochemical information (e.g., glucose and HbA1c) could better reflect T2DM risk than a single established risk factor [29,39-46]. Determination of the levels of several amino acid and other metabolites that showed a marked change after metformin treatment could significantly improve drug response prediction and serve as markers for the diagnosis of T2DM. The present systematic review provides detailed information of metabolite changes of bio fluids after metformin treatment. The results showed glucose, phenylalanine, tyrosine, glutamate, lysine, total cholesterol and LDL cholesterol were significantly changed after metformin therapy.

Especially, aromatic amino acids such as phenylalanine and tyrosine are closely correlated of future development of diabetes in prospective 10-year follow-up cohort study with 429 Chinese participants [43]. Including above two amino acids and a kind of branched chain amino acid isoleucine combination concentration had a much higher risk of developing diabetes (5 to 7 fold higher odds compared with those whose amino acid scores were in the lowest quartile) over the 12-year follow-up cohort study in 201 developed diabetes Swedish population [29]. Several studies suggested that higher plasma amino acid level profile could reflect hyperglycemia odds and incidence of type 2 diabetes mellitus hazards assessment [29,43-45]. Phenylalanine and tyrosine could be a strong predictor of diabetes [46], further theses AAAs are future biomarkers of long-term impaired insulin sensitivity.

Glucose reduction (exact percents are not shown) after metformin treatments identified in 3 articles [22-24] was significantly associated with glycolysis or gluconeogenesis pathway (p=0.0129) in MetPA homo sapiens group. Metformin lowers blood glucose level by inhibiting glycogenolysis. Cho et al. indicated that metformin inhibited adrenocorticotropic hormone (ACTH) stimulation, continually decreased cortisol level, and thus decreased the blood glucose concentration [22]. In addition, glucose was conjugated with

glycated hemoglobin (especially hemoglobin A1c), and there was a positive association between blood glucose concentration and HbA1c levels. Measurement of HbA1c levels is an essential component for the diagnosis and prognosis of patients with T2DM because it used to determine glycemic control stage [40]. Metformin might play a relevant role through glycolysis or gluconeogenesis pathway, undermining the synthesis of glucose, reducing HbA1c levels and finally blood glucose reduction.

The levels of phenylalanine measured using GC/MS, LC/MS, and NMR showed a decrease of approximately 5.58 ± 2.23% in 3 studies [23-25]. The plasma tyrosine levels decreased by 11.50 ± 2.12% after metformin administration [24,26]. Because phenylalanine and tyrosine are the two amino acids that are the most significantly associated with the phenylalanine, tyrosine, and tryptophan biosynthesis pathway (p=0.0112), any changes in their concentrations are closely interconnected. Preiss suggested that metformin, which is an insulin sensitizer, decreased the levels of phenylalanine and tyrosine and increased the levels of alanine in patients with coronary disease. Phenylalanine and tyrosine were involved in a metabolic pathway in which two aromatic amino acids (AAA) were significantly associated with the onset of diabetes and they could predict T2DM [28,29].

The levels of glutamate decreased by 35.49 ± 26.18% in 3 studies [23-25] and the levels of glutamate are regulated by d-glutamine and g-glutamate metabolism (p=0.0046) and it also conjugated with phenylalanine nitrogen metabolism (p=0.0162). Phenylalanine and tyrosine are closely associated with each other and glutamate is associated with the above two AAAs through ammonia. Some studies have suggested that amino acids adjust insulin sensitivity through gluconeogenesis, especially impaired insulin sensitivity related to dysfunction of amino acid metabolism, and it may result in adiposity or diabetes [29,30]. Amino acids could modulate peripheral insulin sensitivity and eventually contribute to T2DM patients with insulin resistance [31]. Although the levels of phenylalanine and tyrosine are closely associated with the hazard of diabetes, metformin increases the sensitivity of insulin, which indicates that metformin may play an important role in the plasma AAA concentrations [25].

The serum levels of lysine analyzed using UPLC and NMR showed a decrease by 25.28 ± 27.90% after metformin treatment [24,27]. Lysine was the most significantly associated with biotin metabolism pathway (p=0.00457), and it was also connected with fatty acid biosynthesis pathway. Some studies showed that the concentration of fatty acids and certain amino acids such as lysine were higher in T2DM than in healthy humans [32,33]. In addition, lysine is associated with glycolysis or gluconeogenesis pathway, and thus, there is a positive association between glucose concentration and change in lysine levels. Metformin could have a favorable effect on decrease in lysine levels with the expected lowering of fatty acid levels in the blood.

The total cholesterol and LDL cholesterol concentration in serum decreased (exact percent not shown) [23,25,26] but those of HDL cholesterol increased by 34% [8,26]. These studies indicate that metformin probably could affect total, LDL, and HDL cholesterol. The levels of total and LDL cholesterol were statistically significantly lower than those of HDL cholesterol after metformin administration, and changes in these levels are relevant to glucose lowering [34].

Impaired insulin secretion and diminished insulin action affect gluconeogenesis in the liver and induce subsequent hyperglycemia in patients with T2DM [35]. Exacerbated insulin resistance plays an important role in increasing glucose production and deteriorating postprandial glucose tolerance [36]. Insulin signaling is associated with the mechanism of metformin's action and the phosphorylation of hepatic CREB binding protein (CBP) gene [36,37]. Insulin reduction is independent effect after metformin treatment but its signal is closely associated with metformin in specific gene site serine 436 [35].

Our study had several limitations deserve comment. Some studies did not indicate the exact change in percent or in any other unit and only specified an increase or a decrease. For a reliable systemic review, exact data from many articles are required for credible statistical analysis. Further, several studies have shown changes in the levels of important metabolites such as cortisol, mannose, arginine, serine, and histidine after metformin therapy; however, the results were not overlapped among the other studies. Thus, we excluded the inconsistent study results although the information of above metabolites is important for the diagnosis of T2DM or predicting metformin response. Despite there were several reports that did not contain the exact information of the extent of changes of metabolites, we could integrate their increase or reduction aspects reflect biological changes induced by metformin administration. The provided metabolites from this systematic review might be the clues for the diagnosis for T2DM or predict the metformin responses when they are accompanied with well-known as biomarkers glucose level or HbA1c score.

Conclusion

Our study demonstrated several metabolic alterations and their mechanisms in T2DM patients after metformin treatment using systematic review and pathway analysis. Even though the evidence of metabolomics markers for metformin treatment is not fully established, the selected metabolites were found that they are related directly or indirectly to glycolysis, gluconeogenesis, or insulin sensitivity. With additional validation studies, these metabolites could be developed as advantageous markers for early diagnosis for T2DM or prediction for metformin treatment. Moreover, this study showed the exploration for metabolomics markers of clinically important disease using systematic review and pathway analysis. These approaches could provide the strength of comprehensive understanding of disease and drug reaction.

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