Metabolic profiling in diabetes

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Abstract

Metabolic profiling, or metabolomics, has developed into a mature science in recent years. It has major applications in the study of metabolic disorders. This review addresses issues relevant to the choice of the metabolomics platform, study design and data analysis in diabetes research, and presents recent advances using metabolomics in the identification of markers for altered metabolic pathways, biomarker discovery, challenge studies, metabolic markers of drug efficacy and off-target effects. The role of genetic variance and intermediate metabolic phenotypes and its relevance to diabetes research is also addressed.

Introduction

Metabolic profiling, or metabolomics, aims at determining all relevant small molecules (metabolites) in a biological sample (Pauling *et al.* 1971). Recent technology advances have allowed the characterization of hundreds of metabolites from a small amount of blood or urine in a single experiment (Nicholson *et al.* 1999). The metabolic profile of an individual patient, a lab animal, or even a cell culture provides a functional readout of its current metabolic state (Griffin *et al.* 2001, Fiehn 2002, Nicholson *et al.* 2002, Bain *et al.* 2009). Metabolomics can thus be viewed as the equivalent to an 'imaging approach' for the biochemical processes that occur within an organism. The biochemical knowledge that has been accumulated in over a century of individual experiments on cells and tissue cultures provides a thorough basis for the interpretation of perturbations in the metabolic state of a patient and of the underlying metabolic connections inside the human body, as they are revealed by studies of metabolic profiling in diabetes (German *et al.* 2005, Wenk 2005). Application of metabolomics approaches to metabolic disorders, especially diabetes, is particularly promising since deregulations of metabolic processes are expected to be directly related to relevant disease end-points. Friedrich (2012) recently reviewed current findings of metabolic research regarding diabetes in animal models and humans in this journal. I therefore first give an introduction to metabolomics with an update on current diabetes studies using metabolomics (Table 1), followed by a focus on study design considerations, current challenges and future directions of metabolic profiling, and how metabolomics can be applied most successfully in diabetes research (Table 2).

Today, two fundamentally different techniques are mainly used to obtain metabolomics data on larger sets of samples: mass spectrometry (MS) and proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy (Nicholson & Lindon 2008). Both techniques have their strengths and weaknesses. MS-based methods are in general more sensitive, but they are also more complex in their implementation with a higher risk of generating technical artifacts and measurement errors. NMR-based methods are more robust and straightforward to implement, cheaper, faster, and especially the reproducibility of NMR spectra is excellent. While MS calls for complex sample extraction methods, NMR requires higher sample volumes.

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Table 1 Selected human metabolomics studies with diabetes-related phenotypes

Metabolomics method	Experimental model	Key results	Reference
Cross-sectional studies			
Targeted metabolomics	Incident type 2 diabetes (T2D)	Plasma phospholipid markers of T2D	Wang *et al*. (2005)
Targeted metabolomics	Lean/obese with insulin resistance	Branched-chain amino acids (BCAAs) differentiate obese and lean individuals and contribute to insulin resistance	Newgard *et al*. (2009)
Non-targeted metabolomics	Euglycemic clamp	α-hydroxybutyrate (AHB) and linoleoylglycerophosphocholine (L-GPC) are early markers for insulin resistance	Gall *et al*. (2010)
Multi-platform metabolomics	Incident T2D	Diabetes-associated perturbations of metabolic pathways are detectable under subclinical conditions	Suhre *et al*. (2010)
Non-targeted metabolomics	Incident T2D	Metabolomic profiles reflective of glucose homeostasis in nondiabetic and T2D obese African–American women	Fiehn *et al*. (2010)
^{1}H NMR metabolomics	HOMA-IR (patients with T2D were excluded)	Metabolic signatures of insulin resistance in young adults	Würtz *et al*. (2012)
Non-targeted metabolomics	Incident T2D and impaired fasting glucose	Confirms the role of catabolism of BCAAs in T2D and impaired fasting glucose+new markers	Menni *et al*. (2013)
Non-targeted metabolomics	Incident T2D, multi-matrix (saliva, plasma, urine)	1,5-anhydroglucitol (1,5-AG) in saliva is a noninvasive marker of short-term glycemic control	Mook-Kanamori *et al*. (2014)
Prospective studies			
Non-targeted metabolomics (targeted for replication)	Individuals who developed T2D over 12 years	BCAAs are predictive of diabetes	Wang *et al*. (2011)
Targeted metabolomics	Individuals who developed insulin resistance or T2D over 7 years	Glycine and L-GPC are predictors of impaired glucose tolerance and of T2D	Wang-Sattler *et al*. (2012)
Non-targeted metabolomics	Two cohorts (3 and 9.5 years follow-up) with diverse diabetes relevant traits	AHB and L-GPC are independent predictors of impaired glucose tolerance	Ferrannini *et al*. (2013)
Targeted metabolomics	Two cohorts with a 7-year follow-up and diabetes relevant traits	Sugar metabolites, amino acids, and choline-containing phospholipids are independently associated with risk of T2D	Floegel *et al*. (2013)
Non-targeted metabolomics	Individuals who developed T2D over 12 years	2-aminoadipic acid is a biomarker for diabetes risk	Wang *et al*. (2013)

MS is most often coupled to additional gas- or liquid-phase chromatography separation steps (GS–MS and LC–MS) that allow for the stratification of complex mixtures. As a consequence, MS-based methods provide an overall larger number of molecules that can be quantified, but they also require longer measurement times. Identification of the biochemical identity of molecules in MS is achieved either based on high mass-resolution (Aharoni *et al*. 2002) or using known fractionation pattern (tandem MS; Evans *et al*. 2009), while in NMR this step requires the deconvolution of complex NMR spectra (Weljie *et al*. 2006). Both methods can also generate data for metabolites of which the identity is initially unknown. These unknowns may eventually be identified using additional analytical methods and thereby can provide new insights into the studied phenotype or disease (Krumsiek *et al*. 2012).

Depending on the objective, targeted or non-targeted metabolomics approaches may be favored. Targeted approaches focus on a specific subset of the metabolome and provide data only on a predefined set of molecules, while non-targeted metabolomics approaches allow for the discovery of new molecules that associate with the phenotype under investigation. Targeted approaches typically attempt to quantify the measured metabolites on an absolute scale, using external or internal standards as a reference, while non-targeted approaches provide only semi-quantitative measures, such as ion counts or areas under a curve using arbitrary units. Targeted methods with absolute quantification have the advantage that measurements from different studies can be easily combined and compared. They typically have lower technical variance since more time can be spent on measuring a smaller set of metabolic features. However, discovery of new processes and pathways is more likely to be done using non-targeted methods, aiming at measuring the widest possible range of metabolites.

**Table 2** Examples of application of metabolomics in diabetes research

Application	Example
Diagnostic biomarker discovery	1,5-AG is a marker of short-term glycemic control (Yamanouchi, *et al*. 1996)
Prognostic biomarker discovery	Branched chain amino acids are predictive of insulin resistance (Wang *et al*. 2011)
Elucidation of disease pathways	Identification of the role of solute carriers in the physiological response to glucose challenge (Deo *et al*. 2010)
Identification of drug side effects	Methylglutaryl carnitine is oppositely affected by rosiglitazone treatment in diabetic and WT mice (Altmaier *et al*. 2008)
Functional biomarker for drug action	Ratios between specific phospholipid species are markers for FABP4 inhibition (Suhre *et al*. 2011a)

The Human Metabolome Database (Wishart *et al*. 2013), which is probably the largest and most comprehensive metabolomics database available to date (http://www.hmdb.ca/), contains over 40 000 entries. Note, however, that this number includes an important number of 'expected' metabolites. These are small molecules that have been predicted to exist based on the metabolic capabilities of known metabolic pathways, but that these 'expected' metabolites have not yet been detected in a biological sample. These 'expected' metabolites comprise, in particular, lipid species that are made up of several fatty acid side chains with different chain lengths and degrees of desaturation that may occur in a combinatorial fashion (Wenk 2005). The number of metabolites becomes much smaller when the scope is limited to confirmed metabolites in a single biological fluid: in an attempt to detect as many known metabolites as possible in a single sample, a multi-platform metabolomic approach using NMR and a set of different types of MS identified 445 and quantified 378 unique metabolites or metabolite species in human urine (Bouatra *et al*. 2013). Overall, the human urine metabolome database describes 2651 confirmed human urine metabolite species (http://www.urinemetabolome.ca). To date, no single existing technique alone can be expected to provide the full picture in any diabetes study with metabolomics. In expectation of future technical improvements, it is thus advisable to store valuable specimens in adequate bio-banks for future re-analysis, ideally aliquoted into small volumes so that no additional thawing cycles are required (Fliniaux *et al*. 2011, Yin *et al*. 2013).

Cross-sectional studies for the identification of altered metabolic pathways

Altered metabolite profiles can be the indicators of changes in disease-relevant metabolic processes. Metabolites that associate with relevant disease phenotypes can be developed into biomarkers that are indicative of a metabolic deregulation. However, the question of whether a perturbation in a metabolite is causal to a disease phenotype, consequential, or just driven by a confounding factor needs to be addressed eventually. Inter-correlations between different metabolites can help with answering this question. The 'simplest' design of a diabetes study with metabolomics is a case–control design, in which blood is collected at a single time point from a group of patients with diabetes and from a 'healthy' group of controls. Statistically significant differences in metabolite concentrations between the groups are then analyzed and interpreted. Cases and controls can be matched to reduce the influence of confounding factors, but modeling of covariates into statistical models appears to be the preferable alternative (Faresjo & Faresjo 2010, de Graaf *et al*. 2011). Often, cross-sectional studies draw their samples from existing larger population studies that have an epidemiological background. A pilot study (Suhre *et al*. 2010), conducted in the general German Cooperative Health Research in the Region of Augsburg (KORA) population (Wichmann *et al*. 2005) using 40 cases of type 2 diabetes (T2D) and 60 controls, showed that diabetes-related complications can already be detected under subclinical conditions by screening over 420 unique small molecules on three different metabolomics platforms (Fig. 1). This study identified diabetes-associated perturbations of metabolic pathways linked to kidney dysfunction (3-indoxyl sulfate), indicators of interaction with the gut microflora (bile acids), and lipid metabolism (glycerophospholipids, free fatty acids). The latter observation replicated early work on plasma phospholipid metabolic profiling by Wang *et al*. (2005), who reported associations of different types of phospholipids with T2D. In a cohort of 399 nondiabetic subjects with a broad spectrum of insulin sensitivity and glucose tolerance and using a nontargeted metabolomics approach, Gall *et al*. (2010) found that α-hydroxybutyrate (AHB) is an early marker for both insulin resistance and
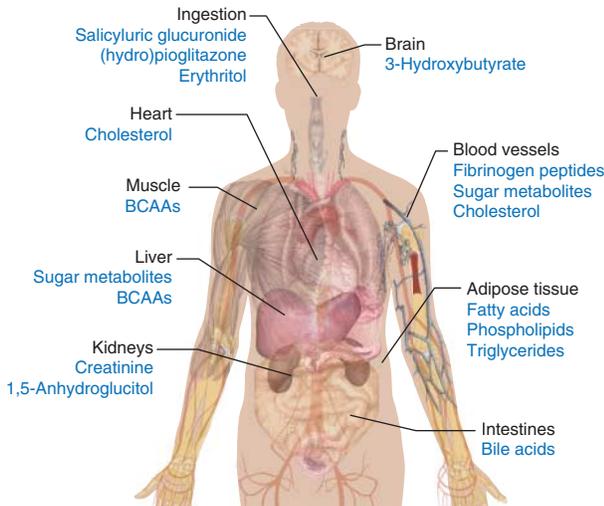
Figure 1

An example of perturbed metabolites and metabolic classes that were found associated with incident diabetes in a metabolomics study and their link to organ-specific processes and pathways (Figure reproduced from Suhre *et al.* (2010)).

impaired glucose regulation. The underlying biochemical mechanisms may involve increased lipid oxidation and oxidative stress. Menni *et al.* (2013) conducted a much larger cross-sectional metabolomics study on diabetes, with a broad nontargeted panel of 447 metabolites in blood plasma from participants of the TwinsUK study (Moayyeri *et al.* 2013). The authors found molecular markers that arise before and after hyperglycemia in a large population based study of 2204 female participants, of whom 115 had T2D and 192 had impaired fasting glucose. In their study, 42 metabolites from three major energy sources, carbohydrates, lipids, and proteins, associate significantly with T2D after adjusting for multiple testing. Twenty-two of these metabolites had already previously been reported in association with T2D or insulin resistance, 20 were newly discovered.

Mook-Kanamori *et al.* (2014) conducted a case–control study to screen for diabetes-relevant metabolic readouts in saliva, using blood and urine as a reference. Using a nontargeted MS platform, they tested 2178 metabolites in saliva, blood plasma, and urine samples from 369 individuals for association with T2D. They observed a strong association of T2D with 1,5-anhydroglucitol (1,5-AG) in saliva that was robust across three different non-Caucasian ethnicities, irrespective of BMI, age, and gender. 1,5-AG in blood has been established in the past as a reliable marker of short-term glycemic control (Kim *et al.* 2012), indicating that 1,5-AG in saliva can be used as a noninvasive marker for screening undiagnosed diabetes.

Prospective studies and biomarker discovery

While cross-sectional case–control studies can provide valuable insights into the mechanisms and processes related to the actual disease state and its co-morbidities, and are particularly interesting for biomarker discovery for incident diabetes, observations taken before disease onset are required in order to identify metabolic biomarkers that are predictive of disease development and its related co-morbidities. Such longitudinal studies require samples to be collected for metabolic analysis even before concrete plans for these measurements had been conceived, which makes such samples particularly valuable. A hallmark study of this design is that of Wang *et al.* (2011). Using a panel of amino acids, amines, and other polar metabolites in a longitudinal setting with 2422 individuals followed for 12 years (201 of whom developed diabetes), the authors found that all three branched chain amino acids (BCAAs) – isoleucine, leucine, valine – and two aromatic amino acids – tyrosine and phenylalanine – are associated with future diabetes. Furthermore, a subset of three of them, with the 'top combination' of isoleucine, phenylalanine, and tyrosine, could be used to predict future diabetes. It is of note here that BCAAs have previously been associated with incident T2D in cross-sectional studies, indicating the importance of perturbed amino acid metabolism in patients with T2D (Newgard *et al.* 2009, Fiehn *et al.* 2010, Gall *et al.* 2010, Suhre *et al.* 2010). Newgard *et al.* (2009) actually tested the effect of a high-fat diet supplemented with BCAA on insulin resistance in rats. Their findings indicated that in the context of a dietary pattern that includes high fat consumption, BCAAs contribute to the development of obesity-associated insulin resistance.

Würtz *et al.* (2012) studied the metabolic signatures of insulin resistance in 7098 young adults. They again confirmed the association of branched-chain and aromatic amino acids, gluconeogenesis intermediates, ketone bodies, and fatty acids with insulin resistance. Moreover, due to the large number of samples, they were able to identify statistically significant sex- and obesity-dependent interactions, showing that leucine, isoleucine, valine, and tyrosine were significant in women only if they were abdominally obese.

Using a lipidomics-oriented panel with 140 metabolites in 4297 fasting serum samples from a longitudinal population-based study, covering a time span of 7 years, Wang-Sattler *et al.* (2012) identified candidate biomarkers for pre-diabetes. Their study indicated that the levels of the three metabolites glycine,

linoleoylglycerophosphocholine (lyso-phosphatidyl-choline 18:2, L-GPC), and acetylcarnitine were altered in individuals with impaired glucose tolerance (IGT). Lower levels of glycine and L-GPC were predictors of IGT and T2D. To test the predictivity of AHB and L-GPC for incident dysglycemia, Ferrannini *et al.* (2013) enrolled 1261 nondiabetic participants from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study and 2580 from the Botnia Prospective Study, with 3- and 9.5-year follow-up data respectively. They found that AHB and L-GPC are independent predictors of degrading glucose tolerance and are physiologically consistent with a joint signature of insulin resistance and β-cell dysfunction. Floegel *et al.* (2013) used a targeted metabolomics approach to identify serum metabolites that are associated with the risk of T2D in a study comprising 800 incident cases of T2D and 2282 controls, randomly drawn from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam. The mean time of follow-up for this study was 7 years. In addition to associations found between several phospholipids, hexoses, and phenylalanine, lower levels of glycine and L-GPC were again found to be associated with an increased risk of developing T2D. In a nested case–control design with 188 cases and 188 matched controls of the Framingham Heart Study, Wang *et al.* (2013) identified 2-aminoadipic acid (2-AAA) as being most strongly associated with the risk of developing diabetes within a 12 years follow-up time. Study participants in the top quartile of 2-AAA concentrations had a four times higher risk of developing T2D.

After identification of an association of a metabolic trait with a disease-related trait, the next challenge is how to develop this knowledge into a clinically applicable biomarker (Batch *et al.* 2014). Milburn & Lawton (2013) present an example of how a set of metabolites that is associated with a T2D-related phenotype can be translated into a fasting blood biomarker for the diagnosis and monitoring of insulin resistance. Cobb *et al.* (2013) conducted a clinical study of the resulting clinical test, called Quantose.

From these cross-sectional and prospective studies, it emerges that diabetes-related perturbations of metabolic pathways involve many different classes of metabolites, including carbohydrates, amino acids, in particular BCAAs and glycine, and certain lipids, such as phospholipids and L-GPC. Many associations were coherently associated with T2D-related phenotypes in several studies. In many cases the specific nature of the metabolic phenotypes provides deeper functional insights into the metabolic

perturbations that may be at the basis of these associations (Newgard 2012). However, most of these studies focused on broad outcomes, such as incident diabetes or insulin resistance. It can be expected that future metabolomics studies on diabetes with more detailed characterization of the patients' diabetes state and a specific focus on diabetes-related co-morbidities, such as skin disorders, micro-vascular, or macro-vascular incidents shall reveal even deeper insights into the pathophysiology of this complex disease.

Challenge studies to investigate physiological perturbations

Metabolomics measurement taken during physiological challenges may allow the identification of metabolic signatures that are not detectable in the fasting state (Shaham *et al.* 2008, Pellis *et al.* 2012, Ho *et al.* 2013). Krug *et al.* (2012) revealed the dynamic range of the human metabolome by submitting 15 young healthy male volunteers to a highly controlled challenge protocol over a 4-day period. The challenges included a 36 h fasting period, oral glucose and lipid tests, controlled liquid test meals, physical exercise, and cold stress challenges. The study resulted in a rich data set of 275 metabolic traits from blood, urine, exhaled air, and breath exhale condensate, measured at up to 56 time points and provides a unique reference for future metabolomics studies. The data collected for this study is freely available at http://metabolomics.helmholtz-muenchen.de/humet/. The authors show that physiological challenges increase the variation that is observed between individuals, revealing metabolic phenotypes that are not observable under baseline (typically overnight-fasting) conditions. Protocols developed within this study were applied by Wahl *et al.* (2014) to elucidate the role of genetic variance in the *FTO* gene, one of the major diabetes and obesity risk genes (Frayling *et al.* 2007). The metabolic response to five defined challenge tests, consisting of an oral glucose tolerance test, a standardized high-fat high-carbohydrate meal, and a lipid tolerance test, as well as an intravenous glucose tolerance test and a euglycemic hyperinsulinemic clamp, was investigated in the plasma samples of 25 homozygous carriers of the *FTO* risk allele (rs9939609 AA genotype) and 31 carriers of the protective TT genotype. Although this study found only minor effects of *FTO* genotype on the metabolite fluxes after standardized metabolic challenges, it highlights how gene and environment interactions can be investigated in future diabetes studies with metabolomics, using well-designed

physiological challenge protocols. Similarly, metabolomics-based challenge studies can be conducted using animal models of diabetes. For instance, transgenic pigs that express a dominant-negative glucose-dependent insulinotropic polypeptide receptor (GIPRdn) develop impaired glucose control and display loss of β-cell mass. These animals provide a unique opportunity for studying metabolic changes that occur before the onset of overt diabetes. Renner *et al.* (2012) report results from a metabolomics study from intravenous glucose tolerance tests of 2.5- and 5-month-old GIPRdn transgenic and control animals. They found that seven amino acids (Phe, Orn, Val, xLeu, His, Arg, and Tyr) were increased in 2.5-month-old pigs, but decreased in 5-month-old GIPRdn transgenic pigs compared with controls, and that certain sphingomyelins and phospholipids were decreased in the plasma of 5-month-old GIPRdn transgenic pigs. The observed changes in metabolite concentrations were associated with gene expression levels of relevant pathways in the liver and provide new insights into diabetes-related pathways that cannot be studied easily in humans.

Identification of metabolic markers of drug efficacy and off-target effects

Currently, the most frequently applied drugs for the therapy of T2D include suppressors of hepatic gluconeogenesis (metformin) (Hundal *et al.* 2000), insulin-sensitizing PPAR agonists (pioglitazone) (Hirose *et al.* 2002), and insulin secretagogues (sulfonylureas) (Groop 1992). However, therapeutics that target the multifactorial nature of T2D to increase drug efficiency are still in an early phase of the development. In this study, metabolomics finds its application in the development and screening of new drugs (pharmacometabolomics) (Kaddurah-Daouk *et al.* 2008) by generating a largely unbiased metabolome-wide view of drug-induced perturbations that can be applied at each level of the therapeutics discovery pipeline from cellular and animal disease models to the preclinical and clinical translation (Robertson & Frevert 2013). Pharmacometabolomics can capture both a drug's pathway of action and unexpected off-target effects of a new drug. In an early metabolomics study in mice, Watkins *et al.* (2002) found that the PPARγ antagonist rosiglitazone induced hypolipidemia and elicited an unusual accumulation of polyunsaturated fatty acids within adipose tissue in mice. Using a similar study design, Altmaier *et al.* (2008) found that methylglutaryl carnitine is oppositely affected by rosiglitazone treatment in healthy and diabetic (*db/db*) mice. They also report a diabetes-related shift in lysophosphatidylcholine to phosphatidylcholine ratios.

Starting from the observation that metabolomics may indicate functionally relevant perturbations in metabolism, the technique can also be used to identify mechanistic markers for drug action, which can then be used for screening and efficacy tests. For instance, the fatty acid-binding protein 4 (FABP4) is part of a family of lipid chaperones that control intracellular fatty acid fluxes. Mice lacking FABP4 are protected against genetic or diet-induced insulin resistance, making FABP4 inhibition a target for anti-diabetes drug development. Using a targeted metabolomics platform with a focus on lipid-related species, Suhre *et al.* (2011*a*) showed that FABP4 inhibition leads to a clear metabolic signature in the form of a shift between lipid species that contain different amounts of mono- and unsaturated C16 and C18 fatty acids (C16:0, C16:1, C18:0, and C18:1). Note that these fatty acids are substrates of FABP4. The biomarker identified in that study was a ratio between two phospholipids and can be implemented in high-throughput screenings of potential FABP4-inhibitory molecules. Metabolite profiling should thus have a firm place in the development of anti-diabetes drugs, be it for the investigation of functional mechanisms, the identification of drug side effects, the development of mechanistic markers of drug efficacy, or the evaluation of changes in metabolic profiles related to treatment response.

The role of genetic variance and the intermediate metabolic phenotypes

Diabetes is a metabolic disorder with a considerable heritable contribution. Studies indicate up to 69% heritability for T2D in patients, with age at onset of 35–60 years (Almgren *et al.* 2011). When investigating metabolic perturbations in the context of diabetes, it is therefore important to consider the role of genetic variance in modification of a patient's body's metabolic capabilities. Genome-wide association studies (GWAS) with metabolic phenotypes have identified many genetic loci that modulate the metabolic phenotype as a function of genotype (reviewed in Suhre & Gieger (2012)). These so-called genetically influenced metabotypes (GIMs) are generally constituted of relatively frequent genetic variants, with minor allele frequencies of 20% and above, show large effect sizes that explain 10–20% of the observed variance in the metabolic trait, and are most often located in or near metabolically active genes, such as enzymes, transporters, and regulators thereof, and in

mostly all cases the associated metabolic phenotypes then match the function of the genes (Suhre *et al.* 2011*b*).

Despite its high heritability, GWAS with T2D have failed to identify genetic variants that explain the larger part of the genetic risk of developing diabetes for an individual. This lack of explained genetic risk is referred to as the 'missing heritability' (Maher 2008) and indicates that more complex interactions between many genetic variants need to be considered. To approach this problem, Kronenberg (2012) suggested investigating intermediate metabolic phenotypes as a means to connect genetic variants with disease end-points. For instance, the first large-scale association study for T2D (WTCCC 2007) found a marginal association of the LIPC locus with T2D (SNP rs4775041; $P=0.061$). At that time, this locus also associated only weakly ($P=0.025$) with blood triglyceride levels (Kathiresan *et al.* 2008). However, the first GWAS with metabolic traits (Gieger *et al.* 2008) identified a nearly genome-wide significant association of several phosphatidylethanolamine species (PE aa C38:6, $P=9.6\times10^{-8}$) at this locus. Subsequent GWAS confirmed the association of the LIPC locus with triglycerides (Teslovich *et al.* 2010) and with phosphatidylethanolamines (Shin *et al.*, *Nature Genetics* (In Press)), and revealed an association with metabolic syndrome (Kraja *et al.* 2011). Despite its low statistical power, the Gieger *et al.* (2008) GWAS with metabolomics could hence identify an intermediate metabolic trait that arguably lies on the pathway between genetic variants of the hepatic lipase LIPC and diabetes-related outcomes, such as triglyceride levels and metabolic syndrome. It is noteworthy herein that phosphatidylethanolamines were also found to be associated with diabetes in a subsequent study (Suhre *et al.* 2010).

A second example where genetics is linked to metabolomics in the study of diabetes is the glucokinase (hexokinase 4) regulator (GCKR) locus. This is a major pleiotropic risk locus associated with diabetes- and cardiometabolic-related traits, such as fasting glucose (Dupuis *et al.* 2010) and insulin levels (Aulchenko *et al.* 2009), triglyceride levels, and chronic kidney disease (Kottgen *et al.* 2010). It is also associated with many metabolic traits (Illig *et al.* 2010, Würtz *et al.* 2012). A recent GWAS for metabolic traits has also found a strong association of the GCKR locus with mannose-to-glucose ratios (Suhre *et al.* 2011*b*). The fasting level of mannose is lower in carriers of the risk allele, as opposed to that of glucose, which is higher. Little is known about the physiological role of mannose, other than its use in protein glycosylation. Mannose enters the cell via a specific transporter that is insensitive to glucose

(Panneerselvam & Freeze 1996), and hepatic glycogen breakdown is implicated in the maintenance of plasma mannose concentrations (Taguchi *et al.* 2005). These observations and the association with GCKR detected in the GWAS study, which is even stronger than that of glucose with GCKR, call for further investigation of the role of mannose as a differential biomarker, or even as a potential point of intervention in diabetes care. A third example of a diabetes-relevant GIM is SNP rs10830963 in the gene encoding the melatonin receptor (MTNR1B) which is associated with fasting glucose (Prokopenko *et al.* 2009). The same SNP in associated with tryptophan-to-phenylalanine ratios in the study reported by Illig *et al.* (2010). Phenylalanine is a precursor of melatonin, therefore indicating a possible functional relationship between this pathway and the regulation of glucose homeostasis (Illig *et al.* 2010).

Beyond inherited genetic variance alone, epigenetic modifications of DNA are suspected to be associated with diabetes phenotypes (Ng *et al.* 2010, Drong *et al.* 2012). A recent epigenome-wide association study (EWAS) of DNA methylation with metabolomics has shown that DNA methylation also plays an important role in human metabolism (Petersen *et al.* 2014). In this study, we briefly highlight one diabetes-relevant example from that study: the thioredoxin-interacting protein (TXNIP) plays a functional role in glucose regulation. DNA methylation levels of CpG locus cg19693031 near the *TXNIP* gene were associated with levels of chylomicrons in blood plasma, and also with known metabolic markers of diabetes, including different phospholipids, hexose, and AHB. In a previous study, *TXNIP* expression was elevated in the muscle of pre-diabetic and diabetic study participants (Parikh *et al.* 2007). However, there was no evidence for association between common genetic variations in *TXNIP* and T2D. Hence, DNA methylation may play a regulatory role of *TXNIP*, resulting in the observed metabolic phenotype. These examples show that by combining information from different types of genetic and disease association studies, it is possible to extract new insights into metabolic pathways relevant to diabetes.

Future directions: study design and data analysis

A number of lessons can be learned from the studies cited in this review. Clearly, metabolomics is one of the methods of choice that should be applied whenever the resources and sample availability permit it.

Existing samples from previous and ongoing epidemiological cohort studies provide easy access to extensive phenotype datasets in cross-sectional and prospective designs and have been the prime source for large-scale metabolomics studies on diabetes so far. Drawbacks of these studies are in particular a relatively coarse characterization of the T2D phenotype, many studies lacking for instance data on glucose tolerance tests, not to mention euglycemic clamp tests or more elaborate metabolic challenges. Moreover, in most cross-sectional studies, participants with T2D constitute a very heterogeneous group. For instance, individuals with well-adjusted diabetes are possibly more similar to members of the control group than some potentially pre-diabetic 'healthy' study participants. Medical treatment and development of co-morbidities vary for every patient. Detailed phenotyping of study participants is hence of utmost importance, where at the same time large participant numbers are required in order to obtain statistically significant association results when testing multiple metabolic traits. In a world of limited resources, this necessarily leads to a trade-off between depth of phenotyping and study size. We shall probably find studies in the future that cover the whole spectrum between large number of participants and exhaustive phenotypic and metabolic characterization, ideally also some combined with physiological challenges. All those studies have their merit and shall complement each other. In particular, we expect to see more extensive metabolic coverage of less commonly studied biological samples, including saliva, stools, tears, sweat, hair, fingernails, and, wherever accessible, tissue from biopsies. This may in particular include combined studies of the gut microbiome (Martin *et al.* 2007) and studies of patients undergoing bariatric surgery (Friedrich *et al.* 2012). New prospective studies should in particular attempt to enroll patients that are newly diagnosed with diabetes and yet untreated, in order to be able to investigate individual responses to treatment from the start. A lesson learned from past epidemiological studies is that one should collect and store as many samples of different kinds of biomaterial as possible for future use, and for as many time points as possible, even if at the time of study design neither plans for analysis nor funding for their analysis are available. Wisely built-up biobanks should be able to attract such funding easily at a later stage when samples are readily available in a freezer (Watts 2012).

This review has focused mostly on human studies, but the value of metabolomics studies in cell culture and animal models should not be underestimated. Here again, a combination of deep metabolic phenotyping with large sample numbers, including biological replicates, multiple conditions, and time points, are key to success. In contrast to studies on human samples, animal studies allow for access to a combination of tissues from several organs, while work on cell culture allows for the variation of a single parameter at a time and potentially even investigation of the metabolome of a single cell (Ibanez *et al.* 2013), possibly limited to specific subcellular organelles. Moreover, although only briefly mentioned in this review, the value of parallel access to complementary large-scale data, such as genome-wide genetic variation, DNA methylation, gene expression, noncoding RNA levels, and proteomics cannot be overestimated. Metabolomics is only one facet of the greater picture that describes a complex disease (albeit one of the most informative ones when it comes to metabolic disorders). One major challenge for the future lies in the integrative interpretation of these datasets, going beyond simple association tests on single phenotypes. New data analysis approaches naturally rely on systems biology methods, including for instance the unbiased analysis of all possible pairs of ratios between metabolite concentrations (Petersen *et al.* 2012), their connection in Gaussian graphical models (Krumsiek *et al.* 2011, 2012), and the interpretation of metabolomic profiles using unbiased pathway models (Deo *et al.* 2010).

References

Aharoni A, Ric de Vos CH, Verhoeven HA, Maliepaard CA, Kruppa G, Bino R & Goodenowe DB 2002 Nontargeted metabolome analysis by use of Fourier Transform Ion Cyclotron Mass Spectrometry. *OMICS* **6** 217–234. (doi:10.1089/15362310260256882)

Almgren P, Lehtovirta M, Isomaa B, Sarelin L, Taskinen MR, Lyssenko V, Tuomi T & Groop L 2011 Heritability and familiality of type 2 diabetes and related quantitative traits in the Botnia Study. *Diabetologia* **54** 2811–2819. (doi:10.1007/s00125-011-2267-5)

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Altmaier E, Ramsay SL, Graber A, Mewes HW, Weinberger KM & Suhre K 2008 Bioinformatics analysis of targeted metabolomics – uncovering old and new tales of diabetic mice under medication. *Endocrinology* **149** 3478–3489. (doi:10.1210/en.2007-1747)

Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, Penninx BW, Janssens AC, Wilson JF, Spector T *et al.* 2009 Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nature Genetics* **41** 47–55. (doi:10.1038/ng.269)

Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM & Newgard CB 2009 Metabolomics applied to diabetes research: moving from information to knowledge. *Diabetes* **58** 2429–2443. (doi:10.2337/db09-0580)

Batch BC, Hyland K & Svetkey LP 2014 Branch chain amino acids: biomarkers of health and disease. *Current Opinion in Clinical Nutrition and Metabolic Care* **17** 86–89. (doi:10.1097/MCO.0000000000000010)

Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P *et al.* 2013 The human urine metabolome. *PLoS ONE* **8** e73076. (doi:10.1371/journal.pone.0073076)

Cobb J, Gall W, Adam KP, Nakhle P, Button E, Hathorn J, Lawton K, Milburn M, Perichon R, Mitchell M *et al.* 2013 A novel fasting blood test for insulin resistance and prediabetes. *Journal of Diabetes Science and Technology* **7** 100–110. (doi:10.1177/193229681300700112)

Deo RC, Hunter L, Lewis GD, Pare G, Vasan RS, Chasman D, Wang TJ, Gerszten RE & Roth FP 2010 Interpreting metabolomic profiles using unbiased pathway models. *PLoS Computational Biology* **6** e1000692. (doi:10.1371/journal.pcbi.1000692)

Drong AW, Lindgren CM & McCarthy MI 2012 The genetic and epigenetic basis of type 2 diabetes and obesity. *Clinical Pharmacology and Therapeutics* **92** 707–715. (doi:10.1038/clpt.2012.149)

Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL *et al.* 2010 New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature Genetics* **42** 105–116. (doi:10.1038/ng.520)

Evans AM, DeHaven CD, Barrett T, Mitchell M & Milgram E 2009 Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. *Analytical Chemistry* **81** 6656–6667. (doi:10.1021/ac901536h)

Faresjo T & Faresjo A 2010 To match or not to match in epidemiological studies – same outcome but less power. *International Journal of Environmental Research and Public Health* **7** 325–332. (doi:10.3390/ijerph7010325)

Ferrannini E, Natali A, Camastra S, Nannipieri M, Mari A, Adam KP, Milburn MV, Kastenmuller G, Adamski J, Tuomi T *et al.* 2013 Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. *Diabetes* **62** 1730–1737. (doi:10.2337/db12-0707)

Fiehn O 2002 Metabolomics – the link between genotypes and phenotypes. *Plant Molecular Biology* **48** 155–171. (doi:10.1023/A:1013713905833)

Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL & Adams SH 2010 Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. *PLoS ONE* **5** e15234. (doi:10.1371/journal.pone.0015234)

Fliniaux O, Gaillard G, Lion A, Cailleu D, Mesnard F & Betsou F 2011 Influence of common preanalytical variations on the metabolic profile of serum samples in biobanks. *Journal of Biomolecular NMR* **51** 457–465. (doi:10.1007/s10858-011-9574-5)

Floegel A, Stefan N, Yu Z, Muhlenbruch K, Drogan D, Joost HG, Fritsche A, Haring HU, Hrabe de Angelis M, Peters A *et al.* 2013 Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes* **62** 639–648. (doi:10.2337/db12-0495)

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW *et al.* 2007 A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316** 889–894. (doi:10.1126/science.1141634)

Friedrich N 2012 Metabolomics in diabetes research. *Journal of Endocrinology* **215** 29–42. (doi:10.1530/JOE-12-0120)

Friedrich N, Budde K, Wolf T, Jungnickel A, Grotevendt A, Dressler M, Volzke H, Bluher M, Nauck M, Lohmann T *et al.* 2012 Short-term changes of the urine metabolome after bariatric surgery. *OMICS* **16** 612–620. (doi:10.1089/omi.2012.0066)

Gall WE, Beebe K, Lawton KA, Adam KP, Mitchell MW, Nakhle PJ, Ryals JA, Milburn MV, Nannipieri M, Camastra S *et al.* 2010 α-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PLoS ONE* **5** e10883. (doi:10.1371/journal.pone.0010883)

German JB, Hammock BD & Watkins SM 2005 Metabolomics: building on a century of biochemistry to guide human health. *Metabolomics* **1** 3–9. (doi:10.1007/s11306-005-1102-8)

Gieger C, Geistlinger L, Altmaier E, Hrabe de Angelis M, Kronenberg F, Meitinger T, Mewes HW, Wichmann HE, Weinberger KM, Adamski J *et al.* 2008 Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genetics* **4** e1000282. (doi:10.1371/journal.pgen.1000282)

de Graaf MA, Jager KJ, Zoccali C & Dekker FW 2011 Matching, an appealing method to avoid confounding? *Nephron. Clinical Practice* **118** c315–c318. (doi:10.1159/000323136)

Griffin JL, Williams HJ, Sang E, Clarke K, Rae C & Nicholson JK 2001 Metabolic profiling of genetic disorders: a multitissue ^{1}H nuclear magnetic resonance spectroscopic and pattern recognition study into dystrophic tissue. *Analytical Biochemistry* **293** 16–21. (doi:10.1006/abio.2001.5096)

Groop LC 1992 Sulfonylureas in NIDDM. *Diabetes Care* **15** 737–754. (doi:10.2337/diacare.15.6.737)

Hirose H, Kawai T, Yamamoto Y, Taniyama M, Tomita M, Matsubara K, Okazaki Y, Ishii T, Oguma Y, Takei I *et al.* 2002 Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. *Metabolism: Clinical and Experimental* **51** 314–317. (doi:10.1053/meta.2002.30506)

Ho JE, Larson MG, Vasan RS, Ghorbani A, Cheng S, Rhee EP, Florez JC, Clish CB, Gerszten RE & Wang TJ 2013 Metabolite profiles during oral glucose challenge. *Diabetes* **62** 2689–2698. (doi:10.2337/db12-0754)

Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, Inzucchi SE, Schumann WC, Petersen KF, Landau BR *et al.* 2000 Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* **49** 2063–2069. (doi:10.2337/diabetes.49.12.2063)

Ibanez AJ, Fagerer SR, Schmidt AM, Urban PL, Jefimovs K, Geiger P, Dechant R, Heinemann M & Zenobi R 2013 Mass spectrometry-based metabolomics of single yeast cells. *PNAS* **110** 8790–8794. (doi:10.1073/pnas.1209302110)

Illig T, Gieger C, Zhai G, Romisch-Margl W, Wang-Sattler R, Prehn C, Altmaier E, Kastenmuller G, Kato BS, Mewes HW *et al.* 2010 A genome-wide perspective of genetic variation in human metabolism. *Nature Genetics* **42** 137–141. (doi:10.1038/ng.507)

Kaddurah-Daouk R, Kristal BS & Weinshilboum RM 2008 Metabolomics: a global biochemical approach to drug response and disease. *Annual Review of Pharmacology and Toxicology* **48** 653–683. (doi:10.1146/annurev.pharmtox.48.113006.094715)

Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS *et al.* 2008 Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nature Genetics* **40** 189–197. (doi:10.1038/ng.75)

Kim WJ, Park CY, Lee KB, Park SE, Rhee EJ, Lee WY, Oh KW & Park SW 2012 Serum 1,5-anhydroglucitol concentrations are a reliable index of glycemic control in type 2 diabetes with mild or moderate renal dysfunction. *Diabetes Care* **35** 281–286. (doi:10.2337/dc11-1462)

Journal of Endocrinology

Kottgen A, Pattaro C, Boger CA, Fuchsberger C, Olden M, Glazer NL, Parsa A, Gao X, Yang Q, Smith AV et al. 2010 New loci associated with kidney function and chronic kidney disease. *Nature Genetics* **42** 376–384. (doi:10.1038/ng.568)

Kraja AT, Vaidya D, Pankow JS, Goodarzi MO, Assimes TL, Kullo IJ, Sovio U, Mathias RA, Sun YV, Franceschini N et al. 2011 A bivariate genome-wide approach to metabolic syndrome: STAMPEED consortium. *Diabetes* **60** 1329–1339. (doi:10.2337/db10-1011)

Kronenberg F 2012 Metabolic traits as intermediate phenotypes. In *Genetics Meets Metabolomics: from Experiment to Systems Biology*, Ed. K Suhre. New York, Heidelberg, Dordrecht, London: Springer.

Krug S, Kastenmuller G, Stuckler F, Rist MJ, Skurk T, Sailer M, Raffler J, Romisch-Margl W, Adamski J, Prehn C et al. 2012 The dynamic range of the human metabolome revealed by challenges. *FASEB Journal* **26** 2607–2619. (doi:10.1096/fj.11-198093)

Krumsiek J, Suhre K, Illig T, Adamski J & Theis FJ 2011 Gaussian graphical modeling reconstructs pathway reactions from high-throughput metabolomics data. *BMC Systems Biology* **5** 21. (doi:10.1186/1752-0509-5-21)

Krumsiek J, Suhre K, Evans AM, Mitchell MW, Mohney RP, Milburn MV, Wagele B, Romisch-Margl W, Illig T, Adamski J et al. 2012 Mining the unknown: a systems approach to metabolite identification combining genetic and metabolic information. *PLoS Genetics* **8** e1003005. (doi:10.1371/journal.pgen.1003005)

Maher B 2008 Personal genomes: the case of the missing heritability. *Nature* **456** 18–21. (doi:10.1038/456018a)

Martin FP, Dumas ME, Wang Y, Legido-Quigley C, Yap IK, Tang H, Zirah S, Murphy GM, Cloarec O, Lindon JC et al. 2007 A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Molecular Systems Biology* **3** 112. (doi:10.1038/msb4100153)

Menni C, Fauman E, Erte I, Perry JR, Kastenmuller G, Shin SY, Petersen AK, Hyde C, Psatha M, Ward KJ et al. 2013 Biomarkers for type 2 diabetes and impaired fasting glucose using a non-targeted metabolomics approach. *Diabetes* **62** 4270–4276. (doi:10.2337/db13-0570)

Milburn MV & Lawton KA 2013 Application of metabolomics to diagnosis of insulin resistance. *Annual Review of Medicine* **64** 291–305. (doi:10.1146/annurev-med-061511-134747)

Moayyeri A, Hammond CJ, Hart DJ & Spector TD 2013 The UK Adult Twin Registry (TwinsUK Resource). *Twin Research and Human Genetics* **16** 144–149. (doi:10.1017/thg.2012.89)

Mook-Kanamori DO, El-Din Selim MM, Takiddin AH, Al-Homsi H, Al-Mahmoud KA, Al-Obaidli A, Zirie MA, Rowe J, Yousri NA, Karoly ED et al. 2014 1,5-anhydroglucitol in saliva is a non-invasive marker of short-term glycemic control. *Journal of Clinical Endocrinology and Metabolism* **99** E479–E483. (doi:10.1210/jc.2013-3596)

Newgard CB 2012 Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metabolism* **15** 606–614. (doi:10.1016/j.cmet.2012.01.024)

Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA et al. 2009 A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metabolism* **9** 311–326. (doi:10.1016/j.cmet.2009.02.002)

Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA & Morris MJ 2010 Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* **467** 963–966. (doi:10.1038/nature09491)

Nicholson JK & Lindon JC 2008 Systems biology: metabonomics. *Nature* **455** 1054–1056. (doi:10.1038/4551054a)

Nicholson JK, Lindon JC & Holmes E 1999 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* **29** 1181–1189. (doi:10.1080/004982599238047)

Nicholson JK, Connelly J, Lindon JC & Holmes E 2002 Metabonomics: a platform for studying drug toxicity and gene function. *Nature Reviews. Drug Discovery* **1** 153–161. (doi:10.1038/nrd728)

Panneerselvam K & Freeze HH 1996 Mannose enters mammalian cells using a specific transporter that is insensitive to glucose. *Journal of Biological Chemistry* **271** 9417–9421. (doi:10.1074/jbc.271.16.9417)

Parikh H, Carlsson E, Chutkow WA, Johansson LE, Storgaard H, Poulsen P, Saxena R, Ladd C, Schulze PC, Mazzini MJ et al. 2007 TXNIP regulates peripheral glucose metabolism in humans. *PLoS Medicine* **4** e158. (doi:10.1371/journal.pmed.0040158)

Pauling L, Robinson AB, Teranishi R & Cary P 1971 Quantitative analysis of urine vapor and breath by gas–liquid partition chromatography. *PNAS* **68** 2374–2376. (doi:10.1073/pnas.68.10.2374)

Pellis L, van Erk MJ, van Ommen B, Bakker GC, Hendriks HF, Cnubben NH, Kleemann R, van Someren EP, Bobeldijk I, Rubingh CM et al. 2012 Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. *Metabolomics* **8** 347–359. (doi:10.1007/s11306-011-0320-5)

Petersen AK, Krumsiek J, Wagele B, Theis FJ, Wichmann HE, Gieger C & Suhre K 2012 On the hypothesis-free testing of metabolite ratios in genome-wide and metabolome-wide association studies. *BMC Bioinformatics* **13** 120. (doi:10.1186/1471-2105-13-120)

Petersen AK, Zeilinger S, Kastenmuller G, Romisch-Margl W, Brugger M, Peters A, Meisinger C, Strauch K, Hengstenberg C, Pagel P et al. 2014 Epigenetics meets metabolomics: an epigenome-wide association study with blood serum metabolic traits. *Human Molecular Genetics* **23** 534–545. (doi:10.1093/hmg/ddt430)

Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y et al. 2009 Variants in MTNR1B influence fasting glucose levels. *Nature Genetics* **41** 77–81. (doi:10.1038/ng.290)

Renner S, Romisch-Margl W, Prehn C, Krebs S, Adamski J, Goke B, Blum H, Suhre K, Roscher AA & Wolf E 2012 Changing metabolic signatures of amino acids and lipids during the prediabetic period in a pig model with impaired incretin function and reduced β-cell mass. *Diabetes* **61** 2166–2175. (doi:10.2337/db11-1133)

Robertson DG & Frevert U 2013 Metabolomics in drug discovery and development. *Clinical Pharmacology and Therapeutics* **94** 559–561. (doi:10.1038/clpt.2013.120)

Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, Carr SA, Thadhani R, Gerszten RE & Mootha VK 2008 Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. *Molecular Systems Biology* **4** 214. (doi:10.1038/msb.2008.50)

Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang TP, et al. (In Press) An atlas of genetic influences on human blood metabolites. *Nature Genetics*.

Suhre K & Gieger C 2012 Genetic variation in metabolic phenotypes: study designs and applications. *Nature Reviews. Genetics* **13** 759–769. (doi:10.1038/nrg3314)

Suhre K, Meisinger C, Doring A, Altmaier E, Belcredi P, Gieger C, Chang D, Milburn MV, Gall WE, Weinberger KM et al. 2010 Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. *PLoS ONE* **5** e13953. (doi:10.1371/journal.pone.0013953)

Suhre K, Romisch-Margl W, de Angelis MH, Adamski J, Luippold G & Augustin R 2011a Identification of a potential biomarker for FABP4 inhibition: the power of lipidomics in preclinical drug testing. *Journal of Biomolecular Screening* **16** 467–475. (doi:10.1177/1087057111402200)

Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, Altmaier E, Deloukas P, Erdmann J, Grundberg E et al. 2011b Human metabolic individuality in biomedical and pharmaceutical research. *Nature* **477** 54–60. (doi:10.1038/nature10354)

Taguchi T, Yamashita E, Mizutani T, Nakajima H, Yabuuchi M, Asano N & Miwa I 2005 Hepatic glycogen breakdown is implicated in the maintenance of plasma mannose concentration. *American Journal of Physiology. Endocrinology and Metabolism* **288** E534–E540. (doi:10.1152/ajpendo.00451.2004)

Published by Bioscientifica Ltd.

Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ *et al.* 2010 Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* **466** 707–713. (doi:10.1038/nature09270)

Wahl S, Krug S, Then C, Kirchhofer A, Kastenmüller G, Brand T, Skurk T, Claussnitzer M, Huth C, Heier M *et al.* 2014 Comparative analysis of plasma metabolomics response to metabolic challenge tests in healthy subjects and influence of the FTO obesity risk allele. *Metabolomics* **10** 386–401. (doi:10.1007/s11306-013-0586-x)

Wang C, Kong H, Guan Y, Yang J, Gu J, Yang S & Xu G 2005 Plasma phospholipid metabolic profiling and biomarkers of type 2 diabetes mellitus based on high-performance liquid chromatography/electrospray mass spectrometry and multivariate statistical analysis. *Analytical Chemistry* **77** 4108–4116. (doi:10.1021/ac0481001)

Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C *et al.* 2011 Metabolite profiles and the risk of developing diabetes. *Nature Medicine* **17** 448–453. (doi:10.1038/nm.2307)

Wang TJ, Ngo D, Psychogios N, Dejam A, Larson MG, Vasan RS, Ghorbani A, O'Sullivan J, Cheng S, Rhee EP *et al.* 2013 2-Aminoadipic acid is a biomarker for diabetes risk. *Journal of Clinical Investigation* **123** 4309–4317. (doi:10.1172/JCI64801)

Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, Heim K, Campillos M, Holzapfel C, Thorand B *et al.* 2012 Novel biomarkers for pre-diabetes identified by metabolomics. *Molecular Systems Biology* **8** 615. (doi:10.1038/msb.2012.43)

Watkins SM, Reifsnyder PR, Pan HJ, German JB & Leiter EH 2002 Lipid metabolome-wide effects of the PPARγ agonist rosiglitazone. *Journal of Lipid Research* **43** 1809–1817. (doi:10.1194/jlr.M200169-JLR200)

Watts G 2012 UK Biobank opens it data vaults to researchers. *BMJ* **344** e2459. (doi:10.1136/bmj.e2459)

Weljie AM, Newton J, Mercier P, Carlson E & Slupsky CM 2006 Targeted profiling: quantitative analysis of ^{1}H NMR metabolomics data. *Analytical Chemistry* **78** 4430–4442. (doi:10.1021/ac060209g)

Wenk MR 2005 The emerging field of lipidomics. *Nature Reviews. Drug Discovery* **4** 594–610. (doi:10.1038/nrd1776)

Wichmann HE, Gieger C & Illig T 2005 KORA-gen – resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* **67**(Suppl 1) S26–S30. (doi:10.1055/s-2005-858226)

Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, Djoumbou Y, Mandal R, Aziat F, Dong E *et al.* 2013 HMDB 3.0 – The Human Metabolome Database in 2013. *Nucleic Acids Research* **41** D801–D807. (doi:10.1093/nar/gks1065)

WTCCC 2007 Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447** 661–678. (doi:10.1038/nature05911)

Würtz P, Makinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, Savolainen MJ, Tammelin T, Viikari JS, Ronnemaa T *et al.* 2012 Metabolic signatures of insulin resistance in 7,098 young adults. *Diabetes* **61** 1372–1380. (doi:10.2337/db11-1355)

Yamanouchi T, Ogata N, Tagaya T, Kawasaki T, Sekino N, Funato H, Akaoka L & Miyashita H 1996 Clinical usefulness of serum 1,5-anhydroglucitol in monitoring glycaemic control. *Lancet* **347** 1514–1518. (doi:10.1016/S0140-6736(96)90672-8)

Yin P, Peter A, Franken H, Zhao X, Neukamm SS, Rosenbaum L, Lucio M, Zell A, Haring HU, Xu G *et al.* 2013 Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. *Clinical Chemistry* **59** 833–845. (doi:10.1373/clinchem.2012.199257)