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Review

Metabolomic biomarkers of impaired glucose tolerance and type 2 diabetes mellitus with a potential for risk stratification in women with polycystic ovary syndrome

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ABSTRACT

There is a need to identify biomarkers of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) risk in women with PCOS to facilitate screening and the development of novel strategies to prevent disease progression. Metabolomic technologies may address this need. All published studies on metabolomic biomarkers of IGT and/or T2DM identified through MEDLINE (1966-December 2010), EMBASE (1980-December 2010) and Cochrane (1993-December 2010) were retrieved. Eligible studies were screened and specific study characteristics recorded including study design, number of participants, selection criteria, type of metabolomic technique used, site of sample collection, and a list of metabolites identified to have been altered in IGT and/or T2DM versus healthy controls was created.

Nine metabolomic biomarkers that could potentially be used to identify women with PCOS at risk of developing IGT and/or T2DM were identified including leucine, isoleucine, citrate, glucose, creatinine, valine, glutamine, alanine and HDL. Of these biomarkers, a panel of four biomarkers were consistently either elevated or reduced including glucose (elevated), valine (reduced), HDL (reduced) and alanine (reduced) in IGT/T2DM compared with controls. These biomarkers may predict the development of IGT/T2DM in young women with PCOS. More studies are required to test this hypothesis and translate the findings into patient benefit by reducing the morbidity/mortality associated with IGT/T2DM in PCOS.

Contents

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1. Introduction

Metabolomics is an emerging technology that achieves advanced separation and detection of the sum of the metabolites involved in a metabolic pathway [1]. An important concept of metabolomics is that an individual's metabolite state is a close representation of his/her overall health status [2]. Metabolomic techniques, especially nuclear magnetic resonance (NMR) and mass spectrometry (MS) have been used for identification of individuals at risk of developing impaired glucose tolerance (IGT) and ultimately type 2 diabetes mellitus (T2DM) [3]. Previous studies that applied metabolomic technology reported an increase in glucose and decline in various amino acids (including citrate and lactate) which could be attributed to impaired glycolysis and tricarboxylic acid cycle (TCA cycle) [4–6]. Furthermore, previous research has reported alterations in lipid metabolism including hypertriglyceridaemia, elevation of LDL and alteration of HDL cholesterol [4–6].

Polycystic ovary syndrome (PCOS) is a common metabolic condition that affects 5–10% of women between the ages of 12 and 45 years [7–9]. The current definition requires two or more of the following requirements: chronic ovulatory disorder (oligo-ovulation to anovulation and amenorrhea), presence of hyperandrogenism manifested either in laboratory tests or in clinical symptoms and ultrasound evidence of polycystic ovaries where other causes of anovulation and hyperandrogenism have been ruled out [8,10,11]. The pathogenesis of PCOS has been linked to the development of insulin resistance and hyperinsulinaemia which in turn progress to long term risks of T2DM with its associated micro- and macro-vascular complications [12–14]. There is however currently no way of determining which young women with PCOS will go on to develop impaired glucose tolerance and T2DM so that appropriate preventive measures can be taken.

Non-targeted metabolomic technology, however, has the potential of identifying novel biomarkers of disease. These biomarkers could predict the development of T2DM in women with PCOS which could allow earlier and more effective intervention. Furthermore, the idea of collecting a blood sample from a newly diagnosed woman with PCOS and being able to predict her likelihood of developing T2DM in one or two decades could have an enormous impact on the management and prognosis of this condition. The aim of this study was to identify metabolomic biomarkers of IGT and T2DM with a potential for risk stratification in women with PCOS.

2. Methods

2.1. Collecting the primary data

In order to identify the relevant primary studies for this systematic review, MEDLINE (1966-October 2010), EMBASE (1980-December 2010) and Cochrane (1993-December 2010) databases were searched using the terms "metabolomics", "metabonomics", "type 2 diabetes mellitus" and "polycystic ovary syndrome". Therefore, inclusion criteria included metabolomic techniques (mainly NMR and MS) in humans with IGT and T2DM. Consequently, studies where metabolomic technology was applied to animals (mainly rodents) were excluded.

The original PDFs of studies obtained from the search were located through direct online links to the files from the search results, for example through "Science Direct" or through indirect links provided by the Electronic Library Resource Gateway of the University of Nottingham. A manual search of references from all the studies was also conducted to identify any other potentially relevant studies. The search criterion ended in December 2010. The search findings were independently double-checked by one of the co-authors (WA).

2.2. The main characteristics of the studies

Selected studies were thoroughly screened and specific study characteristics were recorded. These included: type of study design, number of participants (*N*), selection criteria, type of metabolomic technique used (whether NMR or MS), site of sample collected in each study (whether urine, blood or saliva), and finally a list of metabolites identified to have been altered in IGT and/or T2DM versus normal in individuals was created.

2.3. Methodological quality assessment

The methodological quality of each primary study was determined using the QUADOMICS Tool, an adaptation of QUADAS (a quality assessment tool for use in systematic reviews of the diagnostic accuracy studies) (Fig. 1) which takes into account the particular challenges encountered in "-omics" based techniques [15]. The methodologies of the studies which achieved more than 10/16 on the QUADOMICS Tool were classified as high quality (HQ) whereas those which scored 10/16 or less were classified as low quality (LQ). Screening of the general characteristics of the selected studies and assessment of the methodological quality of the studies were independently double-checked by the one of the co-authors (CI).

3. Results

3.1. Selecting the relevant studies

Fig. 2 demonstrates the selection process of the relevant papers. The initial literature search was done through MEDLINE using the keywords: "metabolomics", "metabonomics", "type 2 diabetes mellitus" and "polycystic ovary syndrome". This initial search yielded 82 articles which included 22 reviews. After screening the titles and abstracts, 12 primary studies were isolated. As well as the 22 reviews, primary studies were excluded if they did not apply metabolomic techniques (NMR or MS) or did not concentrate on IGT or T2DM versus healthy individuals. Six studies involving only animals were excluded, isolating six primary studies [4–6,16–18] but one of these was excluded since the authors presented the metabolic pathways affected in subjects with low insulin sensitivity (e.g. arachidonic acid metabolism and steroid hormone biosynthesis) and not individual biomarkers [18]. Searching through the Cochrane, EMBASE and CINAHL databases and hand searching of the references of relevant manuscripts did not yield additional papers.

No study was found where metabolomic techniques had been used to identify potential biomarkers of IGT and T2DM in women affected with PCOS. However, all five selected studies had investigated metabolomic biomarkers in subjects with IGT and/or T2DM compared with normal subjects. In order to identify metabolomic biomarkers of IGT and T2DM with a potential for risk stratification in women with PCOS, we decided to shortlist a panel of metabolites that had been most frequently affected (in three or more of the five studies) in IGT and/or T2DM individuals against normal subjects (Table 3) and investigate the role of the metabolites that were consistently either over- or under-expressed in this shortlist as potential biomarkers for PCOS.

3.2. General characteristics of the studies included

The five case–control studies identified from the literature involved 456 participants [4–6,16,17]. Three studies compared metabolites in T2DM patients against individuals with normal glucose tolerance (NGT) [6,16,17], one compared patients with both T2DM and IGT against normal people [4], whereas one

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1. Description of selection criteria
2. The spectrum of patients used in each study is representative of the patients who will receive the test in practice
3. Full description of the sample size
4. Adequate description of the procedure and timing of the collection of biological sample with respect to clinical factors
5. Adequate description of handling and pre-analytical procedures- were these the same for the whole sample?
6. The period between the reference standard and the index test is short enough to reasonably guarantee that the target condition did not change between the two tests
7. The reference standard is likely to correctly classify the target condition
8. The whole sample or a random selection of the sample received verification using a reference standard of diagnosis
9. The patients received the same reference standard regardless of the result of the index test
10. The execution of the index test is sufficiently described to its permit replication
11. The execution of the reference standard is sufficiently described to its permit replication
12. The index test results are interpreted without knowledge of the results of the reference standard
13. The reference standard results are interpreted without knowledge of the results of the index test
14. The same clinical data is available when test results are interpreted as it would be when the test is used in practice
15. Any uninterpretable/ intermediate test results are reported
16. The presence of overfitting was most likely avoided

Fig. 1. According to QUADOMICS Tool the following methodological criteria were applied to this review.

compared patients with IGT against individuals with NGT [5]. Selection criteria were adequately described apart from Zhao et al. [5] and van Doorn et al. [16], where inclusion criteria were not described other than the laboratory findings compatible with IGT and T2DM respectively as defined by the World Health Organisation (WHO) [19]. Two studies based their metabolomic investigations on plasma [4,17], two studies used both plasma and spot urine samples [5,16], while Salek et al. [6] used urine samples. Finally, three studies performed their metabolomic studies using NMR [4,6,16], one case–control trial used MS [5], and one group used both metabolomic techniques [17]. The above, along with the metabolites identified in each study, are summarised in Table 1.

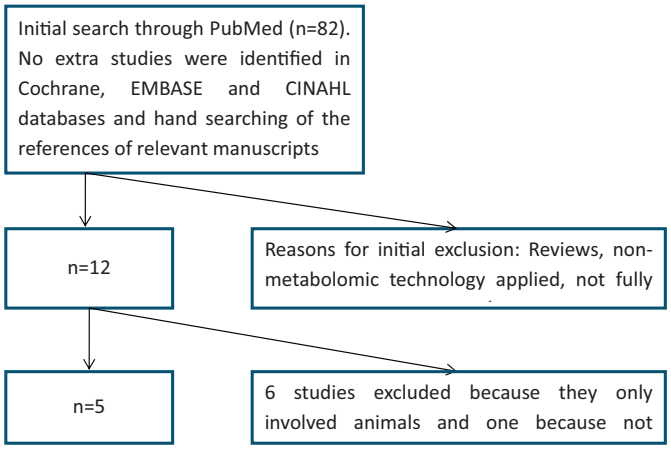

Fig. 2. A flow chart summarising the selection process.

3.3. Isolating the metabolites most frequently affected in the studies screened in this review

From the many metabolites listed in Table 1, the nine which appeared to be affected in most of the studies (i.e. in more than three out of the five studies) were listed in Table 2 to be examined in greater detail. These included: leucine, isoleucine, citrate, glucose, creatinine, valine, glutamine, alanine and HDL. Leucine and isoleucine appeared to be affected in four out of the five studies [4,6,16,17]. Both were implicated in patients diagnosed with T2DM. In three out of these four cases, leucine and isoleucine levels decreased, whereas one group reported elevated levels of these amino acids compared to individuals with NGT.

Citrate, glucose, creatinine, valine, glutamine, HDL and alanine were reportedly affected in three out of the five studies where patients with T2DM and/or IGT were compared to the normal population. Glucose was found to be elevated in all three studies

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Table 1
The main characteristics of each study with the metabolites affected in patients with T2DM and/or IGT compared to normal individuals'.

Study	Study design	Population		Selection criteria		Metabolites Identified	Change Vs normal (↑↓)		Site	Technique used
		N	Mean age ± SD and age range	Inclusion	Exclusion		T2DM	IGT		
Zhang et al. [4]	Case control	231	T2DM: 51 ±9 IGT: 51+10	Han ethnicity, matched for age, gender and BMI	Hypertension, liver and renal dysfunction	HDL	↓		P	NMR
						Isoleucine	↓		P	
						Leucine	↓		P	
						Valine	↓		P	
						Alanine	↓	↓	P	
						Methionine	↓		P	
						Glutamine	↓	↓	P	
						Citrate	↓		P	
						Lysine	↓		P	
						Choline	↓		P	
						Lactate	↓	↓	P	
						Tyrosine	↓	↓	P	
						Phenylalanine	↓	↓	P	
						Histidine	↓	↓	P	
						Glucose	↑		P	
Zhao et al. [5]	Case control	51	IGT: 46.9 ± 11.9	Not described	Participants with disease of the cardiovascular system, the kidney or the liver as well as severe inflammation	2 h Plasma glucose		↑	P	MS
						Glucose (fasting)		↑	P	
						Insulin (fasting)		↑	P	
						HbA1c		↑	P	
						Triglycerides		↑	P	
						Cholesterol		↓	P	
						LDL		↓	P	
						HDL		↓	P	
						C-reactive protein		↑	P	
						Leukocytes		↓	P	
						Total protein		↓	P	
						Creatinine		–	P	
						Uric acid		↑	P	
						Homocysteine		↓	P	
						Total protein		↓	U	
						Albumin		↑	U	
						Creatinine		↑	U	
						pH-value		↑	U	
						Glycochenodeoxycholic acid		↑	P	
						FFAC16: 1[M−1]$^-$		↑	P	
						FFAC20:4[M−1]$^-$		↑	P	
						FFAC22:5[M−1]$^-$		↑	P	
						FFAC18:2[M−1]$^-$		↑	P	
						FFAC20:3[M−1]$^-$		↑	P	
						FFAC22:4[M−1]$^-$		↑	P	
						FFAC16:0[M−1]$^-$		↑	P	
						Isotope of FFA C16:0 [M−1]$^-$		↑	P	
						Isotope of FFA C18:1 [M−1]$^-$		↑	P	
						FFAC18:1[M−1]$^-$		↑	P	
						FFAC20:2[M−1]$^-$		↑	P	
						FFAC18:0[M−1]$^-$		↑	P	
						LPC C18:2 [M−CH_3]$^-$		↑	P	
						LPC C18:2 [M+HCOO]-		↑	P	
						LPCC18:2[M+1]$^+$		↑	P	
						Fragment of LPC C18:2		↑	P	
						LPC C18:2 [M−CH_3]$^-$		↑	P	
						LPC C16:0 [M−CH_3]$^-$		↓	P	

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						LPC C16:0 [M+HCOO]-	↓	P	
						Fragment of LPC C16:0	↓	P	
						LPC C16:0 [M−CH_3]$^-$	↓	P	
						LPC C16:0 [M+HCOO]$^-$	↓	P	
						LPC C16:1 [M−CH_3]$^-$	↓	P	
						LPC C18:1 [M+HCOO]$^-$	↓	P	
						LPC C16:0 [M+H]$^+$	↓	P	
						Fragment of LPC C16:0	↓	P	
						LPC C18:1 [M−CH_3]$^-$	↓	P	
						LPC C18:0 [M+HCOO]$^-$	↓	P	
						Fragment of LPC C18:0	↓	P	
						LPC C18:0 [M+HCOO]$^-$	↑	U	
						Xanthine [M+1]$^+$	↑	U	
						Fragment of tryptophan	↑	U	
						C8:2-OH carnitine [M+1]$^+$	↑	U	
						C10:2-OH carnitine [M+1]$^+$	↓	U	
						Uric acid [M+1]$^+$	↓	U	
						Uric acid [M−1]$^-$	↓	U	
						7-Methylxanthine [M+1]$^+$	↓	U	
						Methyl uric acid [M+1]$^+$	↓	U	
						Methyl uric acid [M−1]$^-$	↓	U	
						3-N ethyl xanthine [M+1]$^+$	↓	U	
						1-Methyl xanthine [M+1]$^+$	↓	U	
						3-Hydroxyhippuric acid [M−1]$^-$	↓	U	
						Fragment of hippuricacid	↓	U	
						Hippuric acid [M−1]$^-$	↓	U	
						Phenylacetyl-glutamine [M+Na]$^+$	↓	U	
Salek et al. [6]	Case control	42	T2DM: 56 ± 9 range: 30–65	Control group: normal physical examination, laboratory studies and ECG. T2DM group: participants suffering from T2DM for at least 3 months, glucose levels controlled by taking only one type of medication	Participants with diabetic complications associated with extreme obesity, extreme weight loss, high blood pressure, cardiovascular disease, renal dysfunction, T1DM, uncorrecte d thyroid dysfunction	Trimethylamine dimethylamine	↑	U	NMR
						N.N-dimethylglycine betainetrim ethylamine-N-oxide	↑	U	
						Creatine	↓	U	
						Creatinine	↓	U	
						Citrate	↑	U	
						Malate	↑	U	
						Fumarate	↑	U	
						Succinate	↑	U	
						2-Oxoglutarate	↓	U	
						Acetate	↓	U	
						Acetoacetate	↑	U	
						n-Butyrate	↑	U	
						α-Hydroxy-n-butyrate	↑	U	
						β-Hydroxybutyrate	↑	U	
						Alanine	↓	U	
						Leucine		U	
						Isoleucine		U	
						Histidine		U	
						Tryptophan		U	
						Glutamine		U	
						Ornithine		U	
						Taurine N-methylnicotinamide Amide		U	
						N-methyl nicotinate acid		U	
						N-methyl-2-pyridone-5-carboxamide	↑	U	
						Allantoin	↓	U	

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Table 1 (*Continued*)

Study	Study design	Population		Selection criteria		Metabolites Identified	Change Vs normal (↑↓)		Site	Technique used
		N	Mean age ± SD and age range	Inclusion	Exclusion		T2DM	IGT		
van Doorn et al. [16]	Case Control	32	T2DM range: 40-75	Not Described	Participants with significant medical history of clinically relevant conditions, or had used any non-steroid a l anti-inflammato ry drugs, TZD or insulin reparation within 2 weeks of the expected start of the study	Leucine	↓		P	NMR
						Isoleucine	↓		P	
						Valine	↓		P	
						3-D-hydroxybutyrate	↓		P	
						Methylene groups from mobile fatty acids in lipoproteins	↑		P	
						Lactate	↑		P	
						Alanine	↓		P	
						Lipid ($C[H_2]–CH_2–CO$)	↑		P	
						Glutamine	↓		P	
						Lipid ($CH_2–CO$)	↑		P	
						Citrate	↓		P	
						Lipid (C=C–CH2–C=C)	↑		P	
						Tyrosine	↓		P	
						Formate	↓			
						Alanine	↑		U	
						Glutamate	↓		U	
						Citrate	↑		U	
						Phenylalanine	↑		U	
						Tyrosine			U	
						Hippurate				
						N-methyl nicotinamide	↑		U	
						phospho(enol) pyruvate				
						Uridine	↓		U	
Suhre et al. [17]	Case control	100	T2DM: 67.7 ± 7.2 age range: 55–79	Participants were taken from the KORA F3 cohort study and were residents of the city and region of Augsburg, Southern Germany	Based on the KORA F3 cohort study	Cholesterol	↓		P	NMR and MS
						HDL	↓		P	
						LDL	↓		P	
						Triglycerides	↓		P	
						1,5 anhydroglucitol	↓		P	
						Pro-hydroxy-pro	↓		P	
						Heptanoate (7:0)	↓		P	
						Cysteine	↓		P	
						Leucine caproate (6:0)	↑		P	
						Arachidonate (20:4n6)	↓		P	
						Valineuridine β hydroxy isovalerate	↓		P	
						Glucose	↑		P	
						3-Indoxyl sulfate	↑		P	
						Phenyl acetyl glutamine	↑		P	
						glutamylvaline creatinine				
						Gamma-glutamyll isoleucine	↑		P	
						erythronate erythritol				
						N-acetylalaninemethylbutyroylcarnitine	↑		P	
						3-Methyl-2-oxovalerate	↑		P	
						Pelargonate	↓		P	
						10-Undecenoate	↓		P	
						Myristate(14:0)	↑		P	
						Palmitate(16:0)	↑		P	
						2-Hydroxopalmitate				
						Margarate (17:0)	↑		P	
						10-Heptadecenoate (17:1n7)				
						Isoleucine	↑		P	
						Stearate (18:0)	↑		P	
						2-Hydroxystearate	↑		P	

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Oleate(18:1n9)	↑	P	
Linoleate(18:2n6)	↑	P	
Linoleamide (18:2n6)	↑	P	
Linolenate (18:3n3 or 6)	↑	P	
Eicosenoate (20:1n9or11)	↑	P	
Dihomo-alpha-linolenate (20:3n3)	↑	P	
Adrenate (22:4n6)	↑	P	

Index: *N* = number of participants, SD = standard deviation, T2DM = type 2 diabetes mellitus, IGT = impaired glucose tolerance, P = plasma, U = urine, NMR = nuclear magnetic resonance, MS = mass spectrometry. For individual abbreviations of metabolites refer to the primary studies.

[4,5,17] whereas valine [4,16,17], HDL [4,5,17] and alanine [4,6,16] were found to be decreased in all the three cases where patients with T2DM and/or IGT were compared against people with NGT. Furthermore, two studies involving urine samples reported elevated levels of citrate [6,16], while one group which used plasma for their metabolomic investigations reported a decrease in citrate in patients with T2DM versus normal individuals [4]. Two studies, one using urine samples from IGT patients [5] and one using plasma from T2DM patients [17], reported elevated levels of creatinine, whereas one group investigating urine from T2DM patients found decreased levels of the amino acid compared to the normal study population [6]. Finally, two studies, one using plasma from both T2DM and IGT patients [4] and one using urine from patients with T2DM [16], reported decreased levels of glutamine compared to normal people. On the other hand, a study investigating urine from T2DM patients demonstrated an elevation of the above amino acid when it was compared to NGT individuals [6].

Of the nine metabolites identified above (Table 2), a panel of four metabolomic biomarkers were consistently either elevated or reduced including glucose (elevated), valine (reduced), HDL (reduced) and alanine (reduced) in IGT/T2DM compared with controls.

3.4. Assessing the quality of the relevant studies

Table 3 summarises the quality assessment process in accordance to the QUADOMICS Tool [15]. Two out of the five studies were classified as "high quality" (HQ) fulfilling 11 out of the 16 criteria [5,6]. The three remaining studies were classified as "low quality" (LQ) achieving 9 or 10 out of the 16 quality criteria [4,16,17]. Of particular note was that none of the studies explicitly stated that the index test results were interpreted without knowledge of the results of the reference standard and vice versa, thus failing to achieve the 12th and 13th criteria of the QUADOMICS [18]. Furthermore, the 2nd quality criterion of the QUADOMICS ("Was the spectrum of patients representative of patients who will receive the test in practice?") [18] was not applicable (N/A) for this review since the studies were conducted on T2DM and/or IGT patients of both sexes.

4. Discussion

This study identified a panel of nine metabolomics biomarkers that could potentially be used to identify women with PCOS who are at risk of developing IGT and T2DM. These include leucine, isoleucine, citrate, glucose, creatinine, valine, glutamine, alanine and HDL. Of these biomarkers, a panel of four metabolomic biomarkers were consistently either over- or under-expressed including glucose, valine, HDL and alanine in IGT/T2DM compared with controls. Shortly after the completion of this study, a paper was published in "Nature Medicine" [20] describing a nested case–control study in the Framingham Offspring Study. Among 2422 normoglycemic individuals followed for 12 years, 201 developed diabetes. Amino acids, amines and other polar metabolites were profiled in baseline specimens by liquid chromatography–tandem mass spectrometry (LC–MS) and cases and controls were matched for age, body mass index and fasting glucose. The study showed that five branched-chain and aromatic amino acids had highly significant associations with future diabetes including isoleucine, leucine, valine, tyrosine and phenylalanine. Three of these five amino acids (isoleucine, leucine and valine) had been identified by our study. Based on our study and the Nature Medicine paper [20] therefore, a total of 11 metabolomic biomarkers (leucine, isoleucine, citrate, glucose, creatinine, valine, glutamine, alanine, HDL tyrosine and phenylalanine) could potentially serve as

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Table 2
The metabolites affected most frequently in the five studies.

Metabolites	Frequency	Study	Main characteristics
Leucine	4/5	Zhang et al. [4]	T2DM, ↓, plasma, NMR
		Salek et al. [6]	T2DM, ↓, urine, NMR
		van Doorn et al. [16]	T2DM, ↓, plasma, NMR
		Suhre et al. [17]	T2DM, ↑, plasma, NMR and MS
Isoleucine	4/5	Zhang et al. [4]	T2DM, ↓, plasma, NMR
		Salek et al. [6]	T2DM, ↓, urine, NMR
		van Doorn et al. [16]	T2DM, ↓, plasma, NMR
		Suhre et al. [17]	T2DM, ↑, plasma, NMR and MS
Citrate	3/5	Zhang et al. [4]	T2DM, ↓, plasma, NMR
		Salek et al. [6]	T2DM, ↑, urine, NMR
		van Doorn et al. [16]	T2DM, ↑, urine, NMR
Glucose	3/5	Zhang et al. [4]	T2DM, ↑, plasma, NMR
		Zhao et al. [5]	IGT, ↑, plasma, MS
		Suhre et al. [17]	T2DM, ↑, plasma, NMR and MS
Creatinine	3/5	Zhao et al. [5]	IGT, ↑, urine, MS
		Salek et al. [6])	T2DM, ↓, urine, NMR
		Suhre et al. [17]	T2DM, ↑, plasma, NMR and MS
Valine	3/5	Zhang et al. [4]	T2DM, ↓, plasma, NMR
		van Doorn et al. [16]	T2DM, ↓, plasma, NMR
		Suhre et al. [17]	T2DM, ↓, plasma, NMR and MS
Glutamine	3/5	Zhang et al. [4]	T2DM and IGT, ↓, plasma, NMR
		Salek et al. [6]	T2DM, ↑, urine, NMR
		van Doorn et al. [16]	T2DM, ↓, urine, NMR
HDL	3/5	Zhang et al. [4]	T2DM, ↓, plasma, NMR
		Zhao et al. [5]	IGT, ↓, plasma, MS
		Suhre et al. [17]	T2DM, ↓, plasma, NMR and MS
Alanine	3/5	Zhang et al. [4]	T2DM and IGT, ↓, plasma, NMR
		Salek et al. [6]	T2DM, ↓, urine, NMR
		van Doorn et al. [16]	T2DM, ↓, plasma, NMR

Index: T2DM = type 2 diabetes mellitus, IGT = impaired glucose tolerance, NMR = nuclear magnetic resonance, MS = mass spectrometry.

Table 3
Methodological quality assessment using the QUADOMICS Tool [15].

Quality criteria	Zhang et al. [4]	Zhao et al. [5]	Salek et al. [6]	van Doorn et al. [16]	Suhre et al. [17]
1	Y	Y	Y	Y	Y
2	N/A	N/A	N/A	N/A	N/A
3	Y	Y	Y	Y	Y
4	Y	Y	Y	Y	Y
5	Y	Y	Y	Y	Y
6	N	Y	Y	N	N
7	Y	Y	Y	Y	Y
8	Y	Y	Y	Y	Y
9	N	Y	Y	Y	Y
10	Y	Y	Y	Y	Y
11	Y	Y	Y	Y	Y
12	N	N	N	N	N
13	N	N	N	N	N
14	?	?	?	?	?
15	N	N	N	N	N
16	Y	Y	Y	N	Y
Total	9/16, LQ	11/16, HQ	11/16, HQ	9/16, LQ	10/16, LQ

Index: 1 = description of selection criteria, 2 = the spectrum of patients used in each study is representative of the patients who will receive the test in practice, 3 = full description of the sample size, 4 = adequate description of the procedure and timing of the collection of biological sample with respect to clinical factors, 5 = adequate description of handling and pre-analytical procedures were these the same for the whole sample? 6 = the period between the reference standard and the index test is short enough to reasonably guarantee that the target condition did not change between the two tests, 7 = the reference standard is likely to correctly classify the target condition, 8 = the whole sample or a random selection of the sample received verification using a reference standard of diagnosis, 9 = the patients received the same reference standard regardless of the result of the index test, 10 = the execution of the index test is sufficiently described to its permit replication, 11 = the execution of the reference standard is sufficiently described to its permit replication, 12 = the index test results are interpreted without knowledge of the results of the reference standard, 13 = the reference standard results are interpreted without knowledge of the results of the index test, 14 = the same clinical data is available when test results are interpreted as it would be when the test is used in practice, 15 = any uninterpretable/intermediate test results are reported, 16 = the presence of overfitting was most likely avoided. Y = criterion achieved, N = criterion not achieved or not mentioned, HQ = high quality, LQ = low quality, N/A = not applicable.

biomarkers for the early detection of IGT and T2DM risk in women with PCOS. This hypothesis, however, will need to be tested in a well-designed prospective longitudinal cohort study of women with PCOS.

We currently do not know the exact levels of fasting sugar or of other metabolomic biomarkers that will be acceptable as a biomarker of IGT and T2DM as this research area is new. However, in the prospective study by Wang et al. [20], logistic regression

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models were used to assess the association between baseline metabolite levels and future diabetes, adjusting for age, sex, BMI and fasting glucose. For the five amino acids of interest, each standard deviation increment in log marker was associated with a 57–102% increased odds of future diabetes ($P = 0.0002–0.002$). Individuals in the top quartile of individual plasma amino acid concentrations had 2- to 3.5-fold higher odds of developing diabetes over the 12-year follow-up period, compared with those whose plasma amino acid levels were in the lowest quartile. Specific and precise levels of fasting sugar or of other metabolomic biomarkers acceptable as biomarkers of impending IGT and T2DM will however be defined only after several future prospective studies to validate and replicate any currently shortlisted biomarkers. The first vital step is the identification and shortlisting of any potentially useful biomarkers for these prospective studies. This is achieved in this study.

It is not possible without further research to explain the exact role these biomarkers may play in IGT and T2DM risk in women with PCOS or whether these associations are a cause or effect. High glucose levels and low HDL cholesterol levels have however been linked with PCOS in previous publications [21]. A patent has also been filed for a metabolomic biomarker profile consisting of citrate elevation, formate elevation, acetoacetate elevation, 3-hydroxy butrate elevation, alanine elevation, glutamine reduction, glutamate reduction, valine elevation, isoleucine elevation, leucine elevation, threonine elevation, lysine elevation; and arginine elevation with PCOS listed as one of several diseases this metabolomic profile could diagnose [22]. The research underpinning this specific claim in relation to PCOS has not been published and was not outlined in the patent application. The pattern of elevation or reduction of metabolite profiles was, however, not consistent with that identified in our study where we found that valine and alanine were consistently reduced and leucine, isoleucine, citrate and glutamine inconsistently elevated and reduced in different studies.

Some of the metabolomic biomarkers have been found in previous studies to be involved in patho-physiological processes which may explain a link to the insulin resistance present in PCOS. Leucine, for example, is a branched-chain α-amino acid and chronic exposure to high leucine has been shown to impair glucose-induced insulin release by lowering the ATP-to-ADP ratio [23] which may be linked to the insulin resistance in PCOS. Isoleucine is also an α-amino acid it has been hypothesized that it, along with other amino acids including alanine, valine, isoleucine and glutamine, decreases glucose oxidation and causes an amino acid-induced insulin resistance [24]. However, as leucine also stimulates insulin release from the pancreas, decreasing blood glucose [25], this contradicts the amino acid-induced insulin resistance hypothesis. More studies are therefore required to clarify the exact role these amino acid may play in the insulin resistance present in PCOS and their potential role as biomarkers of early development of IGT and T2DM in young women with PCOS.

The strengths of our study are in the detailed and rigorous way in which the literature was evaluated to arrive at the shortlist of biomarkers identified. On the other hand, the inconsistent elevation/reduction of some of the biomarkers identified and the fact that some of the studies identified did not score highly on the QUADOMICS quality assessment process is a limitation of the study. Furthermore, we cannot deduce for sure, based on these case–control studies, that the identified biomarkers have predictive value for the development of IGT/T2DM in patients with PCOS and that a prospective study with such patients should be undertaken. We are in the process of collecting these data. However, even after the results from this prospective study have become available, it would still be difficult to deduce for certain that any biomarkers identified will be predictive of the development of development of IGT/T2DM in patients with PCOS unless the findings are independently verified in different populations by several separate research groups. We do, however, see this study as an important hypothesis-generating phase to inform the subsequent phases required in novel biomarker evaluation in this research area [26], and we see great value in informing the scientific community about these research findings at this stage. This is particularly important in the area of "omic" research, where data sharing and collaboration are vital for optimal progress. For example, an independent research group somewhere else with access to stored serum samples from women with PCOS may, based on this paper, decide to independently verify the biomarkers identified in their cohort, which would save time.

In conclusion, this study identified a panel of nine metabolomics biomarkers that could potentially be used to identify women with PCOS who are at risk of IGT and T2DM, including leucine, isoleucine, citrate, glucose, creatinine, valine, glutamine, alanine and HDL. Of these biomarkers, a panel of four metabolomic biomarkers were consistently either elevated or reduced including glucose, valine, HDL and alanine in IGT/T2DM compared with controls. Some of these biomarkers were amino acids which had been previously been linked with insulin resistance, which is present in PCOS. Raised glucose and low HDL levels have also been previously linked with PCOS. More studies are required, however, to test this hypothesis, identify a consistent pattern of either elevation or reduction of these metabolomic biomarkers which would be clinically useful, and explore the exact role these biomarkers may play in the pathophysiology of PCOS. The scope for translating this research theme directly into improved patient benefit lies in the ability to determine which young women with PCOS will go on to develop IGT and T2DM so that appropriate preventative measures can be taken to reduce the morbidity and mortality associated with IGT and T2DM in women with PCOS.

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