Front. Med. 2013, 7(1): 4–13
DOI 10.1007/s11684-013-0248-4

REVIEW

# Metabolomics in human type 2 diabetes research

Jingyi Lu[1], Guoxiang Xie[2,3], Weiping Jia[1], Wei Jia (✉)[2,3]

[1]*Shanghai Diabetes Institute; Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital; Shanghai Key Laboratory of Diabetes Mellitus; Shanghai Clinical Center for Diabetes, Shanghai 200233, China;* [2]*Center for Translational Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China;* [3]*Center for Translational Biomedical Research, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC 28081, USA*



**Abstract** **The high prevalence of diabetes and diabetic complications has caused a huge burden on the modern society. Although scientific advances have led to effective strategies for preventing and treating diabetes over the past several decades, little progress has been made toward curing the disease or even getting it under control, from a public health and overall societal standpoint. There is still a lack of reliable biomarkers indicative of metabolic alterations associated with diabetes and different drug responses, highlighting the need for the development of early diagnostic and prognostic markers for diabetes and diabetic complications. The emergence of metabolomics has allowed researchers to systemically measure the small molecule metabolites, which are sensitive to the changes of both environmental and genetic factors and therefore, could be regarded as the link between genotypes and phenotypes. During the last decade, the progression made in metabolomics has provided insightful information on disease development and disease onset prediction. Recent studies using metabolomics approach coupled with statistical tools to predict incident diabetes revealed a number of metabolites that are significantly altered, including branched-chain and aromatic amino acids, such as isoleucine, leucine, valine, tyrosine and phenylalanine, as diagnostic or highly-significant predictors of future diabetes. This review summarizes the current findings of metabolomic studies in human investigations with the most common form of diabetes, type 2 diabetes.**

**Keywords** metabolomics; type 2 diabetes; metabolic pathway; mass spectrometry; nuclear magnetic resonance (NMR)

## Introduction

Diabetes mellitus is a chronic disease that is characterized by the absolute or relative shortage of insulin, leading to chronic hyperglycemia, which may be due either to the progressive failure of pancreatic β-cell function and consequently a lack of insulin production (type 1 diabetes) or to the development of insulin resistance and subsequently the loss in β-cell function (type 2 diabetes, T2DM). Diabetes mellitus, particularly T2DM, represents one of the most significant global health problems because it is associated with a large economic burden for the health systems of many countries. According to the data from world health organization (WHO), 346 million people are affected by diabetes in 2011 and an estimated 3.4 million people died from consequences of high blood sugar in 2004 and this number was predicted to double between 2005 and 2030 [1]. Due to the broad range of diabetes-related complications, including diabetic nephropathy, peripheral neuropathy and cardiovascular disease, diabetes is a major cause of both morbidity and mortality [2].

It is well known that the development of common forms of diabetes arises from the interplay between environmental and genetic factors. As for T2DM, the predominant cause is related to lifestyle factors including diet, insufficient physical activity, an overweight or obese state and stress [3]. Owe to the enormous effort put into the search of T2DM susceptible genes, more than 60 genetic loci have been identified and widely replicated [4]. However, these variants in total could only account for around 10% of the heritability of T2DM [5], which is called "missing heritability," and the mechanisms underlying the pathogenesis of diabetes are not fully understood.

Received October 9, 2012; accepted December 20, 2012
Correspondence: w_jia@uncg.edu

The diagnosis of diabetes is mainly based on the results of blood tests examining fasting blood glucose or glycated hemoglobin (HbA1c) levels [1]. Whereas the diagnosis and treatment of manifest diabetes have been thoroughly investigated, the identification of novel pathways or early biomarkers indicative of metabolic alterations of T2DM is still not fully understood. Without timely and appropriate management, subjects with diagnosed diabetes have already been affected by one or more macro- or microvascular complications, highlighting the critical importance of early diagnosis of the disease. Metabolomics or metabonomics, the comprehensive and quantitative analysis of all metabolites [6,7], is a rapidly evolving technology by which an entire spectrum of endogenous metabolites in cells, biofluids or tissue following genetic or environmental interventions is measured quantitatively. There is mounting evidence that metabolomics can provide important insight into biomarker discoveries, toxicity evaluation and the pathogenic nature of various diseases [6,8–14] and a great deal of research on diabetes has been conducted with both animal models and clinical human subjects during the last decade. The advantages of metabolomics over other "omics," e.g., genomics, transcriptomics or proteomics, include its high sensitivity and its ability to enable the analysis of relatively few metabolites compared with the unwieldy number of corresponding genes or mRNA molecules. The genome is often referred to as the blueprint of what can happen in our bodies. Pushing this analogy further would suggest that the proteome describes the tools that make things happen and the metabolome would be the end results of that work. The small molecules that compose the metabolome are the final downstream products of the interaction between genes and influences like environmental factors, health behavior or pharmaceutical interventions and their levels reflect the activity of metabolic pathways, which do much of the work in our bodies from signaling transcription to building proteins to create and shuttle energy. Therefore, metabolomics enables the detection of short-term and/or long-term pathophysiological changes in body fluids, cells or tissue and could be a useful tool for disease diagnosis or biomarker detection. The purpose of this review is to summarize the current metabolomic findings on T2DM, the most common form of diabetes, conducted in humans, and give an overview of the perturbed metabolic pathways in T2DM.

## Metabolomics techniques

There are two major high-throughput tools consisting of nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) used in metabolomics study. Both methods enable the comprehensive investigation of metabolic profiles [15] and can provide complementary snapshots of the metabolome of body fluids such as plasma, urine, cells or tissue [16,17].

### Mass spectrometry

MS is the most frequently used technique in metabolic studies and it is a powerful tool for investigating molecular structure as well as for detecting and quantifying metabolites [15]. MS provides mass-to-charge (*m/z*) ratio information, which enables the structure of metabolites to be determined. The greatest advantage of the MS is its high sensitivity, although disadvantages arise from the destruction of the sample and the long sample preparation time required. In addition, MS is often combined with other suitable methods for the analytical separation of compounds, including gas chromatography (GC) or liquid chromatography (LC), to achieve detection of distinct metabolite classes [18] by reducing the complexity of the mass spectra and the matrix effect. Both GC-MS and LC-MS demonstrate high separation efficiency and are excellent tools for metabolic profiling. Since the metabolome consists of a vast array of compounds, most current metabolomic analyses using a single analytical platform can only detect a fraction of the metabolites in a complex biological sample; thus, a multiplatform approach may provide a more comprehensive understanding of metabolic alterations. The combined use of two analytical platforms takes advantage of complementary analytical outcomes and therefore, broadens the "window" of important metabolic variations identified and another advantage of using the platforms in combination (for example, LC-MS and GC-MS) is that we can cross-validate the metabolites mutually detected by these two analytical platforms [14].

### Nuclear magnetic resonance spectroscopy

NMR is another widely used spectroscopic technique for metabolomics that is based on the magnetic properties of the atomic nucleus (e.g., $^{1}H$, $^{13}C$, or $^{31}P$), which enables the identification of metabolites that are otherwise unidentifiable by MS analysis [19]. NMR analysis usually does not require any pretreatment including column chromatography and derivatization. It is non-destructive, non-biased, highly quantitative, and enables the identification of complex unidentified metabolites. The major disadvantage of NMR, relative to MS, is its low sensitivity.

Detailed information regarding NMR theory, its application and typical chemical shift values are available elsewhere [20]. The summarization of NMR spectroscopic applications in modern metabolic research and detailed protocols for biofluid (urine, serum/plasma) and tissue samples can be achieved in literature [16].

## Metabolic variations and metabolic pathways in T2DM patients

Metabolic studies have revealed alterations in metabolites related to pathways involved in the action of insulin,

including lipolysis, ketogenesis, proteolysis and glucose metabolism from either animal studies or oral glucose tolerance test [3]. These results indicate a change from β-oxidation to glycolysis and fat storage in response to glucose ingestion. Furthermore, metabolomics studies conducted on human subjects between diabetic patients and healthy controls revealed many important altered metabolic pathways and metabolic variations. These findings can be summarized as follows.

## Carbohydrate metabolism and tricarboxylic acid (TCA) cycle

Glucose, a primary source of energy for human body, is utilized in cells beginning with glycolysis where glucose is converted into pyruvate. Under aerobic conditions, pyruvate is converted into acetyl coenzyme A (acetyl-CoA), which then could enter the TCA cycle to generate ATP. Under anaerobic conditions, however, lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. We recently conducted a study on diabetes including normal controls and patients with T2DM, type 1 diabetes, fulminant T1DM and diabetic ketoacidosis using metabolomics approach [21] and observed a 9.35 times higher concentration of serum pyruvate in T2DM patients than in normal controls (Table 1 and Fig. 1), suggesting an upregulated glycolysis in T2DM. In addition, the lactate level was also significantly increased. In urine samples, lactate was also found significantly increased in T2DM patients in a study comprising 33 T2DM patients and 20 healthy controls [22].

The TCA cycle is also known as the citric acid cycle, since the formation of citrate via acetyl-CoA is the first step in the cycle and citrate is regenerated by a sequence of reactions. Messana *et al.* [22] demonstrated that T2DM is associated with higher citrate levels compared with a normal condition. However, the perturbations of TCA cycle seem to be more complex than this study suggests: although citrate was observed to be elevated in T2DM in the work by Salek *et al.* [23], three TCA cycle intermediates, namely, malate, fumarate and succinate, were significantly downregulated in urines of diabetic patients in the same study, warranting further studies to address this issue.

1,5-anhydroglucitol (1,5-AG) is a naturally occurring dietary polyol with a similar structure to glucose and is maintained at a steady-state level during normoglycemia [24] through kidney filtration and reabsorption [25]. However, with elevated serum glucose concentrations ( $> 180$ μmol/L), glucose is not completely reabsorbed by the kidney, and the serum 1,5-AG decreased due to the competitive inhibition of renal tubular reabsorption of glucose. Several studies have found 1,5-AG to be a sensitive marker for postprandial hyperglycemia [26–28], which is an independent risk factor for macrovascular complications [29–31]. Many patients who are otherwise well controlled by HbA1c, an indicator of overall blood glucose control, also have significant postprandial hyperglycemia [32]. In agreement with these findings, the serum 1,5-AG levels of normal controls in our study were 10 times as high as those of fulminant type 1 diabetic patients [21], despite the comparable HbA1c ($5.40 \pm 0.31$ vs. $6.30 \pm 0.23$, $P > 0.05$) between the two groups, further supporting the notion that 1,5-AG could be used as an adjunct index to HbA1c for a better glycemic control [26].

## Lipid metabolism

It is well-established that diabetes is often accompanied by dyslipidemia [33], which is a major risk factor of cardiovascular diseases in diabetic patients. The precise pathogenesis of diabetic dyslipidemia remains unknown. Nevertheless, a large body of evidence suggests that increased free fatty acid flux secondary to insulin resistance is the main cause [34,35]. In concert with the findings in references 34 and 35, higher blood levels of fatty acids in T2DM patients were detected in many metabolomic studies [21,36]. For instance, palmitate, heptadecanoate, stearate, oleate and palmitoleate were found to be significantly increased in obese T2DM women in contrast to obese non-diabetic controls [36].

In diabetes, especially under poorly controlled conditions, glucose cannot be efficiently utilized due to absolute or relative shortage of insulin and therefore, the increased flux of fatty acids serves as the major source of energy through β-oxidation, and as a result, ketone bodies including acetone, acetoacetate and 3-hydroxybutyrate are released [37]. Not surprisingly, 3-hydroxybutyrate was higher in plasma [36], serum [21,38] and urine [23] samples of T2DM patients compared to normal subjects in different studies and the concomitant increase of acetoacetate was also observed by Salek *et al.* [23].

Despite the increased availability of lipids, T2DM is associated with a blunted ability of skeletal muscle to oxidize free fatty acids [39–41]. However, the mechanisms that underlie dysfunctional mitochondrial fatty acid oxidation and impaired insulin action are not fully understood. Targeted acylcarnitine profiling with ultra performance liquid chromatography-mass spectrometry (UPLC-MS) in 44 diabetic and 12 non-diabetic subjects demonstrated that acylcarnitines, particularly long-chain acylcarnitines ($C_{10}$-carn, $C_{12}$-carn and $C_{14}$-carn), were significantly increased in T2DM subjects, reflective of incomplete long-chain fatty acid oxidation [42]. Moreover, the proof-of-principle work by Adams *et al.* [42], showed that nuclear factor-κB (NF-κB) can be significantly activated by $C_{12}$-carn and $C_{14}$-carn, which could induce inflammation and plays an important role in the onset of insulin resistance [43,44]. The authors proposed that limited TCA cycle activity relative to mitochondrial fuel delivery contributes to incomplete long-chain fatty acid combustion, in turn promoting accumulation of acylcarnitine by-products that activate NF-κB-associated pathways to inhibit insulin activity. The abnormal accumulation of acylcarnitines was

**Table 1** List of altered metabolic pathways in T2DM patients

| Pathway | Metabolite | Change of direction (vs. healthy control) | Sample | Platform | Reference |
|---|---|---|---|---|---|
| Carbohydrate metabolism and TCA cycle | 1,5-Anhydrogluticol | Down | Serum | NMR, UPLC-MS, GC-MS | [38] |
| | | Down | Serum | GC-MS | [21] |
| | Pyruvate | Up | Serum | GC-MS | [21] |
| | Lactate | Up | Serum | GC-MS | [21] |
| | | Up | Urine | NMR | [22] |
| | | Down | serum | GC-MS | [58] |
| | | Down | Serum | NMR | [57] |
| | Citrate | Up | Urine | NMR | [22] |
| | | Up | Urine | NMR | [23] |
| | | Down | Serum | NMR | [57] |
| | Malate | Down | Urine | NMR | [23] |
| | Fumarate | Down | Urine | NMR | [23] |
| | Succinate | Down | Urine | NMR | [23] |
| Lipid metabolism | 3-Hydroxybutyrate | Up | Plasma | GC-MS | [36] |
| | | UP | Serum | NMR, UPLC-MS, GC-MS | [38] |
| | | Up | Urine | NMR | [23] |
| | | Up | Serum | GC-MS | [21] |
| | Acetoacetate | Up | Urine | NMR | [23] |
| | Fatty acids | Up | Plasma | GC-MS | [36] |
| | | Up | Serum | GC-MS | [21] |
| | LysoPCs | Up | Plasma | UPLC-MS | [46] |
| | LysoPC (18:2) | Down | Serum | LC-MS | [50] |
| | | Down | Serum | LC-MS | [49] |
| | LysoPEs | Up/Down | Plasma | UPLC-MS | [46] |
| | PCs | Up/Down | Serum | LC-MS | [49] |
| | Acetylcarnitines | Up | Plasma | UPLC-MS | [46] |
| | | Up | Plasma | UPLC-MS | [45] |
| | | Up/Down | Plasma | UPLC-MS | [42] |
| | | Down | Plasma | UPLC-MS | [47] |
| Amino acid metabolism | Valine (BCAA) | UP | Serum | NMR, UPLC-MS, GC-MS | [38] |
| | | Up | serum | GC-MS | [58] |
| | | Down | Serum | NMR | [57] |
| | Leucine (BCAA) | UP | Serum | NMR, UPLC-MS, GC-MS | [38] |
| | | Up | Plasma | UPLC-MS | [46] |
| | | Up | Plasma | GC-MS | [36] |
| | | Down | Serum | NMR | [57] |
| | | Down | Urine | NMR | [23] |
| | Isoleucine (BCAA) | UP | Serum | NMR, UPLC-MS, GC-MS | [38] |
| | | Down | Serum | NMR | [57] |
| | | Down | Urine | NMR | [23] |
| | Lysine | Down | Plasma | GC-MS | [36] |
| | | Down | Serum | NMR | [57] |
| | | Down | Serum | GC-MS | [58] |
| | | Up | Plasma | UPLC-MS | [46] |
| | Glycine | Down | Plasma | GC-MS | [36] |
| | | Down | Serum | LC-MS | [50] |
| | | Down | Serum | LC-MS | [49] |
| | Serine | Up | Plasma | UPLC-MS | [46] |
| | Tyrosine | Down | Serum | NMR | [57] |
| | Phenylalanine | Up | Plasma | UPLC-MS | [46] |

(*Continued*)

| Pathway | Metabolite | Change of direction (vs. healthy control) | Sample | Platform | Reference |
|---|---|---|---|---|---|
| | Phenylalanine | Up | Serum | GC-MS | [21] |
| | | Up | Serum | LC-MS | [49] |
| | | Down | Serum | NMR | [57] |
| | Tryptophan | Down | Urine | NMR | [23] |
| | Alanine | Up | Urine | NMR | [22] |
| | | Down | Serum | NMR | [57] |
| | Glutamine | Up | Urine | NMR | [23] |
| | | Up | Serum | GC-MS | [21] |
| | Glutamate | Up | serum | GC-MS | [58] |
| | | Down | Serum | GC-MS | [21] |
| | Methionine | Down | Serum | NMR | [57] |
| | | Up | Serum | GC-MS | [21] |
| | Histidine | Down | Serum | NMR | [57] |
| | | Down | Urine | NMR | [23] |
| | 2-Hydroxybutyrate | Up | Plasma | GC×GC-MS | [62] |
| | | Up | Plasma | GC-MS | [36] |
| | | Up | Serum | GC-MS | [21] |
| | | Up | Plasma | GC-MS | [36] |
| | Hippurate | Up | Urine | NMR | [22] |
| | Taurine | Up | Urine | NMR | [23] |
| Choline metabolism | Betaine | Up | Urine | NMR | [22] |
| | DMA | Up | Urine | NMR | [22] |
| | TMAO | Up | Urine | NMR | [22] |
| | | Up | Urine | NMR | [23] |
| Bile acid metabolism | Cholate | Down | Serum | NMR, UPLC-MS, GC-MS | [38] |
| | Deoxycholate | Up | Serum | NMR, UPLC-MS, GC-MS | [38] |

TCA, tricarboxylic acid cycle; NMR, nuclear magnetic resonance; GC-MS, gas chromatograph-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; UPLC-MS, ultra performance liquid chromatography-mass spectrometry; LysoPC, lysophosphatidylcholine; LysoPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; DMA, dimethylamine; TMAO, trimethylamine N-oxide.

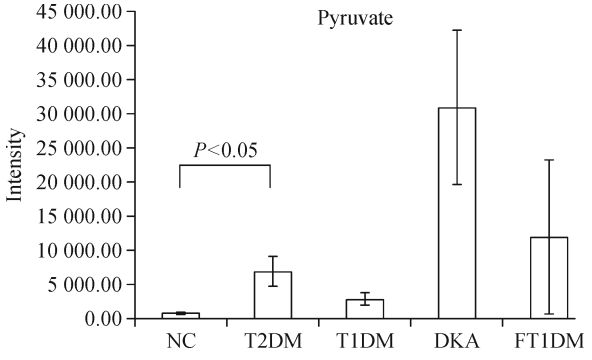


**Fig. 1** The intensity of serum pyruvate measured by GC-MS in different groups. NC, normal control; T2DM, type 2 diabetes; T1DM, type 1 diabetes; DKA, diabetic ketoacidosis; FT1DM, fulminant type 1 diabetes.

replicated in both targeted [45] and non-targeted [46] metabolomic studies. It is noteworthy that a recent study in youth produced contrasting results [47]. Adolescents with T2DM exhibited enhanced mitochondrial function evidenced by similar long-chain acylcarnitines, lower medium- to short-chain acylcarnitines (except $C_8$-carn and $C_{10}$-carn) and higher rates of fat oxidation in comparison to normal controls, possibly suggesting an early metabolic plasticity in youth.

Phospholipids are key components of lipid bilayer of all cells and are implicated in cellular signal transduction [48]. In a recent large prospective study (mean follow-up 7 years) with a targeted metabolomic approach, Floegel *et al.* [49] reported that numerous phospholipids (including sphingomyelin, 9 phosphatidylcholines and lysophosphatidylcholine $C_{18:2}$) at baseline were significantly associated with the risk of T2DM, independent of established risk factors. Likewise, lower levels of lysophosphatidylcholine $C_{18:2}$ were shown to be predictive for T2DM in a prospective population-based cohort by Wang-Sattler *et al.* [50]. Though the precise mechanism underlying such association has yet to be elucidated, these two studies imply that alterations of phospholipids may be an early event in the pathogenesis of T2DM.

### Amino acid metabolism

Branched-chain amino acids (BCAAs) include valine, leucine and isoleucine, which are among the nine essential amino acids for humans. Although the association of BCAAs with insulin and diabetes has been noted for over 60 years [51–53], BCAAs did not gain much research interest until the study done by Newgard *et al.* [9] in 2009. It was found the three BCAAs, isoleucine, leucine, and valine, are correlated with insulin resistance and significantly increased in overweight/obese subjects compared to healthy leans by a metabolomics study with a cohort of 74 obese and 67 lean subjects. BCAAs were further reported to be significantly associated with insulin resistance in both overweight/obese [54] and normal weight subjects [55]. Recently, Wang *et al.* [56] followed 2422 non-diabetic individuals for 12 years and found that the increased branched-chain and aromatic amino acids including isoleucine, leucine, valine, tyrosine and phenylalanine in diabetic patients could be highly-significant predictors for future diabetes. The prospective nature of their study suggests a cause-effect relationship between BCAAs and diabetes. It's noteworthy that decreased levels of BCAAs were reported in both serum [57] and urine samples [23] in diabetic subjects. One possible explanation could be that the T2DM patients recruited in the metabolomic studies mentioned above were not newly-diagnosed and untreated, except those enrolled in the study done by Bao *et al.* [58], in which serum valine was significantly increased in T2DM. Thus the findings observed in references 57 and 23 might not reflect the physiological alterations in BCAA metabolism in T2DM [59].

In two population-based prospective studies [49,50] as mentioned above, baseline serum glycine was found to be inversely correlated with the risk of developing T2DM. Interestingly, a glycine related enzyme, 5-aminolevulinate synthase 1 (ALAS-H), was upregulated in the T2DM group in the same study [50]. Together with the link between insulin and ALAS-H expression [60], it was postulated that the decrease of serum glycine in T2DM may result from insulin resistance. Indeed, Floegel *et al.* [49] observed that glycine was positively associated with insulin sensitivity in their study samples.

Our recent [21] and others' [36,61,62] studies have identified a consistent increase of blood 2-hydroxybutryrate (2-HB) in T2DM patients (Fig. 2A). 2-HB, which could be used to synthesize gluthathione, is released as a byproduct when cystathionine is cleaved to cysteine. Higher level of 2-HB, together with higher cystine [36] in T2DM patients, probably reflected the increased activity of homocysteine transsulfuration pathway secondary to oxidative stress [63,64]. Consistent with the result that 2-HB was negatively correlated with insulin sensitivity [61], we found that the serum 2-HB concentration was positively correlated with HbA1c in our study samples ($r = 0.420$, $P < 0.001$) [21] (unpublished data, Fig. 2B), which is similar with the results achieved by Fiehn *et al.* ($r = 0.455$, $P = 0.001$) [36], raising the possibility that 2-HB could serve as a biomarker of blood glucose control and therefore oxidative stress [65].

### Other altered metabolic pathways

It is well known that bile acids are tightly linked to lipid metabolism. However, there is a growing body of evidence

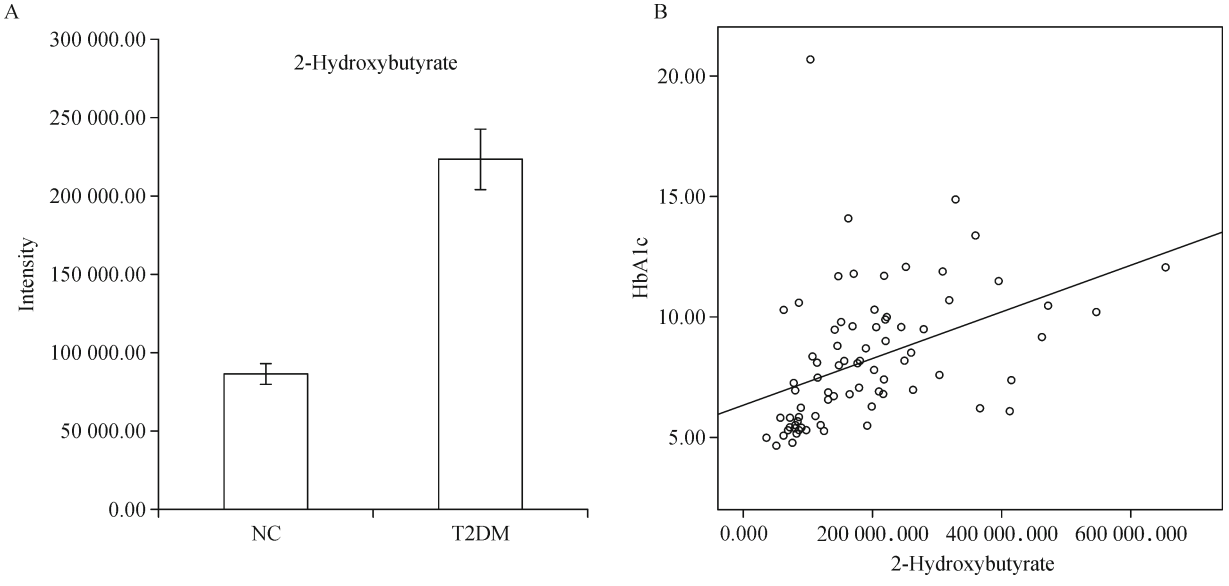


**Fig. 2** The comparison of 2-hydroxybutyrate between nondiabetic and diabetic subjects and the association of 2-hydroxybutyrate with HbA1c. (A) The intensity of serum 2-hydroxybutyrate measured by GC-MS in normal controls and diabetic patients. (B) The Pearson correlation between 2-hydroxybutyrate and HbA1c.

that T2DM is associated with an altered bile acid pool. For example, Bennion *et al.* [66] reported that bile acid pool size and fecal bile acid excretion were significantly higher during uncontrolled hyperglycemia than during relative euglycemia. Bile acid sequestrants can be used to bind bile acids in the intestinal and disrupt the enterohepatic circulation, subsequently modulating the bile acid pool composition. 8-week treatment with colesevelam hydrochloride, a bile acid sequestrant, in diabetic patients was found to significantly improve whole body insulin resistance [67]. Using a metabolomic approach, Suhre *et al.* [38] reported that cholate, one of two primary bile acids, was more frequently detected in controls than in patients with T2DM, while for deoxycholate, a secondary bile acid, was rich in T2DM patients. This phenomenon implies a perturbed composition of bile acid pool in T2DM, and more specifically, a higher rate of conversion from primary to secondary bile acids.

Wang *et al.* [68] recently demonstrated that three metabolites of the dietary lipid phosphatidylcholine—choline, trimethylamine N-oxide (TMAO) and betaine—were able to predict risk for cardiovascular diseases in an independent cohort. Feeding mice with TMAO could promote the development of atherosclerosis. By NMR, Messana *et al.* [22] observed higher urine levels of betaine and TMAO in T2DM patients compared with normal controls. Another metabolomic study with a similar design also reported a significant relationship between TMAO and T2DM in consistency [23]. These findings are of interest in light of the fact that diabetes itself is a major risk factor for CVD in both men and women [69,70].

Additionally, alterations in intestinal microflora-associated metabolites have been detected. Studies using germ-free mice confirmed a critical role of gut microflora in the formation of TMAO from dietary choline [68,71] and recently Swann *et al.* [72] revealed that germ-free and antibiotic-treated mice exhibited lower bile acid diversity and a major increase in the taurine-conjugated bile acids in multiple tissue, confirming the role of gut microflora in the regulation of bile acid pool. Also note that higher urine levels of hippurate, which is mainly produced via gut microbial metabolism, was observed in the metabolomic study done by Messana *et al*. [22]. The human gut microflora has an important role in health, which has been comprehensively discussed by several researchers [73,74]. These studies suggest that the perturbation of gut microflora may underlie some of the metabolic changes in diabetes, thereby modulating diabetes risk.

## Conclusions and future perspectives

The fast-growing application of metabolomics in diabetes has provided researchers much knowledge and the opportunity to gain new insights into metabolic pathways and pathophysiological mechanisms. Several potential metabolic biomarkers and related metabolic pathways have been identified and are currently being investigated and validated in T2DM patients, such as 3-hydroxybutyrate with ketogenesis and altered bile acids, in addition to the BCAAs and AAAs. It is also notable that recent metabolomic studies on T2DM have reported many conflicting results regarding certain metabolites (e.g., BCAAs). Given that human metabolome has been shown to be sensitive to age, sex, diet, drug and other environmental factor, a stringent selection and treatment of samples as well as an optimized metabolomics profiling protocol should always be taken into consideration prior to the study. Nevertheless, metabolomics increased our knowledge of the dysregulated metabolic pathways associated with progression of metabolic diseases and provided potentially new therapeutic strategies targeting these dysregulated pathways, though our understanding about the distinct and complete metabolic footprints of T2DM is still very limited.

## Acknowledgements

This work was supported by grants from the National Basic Research Program of China (973 Program, 2011CB504001) and the National Natural Science Foundation of China (Grant Nos. 81100590 and 81170760).

## References

1. World Health Organization. Diabetes: Fact sheet N°312. 2011
2. National Diabetes Information Clearinghouse (NDIC). Complications of Diabetes. 2012
3. Friedrich N. Metabolomics in diabetes research. J Endocrinol 2012; 215(1): 29–42
4. Pal A, McCarthy M . The genetics of type 2 diabetes and its clinical relevance. Clin Genet 2012 Nov 20. [Epub ahead of print] doi: 10.1111/cge.12055
5. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, Zeggini E, Huth C, Aulchenko YS, Thorleifsson G, McCulloch LJ, Ferreira T, Grallert H, Amin N, Wu G, Willer CJ, Raychaudhuri S, McCarroll SA, Langenberg C, Hofmann OM, Dupuis J, Qi L, Segrè AV, van Hoek M, Navarro P, Ardlie K, Balkau B, Benediktsson R, Bennett AJ, Blagieva R, Boerwinkle E, Bonnycastle LL, Bengtsson Boström K, Bravenboer B, Bumpstead S, Burtt NP, Charpentier G, Chines PS, Cornelis M, Couper DJ, Crawford G, Doney AS, Elliott KS, Elliott AL, Erdos MR, Fox CS, Franklin CS, Ganser M, Gieger C, Grarup N, Green T, Griffin S, Groves CJ, Guiducci C, Hadjadj S, Hassanali N, Herder C, Isomaa B, Jackson AU, Johnson PR, Jørgensen T, Kao WH, Klopp N, Kong A, Kraft P, Kuusisto J, Lauritzen T, Li M, Lieverse A, Lindgren CM, Lyssenko V, Marre M, Meitinger T, Midthjell K, Morken MA, Narisu N, Nilsson P, Owen KR, Payne F, Perry JR, Petersen AK, Platou C, Proença C, Prokopenko I, Rathmann W, Rayner NW, Robertson NR, Rocheleau G, Roden M, Sampson MJ, Saxena R, Shields BM, Shrader P, Sigurdsson G, Sparsø T, Strassburger K, Stringham HM, Sun Q, Swift AJ, Thorand B, Tichet J, Tuomi T, van Dam RM, van Haeften TW, van Herpt T, van Vliet-Ostaptchouk JV, Walters GB, Weedon MN, Wijmenga C, Witteman J, Bergman RN,

Cauchi S, Collins FS, Gloyn AL, Gyllensten U, Hansen T, Hide WA, Hitman GA, Hofman A, Hunter DJ, Hveem K, Laakso M, Mohlke KL, Morris AD, Palmer CN, Pramstaller PP, Rudan I, Sijbrands E, Stein LD, Tuomilehto J, Uitterlinden A, Walker M, Wareham NJ, Watanabe RM, Abecasis GR, Boehm BO, Campbell H, Daly MJ, Hattersley AT, Hu FB, Meigs JB, Pankow JS, Pedersen O, Wichmann HE, Barroso I, Florez JC, Frayling TM, Groop L, Sladek R, Thorsteinsdottir U, Wilson JF, Illig T, Froguel P, van Duijn CM, Stefansson K, Altshuler D, Boehnke M, McCarthy MI ; MAGIC investigators; GIANT Consortium. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010; 42(7): 579–589

6. Fiehn O. Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. Comp Funct Genomics 2001; 2(3): 155–168
7. Nicholson JK, Lindon JC, Holmes E. "Metabonomics": understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica1999; 29(11): 1181–1189
8. Lucio M, Fekete A, Weigert C, Wägele B, Zhao X, Chen J, Fritsche A, Häring HU, Schleicher ED, Xu G, Schmitt-Kopplin P, Lehmann R. Insulin sensitivity is reflected by characteristic metabolic fingerprints—a Fourier transform mass spectrometric non-targeted metabolomics approach. PLoS ONE 2010; 5(10): e13317
9. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009; 9(4): 311–326
10. Qiu Y, Cai G, Su M, Chen T, Zheng X, Xu Y, Ni Y, Zhao A, Xu LX, Cai S, Jia W. Serum metabolite profiling of human colorectal cancer using GC-TOFMS and UPLC-QTOFMS. J Proteome Res 2009; 8 (10): 4844–4850
11. Lanza IR, Zhang S, Ward LE, Karakelides H, Raftery D, Nair KS. Quantitative metabolomics by H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. PLoS ONE 2010; 5(5): e10538
12. Bernini P, Bertini I, Luchinat C, Tenori L, Tognaccini A. The cardiovascular risk of healthy individuals studied by NMR metabonomics of plasma samples. J Proteome Res 2011; 10(11): 4983–4992
13. Xie G, Zheng X, Qi X, Cao Y, Chi Y, Su M, Ni Y, Qiu Y, Liu Y, Li H, Zhao A, Jia W. Metabonomic evaluation of melamine-induced acute renal toxicity in rats. J Proteome Res 2010; 9(1): 125–133
14. Chen T, Xie G, Wang X, Fan J, Qiu Y, Zheng X, Qi X, Cao Y, Su M, Xu LX, Yen Y, Liu P, Jia W. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. Mol Cell Proteomics 2011;10(7):M110.004945
15. Lenz EM, Wilson ID. Analytical strategies in metabonomics. J Proteome Res 2007; 6(2): 443–458
16. Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, Nicholson JK. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2007; 2(11): 2692–2703
17. Bictash M, Ebbels TM, Chan Q, Loo RL, Yap IK, Brown IJ, de Iorio M, Daviglus ML, Holmes E, Stamler J, Nicholson JK, Elliott P. Opening up the "Black Box": metabolic phenotyping and metabolome-wide association studies in epidemiology. J Clin Epidemiol 2010; 63(9): 970–979
18. Büscher JM, Czernik D, Ewald JC, Sauer U, Zamboni N. Cross-platform comparison of methods for quantitative metabolomics of primary metabolism. Anal Chem 2009; 81(6): 2135–2143
19. Kim HK, Choi YH, Verpoorte R. NMR-based plant metabolomics: where do we stand, where do we go? Trends Biotechnol 2011; 29(6): 267–275
20. Blumich B. Essential NMR: For Scientists and Engineers. Springer-Verlag Berlin and Heidelberg GmbH & Co. K, 2005
21. Lu J, Zhou J, Bao Y, Chen T, Zhang Y, Zhao A, Qiu Y, Xie G, Wang C, Jia W, Jia W. Serum metabolic signatures of fulminant type 1 diabetes. J Proteome Res 2012; 11(9): 4705–4711
22. Messana I, Forni F, Ferrari F, Rossi C, Giardina B, Zuppi C. Proton nuclear magnetic resonance spectral profiles of urine in type II diabetic patients. Clin Chem 1998; 44(7): 1529–1534
23. Salek RM, Maguire ML, Bentley E, Rubtsov DV, Hough T, Cheeseman M, Nunez D, Sweatman BC, Haselden JN, Cox RD, Connor SC, Griffin JL. A metabolomic comparison of urinary changes in type 2 diabetes in mouse, rat, and human. Physiol Genomics 2007; 29(2): 99–108
24. Yamanouchi T, Tachibana Y, Akanuma H, Minoda S, Shinohara T, Moromizato H, Miyashita H, Akaoka I. Origin and disposal of 1,5-anhydroglucitol, a major polyol in the human body. Am J Physiol 1992; 263(2 Pt 1): E268–E273
25. Yamanouchi T, Akanuma H, Asano T, Konishi C, Akaoka I, Akanuma Y. Reduction and recovery of plasma 1,5-anhydro-D-glucitol level in diabetes mellitus. Diabetes 1987; 36(6): 709–715
26. Dungan KM, Buse JB, Largay J, Kelly MM, Button EA, Kato S, Wittlin S. 1,5-anhydroglucitol and postprandial hyperglycemia as measured by continuous glucose monitoring system in moderately controlled patients with diabetes. Diabetes Care 2006; 29(6): 1214–1219
27. Yamanouchi T, Inoue T, Ogata E, Kashiwabara A, Ogata N, Sekino N, Yoshimura T, Ichiyanagi K, Kawasaki T. Post-load glucose measurements in oral glucose tolerance tests correlate well with 1,5-anhydroglucitol, an indicator of overall glycaemic state, in subjects with impaired glucose tolerance. Clin Sci (Lond) 2001; 101(3): 227–233
28. Yamanouchi T, Ogata N, Tagaya T, Kawasaki T, Sekino N, Funato H, Akaoka L, Miyashita H. Clinical usefulness of serum 1,5-anhydroglucitol in monitoring glycaemic control. Lancet 1996; 347 (9014): 1514–1518
29. Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, Ziegelasch HJ, Lindner J. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. Diabetologia 1996; 39(12): 1577–1583
30. Muggeo M, Zoppini G, Bonora E, Brun E, Bonadonna RC, Moghetti P, Verlato G. Fasting plasma glucose variability predicts 10-year survival of type 2 diabetic patients: the Verona Diabetes Study. Diabetes Care 2000; 23(1): 45–50
31. Temelkova-Kurktschiev TS, Koehler C, Henkel E, Leonhardt W, Fuecker K, Hanefeld M. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis

than fasting glucose or HbA1c level. Diabetes Care 2000; 23(12): 1830–1834
32. Erlinger TP, Brancati FL. Postchallenge hyperglycemia in a national sample of U.S. adults with type 2 diabetes. Diabetes Care 2001; 24 (10): 1734–1738
33. Kannel WB. Lipids, diabetes, and coronary heart disease: insights from the Framingham Study. Am Heart J 1985; 110(5): 1100–1107
34. Krauss RM, Siri PW. Dyslipidemia in type 2 diabetes. Med Clin North Am 2004; 88(4): 897–909, x
35. Taskinen MR. Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia 2003; 46(6): 733–749
36. Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH. Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. PLoS ONE 2010; 5(12): e15234
37. Balasse EO, Féry F. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab Rev 1989; 5(3): 247–270
38. Suhre K, Meisinger C, Döring A, Altmaier E, Belcredi P, Gieger C, Chang D, Milburn MV, Gall WE, Weinberger KM, Mewes HW, Hrabé de Angelis M, Wichmann HE, Kronenberg F, Adamski J, Illig T. Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PLoS ONE 2010; 5(11): e13953
39. Blaak EE, van Aggel-Leijssen DP, Wagenmakers AJ, Saris WH, van Baak MA. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. Diabetes 2000; 49(12): 2102–2107
40. Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. J Clin Invest 2005; 115(7): 1699–1702
41. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 1999; 277(6 Pt 1): E1130–E1141
42. Adams SH, Hoppel CL, Lok KH, Zhao L, Wong SW, Minkler PE, Hwang DH, Newman JW, Garvey WT. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. J Nutr 2009; 139(6): 1073–1081
43. Kramer HF, Goodyear LJ. Exercise, MAPK, and NF-kappaB signaling in skeletal muscle. J Appl Physiol 2007; 103(1): 388–395
44. Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. Mol Med 2008; 14(3–4): 222–231
45. Mihalik SJ, Goodpaster BH, Kelley DE, Chace DH, Vockley J, Toledo FG, DeLany JP. Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity (Silver Spring) 2010; 18(9): 1695–1700
46. Ha CY, Kim JY, Paik JK, Kim OY, Paik YH, Lee EJ, Lee JH. The association of specific metabolites of lipid metabolism with markers of oxidative stress, inflammation and arterial stiffness in men with newly diagnosed type 2 diabetes. Clin Endocrinol (Oxf) 2012; 76 (5): 674–682
47. Mihalik SJ, Michaliszyn SF, de las Heras J, Bacha F, Lee S, Chace DH, DeJesus VR, Vockley J, Arslanian SA. Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation. Diabetes Care 2012; 35(3): 605–611
48. Cole LK, Vance JE, Vance DE. Phosphatidylcholine biosynthesis and lipoprotein metabolism. Biochim Biophys Acta 2012; 1821(5): 754–761
49. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG, Fritsche A, Häring HU, Hrabe de Angelis M, Peters A, Roden M, Prehn C, Wang-Sattler R, Illig T, Schulze MB, Adamski J, Boeing H, Pischon T. Identification of Serum Metabolites Associated With Risk of Type 2 Diabetes Using a Targeted Metabolomic Approach. Diabetes 2012 Oct 4. [Epub ahead of print] doi: 10.2337/db12-0495
50. Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, Heim K, Campillos M, Holzapfel C, Thorand B, Grallert H, Xu T, Bader E, Huth C, Mittelstrass K, Döring A, Meisinger C, Gieger C, Prehn C, Roemisch-Margl W, Carstensen M, Xie L, Yamanaka-Okumura H, Xing G, Ceglarek U, Thiery J, Giani G, Lickert H, Lin X, Li Y, Boeing H, Joost HG, de Angelis MH, Rathmann W, Suhre K, Prokisch H, Peters A, Meitinger T, Roden M, Wichmann HE, Pischon T, Adamski J, Illig T. Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol 2012; 8: 615
51. Felig P, Marliss E, Cahill GF Jr. Plasma amino acid levels and insulin secretion in obesity. N Engl J Med 1969; 281(15): 811–816
52. Felig P, Wahren J, Hendler R, Brundin T. Splanchnic glucose and amino acid metabolism in obesity. J Clin Invest 1974; 53(2): 582–590
53. Luetscher JA Jr. The Metabolism of Amino Acids in Diabetes Mellitus. J Clin Invest 1942; 21(3): 275–279
54. Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, Tanner CJ, Kuchibhatla M, Houmard JA, Newgard CB, Kraus WE. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 2009; 32(9): 1678–1683
55. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, Ilkayeva OR, Wenner BR, Bain JR, Lee JJ, Lim SC, Khoo CM, Shah SH, Newgard CB. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia 2010; 53(4): 757–767
56. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes. Nat Med 2011; 17(4): 448–453
57. Zhang X, Wang Y, Hao F, Zhou X, Han X, Tang H, Ji L. Human serum metabonomic analysis reveals progression axes for glucose intolerance and insulin resistance statuses. J Proteome Res 2009; 8 (11): 5188–5195
58. Bao Y, Zhao T, Wang X, Qiu Y, Su M, Jia W, Jia W. Metabonomic variations in the drug-treated type 2 diabetes mellitus patients and healthy volunteers. J Proteome Res 2009; 8(4): 1623–1630
59. Mochida T, Tanaka T, Shiraki Y, Tajiri H, Matsumoto S, Shimbo K, Ando T, Nakamura K, Okamoto M, Endo F. Time-dependent changes in the plasma amino acid concentration in diabetes mellitus. Mol Genet Metab 2011; 103(4): 406–409
60. Phillips JD, Kushner JP. Fast track to the porphyrias. Nat Med 2005; 11(10): 1049–1050
61. Gall WE, Beebe K, Lawton KA, Adam KP, Mitchell MW, Nakhle PJ, Ryals JA, Milburn MV, Nannipieri M, Camastra S, Natali A, Ferrannini E; RISC Study Group. alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. PLoS ONE 2010; 5(5): e10883
62. Li X, Xu Z, Lu X, Yang X, Yin P, Kong H, Yu Y, Xu G.

Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: Biomarker discovery for diabetes mellitus. Anal Chim Acta 2009; 633(2): 257–262

63. Mosharov E, Cranford MR, Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry 2000; 39(42): 13005–13011
64. Persa C, Pierce A, Ma Z, Kabil O, Lou MF. The presence of a transsulfuration pathway in the lens: a new oxidative stress defense system. Exp Eye Res 2004; 79(6): 875–886
65. Kostolanská J, Jakus V, Barák L. HbA1c and serum levels of advanced glycation and oxidation protein products in poorly and well controlled children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2009; 22(5): 433–442
66. Bennion LJ, Grundy SM. Effects of diabetes mellitus on cholesterol metabolism in man. N Engl J Med 1977; 296(24): 1365–1371
67. Schwartz SL, Lai YL, Xu J, Abby SL, Misir S, Jones MR, Nagendran S. The effect of colesevelam hydrochloride on insulin sensitivity and secretion in patients with type 2 diabetes: a pilot study. Metab Syndr Relat Disord 2010; 8(2): 179–188
68. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011; 472(7341): 57–63
69. McGill HC Jr, McMahan CA. Determinants of atherosclerosis in the young. Am J Cardiol 1998; 82(10B): 30T–36T
70. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97(18): 1837–1847
71. al-Waiz M, Mikov M, Mitchell SC, Smith RL. The exogenous origin of trimethylamine in the mouse. Metabolism 1992; 41(2): 135–136
72. Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci USA 2011; 108(Suppl 1): 4523–4530
73. Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. Nat Rev Microbiol 2005; 3(5): 431–438
74. Fujimura KE, Slusher NA, Cabana MD, Lynch SV. Role of the gut microbiota in defining human health. Expert Rev Anti Infect Ther 2010; 8(4): 435–454