THEMATIC REVIEW

Perspectives for metabolomics in testosterone replacement therapy

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Abstract

Testosterone is the major circulating androgen in men but exhibits an age-related decline in the ageing male. Late-onset hypogonadism or androgen deficiency syndrome (ADS) is a 'syndromic' disorder including both a persistent low testosterone serum concentration and major clinical symptoms, including erectile dysfunction, low libido, decreased muscle mass and strength, increased body fat, decreased vitality or depressed mood. Given its unspecific symptoms, treatment goals and monitoring parameters, this review will outline the various uncertainties concerning the diagnosis, therapy and monitoring of ADS to date. Literature was identified primarily through searches for specific investigators

in the PubMed database. No date or language limits were applied in the literature search for the present review. The current state of research, showing that metabolomics is starting to have an impact not only on disease diagnosis and prognosis but also on drug treatment efficacy and safety monitoring, will be presented, and the application of metabolomics to improve the clinical management of ADS will be discussed. Finally, the scientific opportunities presented by metabolomics and other -omics as novel and promising tools for biomarker discovery and individualised testosterone replacement therapy in men will be explored.

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Testosterone serum concentrations in men: physiology and epidemiology

Testosterone is the major circulating androgen in men and is essential for the development and maintenance of specific reproductive tissues, such as the testis, and other characteristic male properties, including increased muscle strength, bone mass and hair growth (Mooradian *et al.* 1987). In serum, most of the circulating testosterone (50–60%) is bound to sex hormone-binding globulin (SHBG), while a smaller fraction (40–50%) is loosely bound to albumin, leaving only 1–3% to circulate unbound as 'free' testosterone (Kaufman & Vermeulen 2005). To maintain testosterone at appropriate concentrations, a dynamic network of different interacting factors involved in the excretion and metabolic clearance of testosterone must be in balance. Briefly, the testosterone action in target cells depends on the amount of steroid that can penetrate into the cells, the extent of metabolic conversions within the cells, its interactions with the receptor proteins and finally the action of the androgen receptors at the genomic level (Rommerts 2004).

Based on the findings from various prospective epidemiological studies (Morley *et al.* 1997, Zmuda *et al.* 1997, Harman *et al.* 2001, Feldman *et al.* 2002, Haring *et al.* 2010), it is now well established that total testosterone (TT) concentration shows an age-related decline, with mean serum TT concentration at the age of 75 years being approximately two-thirds of that at age 25 years (Vermeulen *et al.* 1996). Over and above this established age-related decline at the population level, a considerably larger inter-individual variability of TT concentrations can be observed at any age. Dependent on the genetic background, accompanying comorbidities, medications or adverse lifestyle behaviours, individual TT concentrations could be either well preserved against this physiological decline or decrease even more progressively (Haring *et al.* 2010). Although the physiological basis and the extent of the suggested cofactors underlying the large inter-individual variability in TT concentrations are not yet fully elucidated, disturbances in the biosynthesis and actions of testosterone caused by acute illness or chronic diseases are well known (Kaufman & Vermeulen 2005).

Conversely, prospective epidemiological cohort studies have accumulated evidence suggesting that low TT concentration is an independent predictor of various cardiovascular risk factors, including obesity (Svartberg *et al.* 2004), subclinical inflammation (Haring *et al.* 2011a), dyslipidaemia

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(Haring *et al.* 2011b), hypertension (Torkler *et al.* 2011), metabolic syndrome (Laaksonen *et al.* 2004, Kupelian *et al.* 2006, Haring *et al.* 2009) and type 2 diabetes (Vikan *et al.* 2010, Schipf *et al.* 2011). Prospective population studies have also repeatedly observed that low TT is independently associated with an increased mortality risk (Araujo *et al.* 2011). However, although numerous prospective studies have shown that low TT precedes the onset of various cardiovascular risk factors, others have found reduced TT in men with type 2 diabetes (Barrett-Connor *et al.* 1990), metabolic syndrome (Laaksonen *et al.* 2003), obesity (Kaplan *et al.* 2006) and comorbidity (Wu *et al.* 2008).

Given the bidirectional nature of the observed low TT–cardiovascular risk factor associations, it is still unclear whether low TT is a risk factor (causal) or a risk marker (secondary) of mechanisms that ultimately lead to a higher cardiovascular risk factor burden; therefore, reverse causality remains a possibility (Box 1). In addition, neither case–control studies nor prospective cohort studies have observed an independent association between low TT and incident fatal or nonfatal cardiovascular disease (CVD) events (Ruige *et al.* 2010), providing further evidence for the role of low TT as a risk marker. In conclusion, recent epidemiological data suggest that serum TT concentration might be considered as a biomarker of good health and overall well-being in men (McLachlan 2010). Circulating TT shows a physiological decline in conjunction with ageing, cardiovascular comorbidity, obesity, medications and depression. Therefore, testosterone assessment may play a role as a personalised risk marker rather than an independent causal risk factor (Maggio & Basaria 2009).

Androgen deficiency syndrome in men: current practice

The primary clinical use of testosterone replacement therapy (TRT) is to diagnose primary or secondary hypogonadism caused by ‘classical’ disorders, such as Klinefelter syndrome, Kallmann syndrome or pituitary insufficiency (Nieschlag & Behre 2004). There is no doubt that these patients should receive TRT (Isidori & Lenzi 2007). However, in the

majority of patients, the diagnosis of low TT parallels with advanced age, accompanying acute or chronic diseases, medication use or adverse health-related lifestyle (Travison *et al.* 2007, Snyder 2008). Thus, testosterone deficiency is a frequent diagnosis in ageing men, with a prevalence of 10–20%, depending on the applied cut-off and studied population (Araujo *et al.* 2004, Haring *et al.* 2010). Androgen deficiency syndrome (ADS) is a ‘syndromic’ disorder including both clinical symptoms and persistently low TT. Thus, TT assessment is a crucial diagnostic criterion requiring proper evaluation and interpretation.

Laboratory diagnosis of testosterone deficiency

Current guidelines unequivocally highlight the measurement of morning serum TT by a reliable assay as the initial diagnostic test to assess the male androgen status (Wang *et al.* 2009a, Bhasin *et al.* 2010, McLachlan 2010). The presently used, extensively automated procedures for the analyses of TT concentration in routine diagnostics are commercial platform-based immunoassays. However, given a lack of specificity (Wang *et al.* 2004) and substantial inter-assay as well as inter-laboratory differences in measured absolute TT concentrations (Dorgan *et al.* 2002, Wang *et al.* 2004, Hsing *et al.* 2007), immunological procedures are considered insufficient for the low concentration range of TT in elderly comorbid men (Taieb *et al.* 2003). Hence, the more precise mass spectroscopic procedures, demonstrating considerably lower intra- and inter-laboratory variability (Vesper *et al.* 2009), are increasingly perceived as the gold standard for the measurement of TT (Wang *et al.* 2004, 2009a, Thienpont *et al.* 2008, Bhasin *et al.* 2010, Stanczyk & Clarke 2010). Beyond these analytical factors, several pre-analytical and physiological cofactors also need to be considered for the proper interpretation of TT measurements (Wheeler & Barnes 2008, Haring *et al.* 2011d). Taken together, TT measurements must be evaluated cautiously not only because of the insufficient diagnostic and analytic quality of immunological measurements in the low concentration range but also for the reasons of pre-analytical and physiological influencing factors.

Box 1 Outstanding questions

- Does a male andropause exist, or are testosterone concentrations well preserved in metabolically healthy ageing men over time?
- Which symptoms are specific to low testosterone concentrations?
- Which treatment goal meets an individual’s metabolic needs, or what is a ‘normal’ testosterone concentration at different ages?
- How can TRT take into consideration the large inter-individual variability in testosterone concentrations?
- Is there an optimal dose or duration of TRT to induce clinically relevant benefits with regard to cardiovascular risk, frailty or quality of life?
- Is it possible to predict which individuals will most likely benefit from TRT?
- Which parameters should be monitored to optimise the effectiveness and safety of TRT?

Clinical diagnosis of ADS

Even more uncertainty exists with regard to the clinical symptoms and signs presumably related to low serum TT. Conditions such as erectile dysfunction, low libido, decreased muscle mass and strength, increased body fat, decreased bone density, decreased vitality and depressed mood are suggested to be related to testosterone (Morales *et al.* 2010), but none of these symptoms are specific to low TT concentrations in men (Wang *et al.* 2009a). Therefore, one or more of these symptoms must be corroborated with repeatedly measured low morning TT to constitute the diagnosis of ADS and consider the initiation of TRT (Fig. 1). Similarly, an observational study of 3369 men aged 40–79 years showed that among the various symptoms, only sexual symptoms had a syndromic association with low TT (Wu *et al.* 2010). In contrast, a meta-analysis of 17 randomised placebo-controlled trials showed that TRT only moderately improved the number of sexual symptoms and had no effect on erectile function (Isidori *et al.* 2005). In conclusion, the clinical conditions related to low TT are of a non-specific and only suggestive nature, not diagnostic of ADS (Box 1). This absence of definite clinical correlates of ADS contributes to the uncertainties surrounding TRT in men (Sadovsky *et al.* 2007) and is reflected by the difficulties guideline expert panellists face in issuing firm recommendations and criteria for the initiation of TRT (Wang *et al.* 2009a, Bhasin *et al.* 2010).

Current therapy for ADS

Once a treatment decision has been made, improvements in signs and symptoms of ADS together with serum TT concentrations in the middle to lower range of young adult males should be sought (Wang *et al.* 2009a, Bhasin *et al.* 2010). To achieve these therapeutic goals, injectable, oral and transdermal preparations of natural testosterone are currently available. Due to inadequate data, much discussion exists regarding the critical threshold to determine a definite cut-off for the optimal serum TT concentration in terms of efficacy and safety, as well as a risk–benefit equation for intervention. A review and meta-analysis of 51 treatment studies concluded that the safety of TRT and its adverse cardiovascular effects are still unknown (Fernandez-Balsells *et al.* 2010). The observed increases in haemoglobin or haematocrit and small decreases in HDL cholesterol were of unknown clinical significance. The results from a randomised, double-blinded, placebo-controlled trial in 274 frail elderly men aged 65–90 years showed that the effects of 6-month TRT on muscle strength, lean mass and quality of life were not maintained 6 months post-treatment (O'Connell *et al.* 2011). Furthermore, the time course of the effects induced by TRT from their first manifestation to the maximum effects shows considerable variation (Saad *et al.* 2011), raising the question of optimal treatment duration. The potential risks of over-supplementation during TRT were exemplified by a discontinued trial among elderly men (mean age 74 years)

Figure 1 Standard vs individualised testosterone therapy in men. Instead of relying solely on a single biomarker (fixed testosterone cut-off) and self-reported symptoms for the diagnosis of ADS and monitoring of testosterone therapy, novel diagnostic techniques (multi-omics) promise to advance our understanding of the pathophysiology of ADS (leading to a metabolically healthy state) and thereby enable individualised treatment concepts for the improved diagnosis, therapy and monitoring of ADS in men.

with mobility limitations after testosterone replacement was associated with an increased risk of adverse cardiovascular events in the intervention group (Basaria *et al.* 2010). The starting doses applied in this trial were higher than those recommended by the manufacturer, and the treatment goal in these patients (34.7 nmol/l) was considerably higher than that recommended by the Endocrine Society (13.9–17.4 nmol/l; Bhasin *et al.* 2010).

Taken together, the current evidence about the safety of TRT in men with regard to important patient outcomes is of low quality and limited by short follow-up periods (Fernandez-Balsells *et al.* 2010). Thus, TRT should only be initiated in the presence of TT concentrations clearly below the lower normal limit for younger men, together with unequivocal signs and symptoms of TT deficiency, in the absence of other reversible causes of decreased TT and after screening for contraindications (Nieschlag & Behre 2004). Once initiated, TRT should induce and maintain secondary sex characteristics and improve sexual function, sense of well-being, muscle mass and strength and bone mineral density (Bhasin *et al.* 2010). Accordingly, the response to TRT should be assessed and monitored by patients' well-being, sexual activity and occasional measurement of serum TT, haemoglobin and haematocrit, bone density and prostate parameters (Nieschlag & Behre 2004, Wang *et al.* 2009a).

As stated earlier, the presumed syndromic nature of testosterone deficiency is often difficult to disentangle because symptoms and signs suggestive of ADS are readily accounted for by comorbidities and because borderline TT deficiency is a frequent biochemical accompaniment of systemic disease, which are the reasons why 'pure' ADS is quite uncommon. Furthermore, we do not yet have an operational definition for 'normal' testosterone concentrations at different ages, nor have we identified specific signs and symptoms to accurately discriminate between those who need treatment and those who do not (Isidori & Lenzi 2007). Thus, the conjunction between low TT and several non-specific symptoms, constituting the diagnosis of ADS or late-onset hypogonadism, remains a controversial concept with several outstanding questions (Box 1).

Metabolomics for the improved diagnosis, therapy and monitoring of ADS

The principle techniques of metabolomics

Against the background of the various uncertainties surrounding the diagnosis, therapy and monitoring of ADS described earlier, the application of metabolomics offers a variety of scientific opportunities to improve the clinical management of testosterone deficiency in men. Metabolomics or metabonomics addresses the comprehensive measurement of the total low-molecular weight or metabolite content of a cell, tissue or body fluid. Because small molecules are the end result of all regulatory and metabolic processes at

the cellular level within tissues in all organisms, metabolomics gives a view into whole-system biochemistry (Nicholson & Wilson 2003). With the global analysis of all or nearly all these cellular metabolites, metabolomics offers molecular information that is closest to the phenotype in that metabolites are an ultimate product of gene, mRNA and protein activity (Nordstrom & Lewensohn 2010).

There are two major complementary approaches in metabolomics: targeted analysis is used to measure the concentrations of a limited number of precisely known metabolites (Shulaev 2006), and the complementary non-targeted approach of metabolic profiling measures a large set of metabolites in a global semi-quantitative manner (Lindon & Nicholson 2008). Although a wide range of techniques can be used for metabolomics, the two principal analytical platforms used to analyse metabolites in body fluids, such as urine and plasma, are nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography coupled to mass spectrometry (LC-MS; Lindon & Nicholson 2008). MS-based metabolomics is currently the most developed technique to quantitatively analyse specific metabolites or a defined set of metabolites with a high sensitivity and throughput. Nevertheless, a recent inter-laboratory study showed that ultra-performance LC-MS of human urine is a novel technique for global metabolic profiling, yielding metabolite measurements that are highly reproducible both within and between different laboratories (Benton *et al.* 2012). Hence, the rapid technical progress increasingly enables an analytical flexibility that makes MS-based metabolomics amenable to targeted as well as untargeted metabolomics approaches.

Addressing the basic idea that specific molecules or metabolites for a certain disease or drug intervention are detectable and quantifiable in body fluids, NMR and MS-based metabolomics have been used for biomarker screening, pathway discovery and monitoring of metabolic changes in response to physiological and therapeutic perturbations or environmental stimuli (Clayton *et al.* 2006, Holmes *et al.* 2008a, O'Sullivan *et al.* 2011). Below, I will present the current state of research of these different metabolomics applications with a special focus on their potential to improve diagnosis, therapy and monitoring of ADS in men.

Metabolomics for biomarker discovery

The main application of metabolomics, by far, lies in the discovery of biomarkers in the clinical and pharmaceutical research setting. A biomarker is a biological characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention (Biomarkers Definitions Working Group 2001). An ideal biomarker is quantifiable, reproducible and analytically simple; it is also inexpensive to measure, its concentration or level does not vary widely, it is specific to the condition

of interest and it is not affected by comorbid factors (Vasan 2006).

One of the first applications of metabolomics for biomarker discovery was the diagnosis of inborn errors of metabolism (Moolenaar *et al.* 2003). The most prominent metabolomics biomarker discovery studies are presently conducted in the field of cancer research. Metabolomic analysis of plasma, tissue and urine from prostate cancer patients has identified and validated sarcosine as a potential candidate for early disease detection and aggressivity prediction in prostate cancer (Sreekumar *et al.* 2009). Further exploratory metabolomics studies have been conducted to identify biomarkers in serum and urine from various patient groups and cancer types, including lung, bladder, kidney, colon, oral, breast, ovarian, leukaemia and cervical cancer (Mamas *et al.* 2011).

CVD patients have been investigated to identify potential metabolic biomarkers for improved disease prediction, risk stratification and individualised diagnosis (Nordstrom & Lewensohn 2010). MS-based metabolomics on 36 patients (18 cases showing inducible ischaemia and 18 controls) revealed significant discordant regulation of multiple metabolites after exercise stress testing in cases but not in controls (Sabatine *et al.* 2005). Another serum metabolomics study revealed branched-chain amino acid metabolites and urea cycle metabolites associated with the presence of coronary artery disease (CAD), whilst dicarboxylic acylcarnitines predicted death/myocardial infarction outcomes (Shah *et al.* 2010). Wang *et al.* (2009b) demonstrated that metabolites of arginine methylation provide independent risk prediction for both obstructive CAD and incident major adverse cardiac events in stable patients undergoing cardiac evaluation. Serum metabolomics has correctly diagnosed not only the presence but also the severity of CAD (Brindle *et al.* 2002). Taken together, NMR- and MS-based metabolomics have shown much potential for the identification of novel biomarkers for a range of pathological processes affecting the heart (Barderas *et al.* 2011) and have been proposed as a novel tool for cardiac research (Griffin *et al.* 2011). Metabolomics-based biomarker discovery has also been applied to clinical conditions such as osteoarthritis (Lamers *et al.* 2005), diabetes mellitus (Bain *et al.* 2009) and neurological diseases (Madsen *et al.* 2010). Furthermore, recent metabolomics studies on human urine and serum samples identified numerous metabolites associated with cardiovascular risk factors (Barderas *et al.* 2011), including blood pressure (Holmes *et al.* 2008a), insulin resistance (Wang *et al.* 2011b) and atrial fibrillation (Mayr *et al.* 2008). Furthermore, metabolomics studies demonstrated the phenotypic heterogeneity of CVD and the limitations of single diagnostic biomarkers (Makinen *et al.* 2008).

Most of the metabolomics studies described earlier have been performed in small patient samples and have not yet been validated in larger external samples. Thus, large-scale validation is required to reassess the discovered biomarkers in prospective studies with well-phenotyped clinical outcomes. For this goal, metabolome-wide association studies (MWAS)

have been proposed as a powerful new approach not only to replicate discovered biomarkers but also to identify novel biomarkers of disease risk (Nicholson *et al.* 2008). Enabled by rapid technological advances, high-throughput metabolic profiling used in large-scale molecular epidemiology has proved its potential to detect associations between metabolic phenotypes and disease risk. Holmes *et al.* (2008a) demonstrated this population-level approach for the identification of discriminatory metabolic biomarkers for high blood pressure. Another MWAS identified urinary metabolites that discriminate between southern and northern Chinese study samples to explain the observed regional differences in CVD risk (Yap *et al.* 2010). Although a number of novel biomarkers have been discovered, external validation through MWAS is currently under investigation, and methodological challenges will be addressed (Dunn *et al.* 2011), none of the discovered biomarkers have currently made the transition to routine use in clinical practice (Mamas *et al.* 2011).

However, the described findings fuel confidence that metabolomics may address some of the outstanding questions related to TRT in men (Box 1). To date, the key area of controversy surrounding the diagnosis of ADS relates to 1) the biochemical determination of low TT and 2) the unspecific symptoms of ADS (Traish *et al.* 2011). The diagnostic information obtained from hypothesis-free metabolic profiling complemented with a targeted approach is likely to yield a biomarker set of multiple metabolites that could provide comprehensive insights into pathophysiological metabolic processes specific to the onset and progression of ADS (Fig. 1) that were previously not assessable with traditional single biomarkers such as TT alone (Wopereis *et al.* 2009). Thus, metabolic profiling may help to overcome the single-biomarker conservatism by analysing several biomarkers or biomarker combinations (Kenny *et al.* 2010).

Furthermore, metabolic signatures have the unique potential to disclose linkages between physiological, behavioural and environmental characteristics and could therefore offer a promising approach to account for the unspecific nature of the symptoms related to ADS and the existing doubts about the postulated syndromic nature of low TT (Gould *et al.* 2000, Morley & Perry 2003, Seidman 2006, Perheentupa & Huhtaniemi 2007). Thus, the discovery of metabolic disorders specific to low TT would considerably advance the diagnostic reliability of ADS. Because there is no specific cut-off value for serum testosterone concentration that clearly distinguishes between men with and without testosterone deficiency, one could envision a small case-control metabolomics study to reveal a potential metabolic biomarker that provides suitable discrimination. Furthermore, the identified biomarker could offer potential links to symptoms specifically related to low TT. Surely, metabolites identified from these early studies will need to be externally validated in larger, prospective clinical cohorts and MWAS for their future use as biomarkers.

Metabolomics for the definition of 'normal'

Before the application of metabolomics to study disease onset and progression or the effects of any drug intervention, it is first necessary to define the metabolic range covered by normal physiological variation. Thus, various metabolomics studies have been conducted to investigate the inherent metabolic variability based on intrinsic (gender, age, comorbidity and genetics) and extrinsic (diet, sleep, stress and temperature) factors (Aardema & MacGregor 2002, Lindon *et al.* 2003). Furthermore, each biological tissue or fluid has its own unique metabolic signature (see Fig. 1; Holmes & Nicholson 2007). Because changes in these factors could potentially mask molecular changes caused by the disease or intervention, defining a physiologically normal metabolic pattern is crucial to the identification of robust and specific biomarkers. In contrast to animal studies that have controlled for confounding factors, such as age, gender, diet or stress levels, human populations show a comparably greater variability. Thus, it is clearly necessary to demonstrate that it would be possible to detect the effects of disease progression or drug therapy above that inherent inter-individual variability.

A recent study investigating gender-specific differences in serum metabolite concentrations and their underlying genetic determination revealed significant differences in metabolite profiles between males and females (Mittelstrass *et al.* 2011). Among more than 3000 participants of a population-based epidemiological study, significant differences were shown for 101 of the 131 investigated metabolites. Based on these results, gender-specific therapies that would assign men and women to different categories and predictive biomarkers depending on gender were postulated (Mittelstrass *et al.* 2011). NMR-based metabolomics of urine and plasma profiles from 150 healthy humans showed similar gender-specific metabolic differences (Kochhar *et al.* 2006). Further urine metabolomics studies showed that inter-individual metabolic differences were related to a significant extent to gender and to a lesser extent to age (Psihogios *et al.* 2008) and uncovered additional population-wide metabolic differences related to gender, diurnal variation and age (Slupsky *et al.* 2007). The issue of the comparative effects of different influencing factors was addressed in a unique study that prospectively collected urine and plasma samples from 154 post-menopausal female twins to analyse NMR spectra and statistically decompose metabolic variability into familial (genetic and common environmental), individual environmental and longitudinally unstable components. The main result was the identification and quantification of a substantive proportion of stable biological variation (sum of genetic and environmental variation) in the plasma (60%) and urine metabolomes (47%) (Nicholson *et al.* 2011).

Because metabolic profiles are subject to a large inter-individual variability based on the above-described confounding factors, the importance of a standardised protocol for the application of metabolomics to clinical

research should not be underestimated. Analysing NMR-based metabolomics data of urine and plasma samples drawn from 12 healthy men on two separate occasions 14 days apart, the results of one study emphasise the possibility of collecting consistent metabolomics data in clinical studies (Lenz *et al.* 2003). In summary, metabolomics has been used in a number of studies to define a 'normal' biochemical profile and has proved capable of significantly differentiating effects related to age, gender, diet, comorbidities and drugs. These results offer valuable insights into the sources of human metabolic variability and will have implications for the effective design of biomarker discovery studies.

As stated earlier, there are still considerable uncertainties with regard to the definition of normal TT concentrations at different ages. Our understanding of individual set points for circulating TT concentrations (below which one, but not another, individual may develop metabolic changes indicative of testosterone deficiency) or the concept of reserve capacity (the possibility that men with TT concentrations below the fixed cut-off still may have adequate concentrations to meet their metabolic needs) is very limited. The two current guidelines (Wang *et al.* 2009a, Bhasin *et al.* 2010) provide little direction and arbitrarily defined fixed TT cut-offs respectively. Thus, there is currently no consensus about the definition of normal TT concentrations at different ages, further diluting any efforts to transparently define treatment goals for men under TRT.

Here, metabolomics offers the potential to measure testosterone metabolites and related molecules in urine and serum to provide a broader metabolic picture for the evaluation of the single, absolute serum TT measurement. In particular, the urinary steroid profile, which is mainly based on testosterone, reflects the metabolic pathways of androgenic compounds and is essential for the diagnosis of diseases related to steroid secretion (Mareck *et al.* 2008). Recently, MS-based metabolomics was applied to directly analyse and quantify major urinary metabolites as markers of exogenous steroid administration in routine doping controls (Badoud *et al.* 2011). This method was subsequently validated and applied to a clinical TRT trial comparing a group of healthy male volunteers with a placebo group (Badoud *et al.* 2011). Similar studies replicated this method, establishing a fast and sensitive analytical procedure for the simultaneous separation, determination and quantification of testosterone derivatives in human urine (Bean & Henion 1997, Strahm *et al.* 2008).

By incorporating detailed information on an individual's metabolic status, metabolomics offers the potential to obtain a multi-metabolite characterisation of the metabolic state of the patient for the diagnosis of ADS and monitoring of TRT in men (Fig. 1). Hence, assessment of the individual baseline metabolic profile allows changes in response to TRT to be monitored and treatment goals to be defined in accordance with an individual's metabolic needs. Furthermore, deviations from that 'normal' metabolic profile may elucidate the mechanisms by which TRT alters the metabolic profile of

an individual so that we can better understand and maintain a healthy metabolic profile (Fig. 1). Based on the metabolic profile of a pre-dose baseline urine sample, Clayton *et al.* (2009) demonstrated the feasibility of predicting acetaminophen metabolism and excretion as well as acetaminophen-induced hepatotoxicity in humans. These proof-of-principle studies show that defining a metabolic starting point or baseline is key to predicting the outcome of any drug or lifestyle intervention.

Metabolomics in the investigation of physiological variation and rhythms

Based on a large body of evidence from experimental research, it is now clear that metabolomics has the requisite reproducibility and sensitivity to characterise inter-individual variability in animals as well as humans and to distinguish between various normal physiological states and their changes due to environmental or drug-related influences (Bollard *et al.* 2005). The acquired metabolic spectra of a body fluid, such as urine, serum or saliva, reflects the metabolic status of the organism, which changes in response to stressors to maintain a homeostatic balance. The potential value of metabolomics in the investigation of physiological variation and rhythms important in TRT became apparent with the description of a hormonal cycle based on urine metabolomics (Bollard *et al.* 2001). In that particular study, Bollard *et al.* used NMR spectra of female rat urine to examine the influence of hormonal fluctuations over a 10-day period. Furthermore, hormonal variation was taken into account by analysing metabolite fluctuations and metabolic trajectories. Before that, an insightful approach was used to elucidate time-related effects of toxins on endogenous rat urine profiles (Holmes *et al.* 1992, Beckwith-Hall *et al.* 1998). Metabolomics was also demonstrated to be a non-invasive, sensitive and relatively fast method for assessing the individual changes in metabolic profiles of urine from rats following chronic exposure to pesticides (Wang *et al.* 2011a). Metabolomic phenotyping also revealed novel urinary metabolic markers in response to surgical trauma in Wistar rats (Kinross *et al.* 2011). However, to date, metabolomics has mainly been applied to experimental animal studies conducted in laboratory models of disease or toxicity under controlled genetic and environmental conditions (Gavaghan *et al.* 2000, 2001, 2002, Bollard *et al.* 2001). To investigate the translatability of the experimental data to humans, an NMR-based system-wide characterisation of the urine, serum, liver and kidney metabolomes of the pig showed that the metabolites observed in each of these biological compartments were qualitatively comparable to the metabolic signature of the same biological matrices in humans and rodents (Merrifield *et al.* 2011).

Hence, it is now appropriate to extend those methods to investigate human metabolism and its responses to controlled interventions (Holmes & Nicholson 2007). However, understanding the individual metabolic baseline profile and its inter-individual variability is only the first step, although it

already holds numerous scientific opportunities for the improved diagnosis, treatment and monitoring of ADS in men (Box 1). The aforementioned studies suggest that metabolomics can be an efficient tool for the individualised diagnosis of ADS in men. Metabolomics could help to explain the observed large inter-individual variability in TT concentrations by not only defining an individual metabolic baseline profile but also by uncovering homeostatic mechanisms in response to nutritional or environmental effects. However, ultimately, the potential of metabolomics lies in the ability to assess the response to a medical therapy or drug intervention, including TRT.

Metabolomics for drug response characterisation in time

As described in the previous sections, metabolomics encompasses the comprehensive and systematic profiling of metabolite levels as well as temporal changes in response to stimuli related to lifestyle, environment or drugs. Another major application of metabolomics will be to predict an individual's response to pharmaceutical treatment, perform drug safety studies and obtain drug effect endpoints – a concept termed pharmacometabolomics (Lindon *et al.* 2006). Pharmacometabolomics is envisioned to provide real-time metabolic profiles as dynamic markers reflecting the individual treatment response and to reveal indicators of treatment efficacy. Thus, sequentially collected urine or serum samples could be used to assess changes in the metabolic profile and investigate drug-related effects or disease processes over time (Holmes *et al.* 1998, Nicholson *et al.* 1999). To employ pharmacometabolomics to detect effects specifically related to a drug intervention such as TRT, we must first distinguish alterations occurring in response to a drug from those resulting from other sources of variability, as discussed earlier.

Because perturbations of the metabolic state of an individual generally manifest as particular patterns of metabolites, these metabolic signatures could be used to monitor individuals under TRT. To control for the large inter-individual variability in drug response and for potential confounders, serial sampling can be performed so that each patient serves as his own control. As a proof of principle, studies on patients undergoing controlled interventions, such as the exercise stress test (Sabatine *et al.* 2005) or oral glucose challenge (Shaham *et al.* 2008, Wopereis *et al.* 2009), showed that most metabolites displayed concordant changes in cases and controls, while the metabolites with significant discordant changes in cases remained unchanged in controls. In prospective biomarker studies, metabolomics has identified, categorised and profiled kinetic patterns of early metabolic biomarkers of planned and spontaneous myocardial infarction (Lewis *et al.* 2008b, Baumgartner *et al.* 2010). Based on the plasma samples from 36 patients undergoing a planned myocardial infarction, MS-based metabolomics identified different metabolic profiles in the early phase of myocardial injury (Lewis *et al.* 2008b). In animal studies, urine profiles

were used to predict individual drug metabolism and susceptibility to side effects (Nicholson & Lindon 2008).

A similar approach and another area of intense research is nutrimetabolomics for the investigation of the effects of dietary interventions on metabolic phenotypes (Rezzi *et al.* 2007). Combining untargeted metabolic profiling of urine specimens with cross-validation in large-scale epidemiological data, Heinzmann *et al.* (2010) presented a novel strategy for food biomarker discovery to objectively evaluate the effect of diet on health. In the first step, a standardised diet was administered to eight individuals to reveal putative urinary biomarkers of fruit consumption. Secondly, candidate biomarkers were validated using urinary NMR spectra from 499 UK participants of the International Collaborative Study of Macronutrients, Micronutrients, and Blood Pressure (INTERMAP). This approach identified urinary excretion of proline betaine as a specific and sensitive biomarker of citrus fruit intake (Heinzmann *et al.* 2010). Another short-term diet-controlled study investigated the contribution of metabolic baseline differences in healthy individuals to metabolomic outcomes under various dietary modulations and showed that over and above inter-individual metabolic differences, clear biochemical effects of single-type dietary interventions, animal protein and fruit and wine intake are detectable (Heinzmann *et al.* 2012).

Applying this strategy of untargeted metabolic profiling in a small clinical TRT trial and the subsequent validation of candidate biomarkers using large-scale epidemiological data promises valuable insights for TRT in men. First, investigating relative differences in biological processes rather than absolute treatment targets supersedes fixed TT cut-offs to define treatment goals in men under TRT. Secondly, specific metabolic biomarkers of treatment response may highlight the dynamic metabolic status of an organism in response to drug stimuli (Bollard *et al.* 2005) and could be applied to assess the efficacy of TRT. Finally, metabolomics provides excellent analytical and biological reproducibility with low costs per sample and analyte, allowing frequent monitoring to address time-dependent fluctuations of metabolites that occur in response to TRT. Overall, metabolomics offers a huge potential to accurately assess drug-induced changes and to individually initiate as well as monitor TRT in men.

Metabolomics for measuring drug effectiveness and safety

Pharmacometabolomics is also being embraced to take into account important environmental influences on drug absorption, distribution, metabolism and excretion and to thereby achieve maximal efficacy and avoid adverse drug reactions. The proof of principle of the pharmacometabolomics concept for the prediction of drug toxicity was shown in the rat (Coen *et al.* 2003, Lenz *et al.* 2005) and for drug metabolism in humans (Clayton *et al.* 2006). In a ground-breaking study by Clayton *et al.* (2006), the prediction of the metabolism and toxicity of a dosed substance was performed based solely on the analysis and modelling of

a pre-dose metabolic profile, offering an alternative approach to understand the inter-individual variability in drug response. After administration of paracetamol (acetaminophen) to rats, urinary drug metabolite profiles showed an association between pre-dose urinary composition and the extent of liver damage sustained after paracetamol administration (Clayton *et al.* 2006). This approach was subsequently tested in a clinical study on healthy adults receiving 4 g/day acetaminophen for 7 days, which identified predictive urine metabolite profiles that could distinguish responders from non-responders for liver injury (Winnike *et al.* 2010). Using NMR spectra of pre- and post-dose urine samples after a standard dose of acetaminophen, a human-gut microbiome cometabolite was identified whose high pre-dose urinary level was associated with a low post-dose urinary ratio of acetaminophen sulphate to acetaminophen glucuronide (Clayton *et al.* 2009). This finding adds to the rapidly growing recognition of the multiple metabolic interactions between humans and their gut microbiome (Arumugam *et al.* 2011, Kau *et al.* 2011) and of the potential significance of the latter with regard to disease, drug efficacy and adverse drug reactions (Clayton *et al.* 2009).

Another study used an untargeted MS-based metabolic profiling approach in prostate cancer cell lines to investigate androgen-induced metabolomic alterations in prostate cancer cells. Its findings indicate that androgen exposure results in elevated amino acid metabolism and altered methylation potential in prostate cancer cells. Further, metabolic phenotyping studies confirm higher flux through pathways associated with amino acid metabolism in prostate cancer cells treated with androgens. These findings provide valuable insights into the potential biochemical processes regulated by androgen signalling in prostate cancer (Putluri *et al.* 2011), especially given the hypothesised influence of serum sex hormones on prostate cancer risk (Roddam *et al.* 2008).

For these reasons, pharmacometabolomics is now recognised as an independent and widely used technique to evaluate the inter-individual variability of the beneficial and adverse effects of a drug intervention. Taking into account the genetic and modifying environmental influences that determine the individual metabolic fingerprint, together with drug-induced metabolomics changes, the pharmacometabolomics approach promises various potential applications for the improved treatment and monitoring of ADS in men.

Metabolomics and other omics for individualised TRT in men

The benefits of attaining knowledge about the metabolic state of an individual and the potential of this knowledge to help improve TRT in men have been discussed in the present review. In addition, general advances in molecular profiling (omics) methods, including genomics, transcriptomics, proteomics and metabolomics, coupled with statistical data integration, allows us to analyse different omic levels, their

interactions and finally a global model of the system characteristics. This integrative research strategy, named systems biology (Aderem 2005), is designed to address the complexity of biological systems at all levels of organisation, from molecules, cells and organs to organisms and ecosystems, using multi-omics data sets from high-throughput experimental and computational technologies (Auffray *et al.* 2009). The described advances in 'omics' technology also allow for the incorporation of novel biomarkers at multiple omic levels into integrative personal omics profiles (Chen *et al.* 2012) as well as into large-scale observational studies, which is a concept we previously referred to as systems epidemiology (Haring & Wallaschofski 2012).

This integration of multiple 'omics' data has been initially performed in small-scale animal studies integrating metabolic profiles with quantitative trait locus data in a diabetic rat model (Dumas *et al.* 2007) and in combined metabolic and proteomic data of a mouse model of prostate cancer (Rantalainen *et al.* 2006). With the availability of increasingly powerful high-throughput technologies, computational tools and integrated knowledge bases, multiple 'omics' integration is now being applied to large-scale human clinical and population studies. For example, an integrated analysis of metabolic and genome-wide data revealed new functional relationships between disease-associated variants (Suhre *et al.* 2011). Chen *et al.* (2012) presented an integrated personal omics profile that combined longitudinal genomic, transcriptomic, proteomic, metabolomic and autoantibody data from a single individual over a 14-month period, revealing several dynamic risk markers for various conditions, including type 2 diabetes. Another study analysed the genetic variance of human metabolism and identified 31 genetic variants associated with levels of circulating metabolites (Kettunen *et al.* 2012). Brockmoller *et al.* (2012) integrated the analysis of the lipidome and metabolome with protein and gene expression data from a cohort of breast cancer patients to underline the general relevance of metabolic changes in cancer pathogenesis and tumour progression. Altogether, there is an increasing consensus that systems biology principles will play important roles in tuberculosis (Comas & Gagneux 2011), cancer (Lund & Dumeaux 2008), type 2 diabetes mellitus (Hu 2011) and CVD research (MacLellan *et al.* 2012).

The application of metabolomics together with complementary omics promises to have far-reaching implications for the development of an individualised treatment concept for TRT in men (Fig. 1). For example, a recent genome-wide association study on 14 429 men investigated the genetic determinants of serum TT concentration in men and found that genetic variants in the *SHBG* locus and on the X-chromosome are associated with a substantial variation in TT concentration and increased risk of low TT (Ohlsson *et al.* 2011). Longitudinal epidemiological data from 1859 men aged 20–79 years also suggested genomic effects of the CAG repeat length on overall TT concentration and testosterone-related cardiometabolic effects (Haring *et al.* 2011c). These findings

proved helpful in elucidating the genetic basis of the observed inter-individual variability of TT concentrations in men. A MWAS of TT concentrations in men is currently underway and promises to provide novel insights into the aetiology, biological mechanisms and pathways of testosterone metabolism in men. The broader applications of metabolomics in mammalian systems biology for the study of the health–disease continuum, drug efficacy and drug toxicity were recently reviewed (Dunn *et al.* 2011).

Thus, systems approaches can be used to describe the changes in metabolism in different body compartments affected by exposure to, for example, TRT. Such multi-omic profiles are themselves characteristic of particular types and mechanisms of pathology and can be used to provide a more complete description of the biochemical consequences than can be obtained from one omic-level analysis alone. For example, the evaluation of transcriptomics as well as metabolic changes after the administration of bromobenzene provides a more sensitive approach for detecting the effects of the toxin (Heijne *et al.* 2005). However, although omics-based biomarkers and signatures identified from population-based studies can potentially be used to identify high-risk groups for targeted prevention and treatment, the translation of this knowledge into interventions has proved more difficult than anticipated (Lenfant 2003). It still needs to be determined whether the identified molecular biomarkers (genetic variant, protein and metabolite) confer effect sizes above and beyond traditional risk factors, such as obesity, smoking or family history, and thereby add to the clinical prediction. However, these high-throughput technologies are subject to rapid technological development, and their application to large-scale cohort studies has just begun. Thus, future technological advances in high-throughput methods with improved analytical sensitivity and reproducibility, enhanced bioinformatics and reduced costs will promote the widespread use of these techniques in medical research (Hu 2011), which is expected to foster notable successes in clinical practice (O'Donnell & Nabel 2011). Compared with other molecular profiling techniques, metabolomics represents the omic level closest to the phenotype because it not only provides a direct and dynamic snapshot of the current physiological status of an individual but also molecular information about metabolites that are the ultimate products of gene, mRNA and protein activity. Therefore, metabolomics is considered the omic level most capable of reflecting the non-linear impact of environmental and lifestyle factors on disease risk.

The present review highlights the application of metabolomics and other omics to open exciting avenues for biomarker discovery and individualised TRT in men. This review is based on literature search for specific investigators in the PubMed database without applying any date or language limits for the searches. Identifying markers that can be measured before treatment to individualise the type of therapy, relevant drug dose and treatment scheme plays a crucial role in the framework of personalised medicine.

Metabolomics is starting to have an impact not only on disease diagnosis and prognosis but also on drug treatment efficacy and safety monitoring. With the projected future growth of metabolomics and other omics techniques, systems biology principles are anticipated to contribute significantly to the advent of personalised medicine (Holmes *et al.* 2008b, Lewis *et al.* 2008a, Nicholson & Lindon 2008). Studies published to date have illustrated the potential for applying metabolomics to the field of andrology, and it becomes increasingly clear that systems approaches including multiple omic levels will be essential to address the outlined uncertainties in the current diagnosis and therapy of ADS (Box 1). Thus, the application of the described omics approaches in large, long-term, randomised, placebo-controlled trials of TRT in symptomatic middle-aged and elderly men with well-documented testosterone deficiency would be of great value to improve the diagnosis, therapy and monitoring of ADS in men and the clinical management of TRT.

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References

- Aardema MJ & MacGregor JT 2002 Toxicology and genetic toxicology in the new era of "toxicogenomics": impact of "-omics" technologies. *Mutation Research* **499** 13–25.
- Aderem A 2005 Systems biology: its practice and challenges. *Cell* **121** 511–513. (doi:10.1016/j.cell.2005.04.020)
- Araujo AB, O'Donnell AB, Brambilla DJ, Simpson WB, Longcope C, Matsumoto AM & McKinlay JB 2004 Prevalence and incidence of androgen deficiency in middle-aged and older men: estimates from the Massachusetts Male Aging Study. *Journal of Clinical Endocrinology and Metabolism* **89** 5920–5926. (doi:10.1210/jc.2003-031719)
- Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT & Wittert GA 2011 Endogenous testosterone and mortality in men: a systematic review and meta-analysis. *Journal of Clinical Endocrinology and Metabolism* **96** 3007–3019. (doi:10.1210/jc.2011-1137)
- Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM *et al.* 2011 Enterotypes of the human gut microbiome. *Nature* **473** 174–180. (doi:10.1038/nature09944)
- Auffray C, Chen Z & Hood L 2009 Systems medicine: the future of medical genomics and healthcare. *Genome Medicine* **1** 2. (doi:10.1186/gm2)
- Badoud F, Grata E, Boccard J, Guillaume D, Veuthey JL, Rudaz S & Saugy M 2011 Quantification of glucuronidated and sulfated steroids in human urine by ultra-high pressure liquid chromatography quadrupole time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry* **400** 503–516. (doi:10.1007/s00216-011-4779-8)
- 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)
- Barderas MG, Laborde CM, Posada M, de la Cuesta F, Zubiri I, Vivanco F & Alvarez-Llamas G 2011 Metabolomic profiling for identification of novel potential biomarkers in cardiovascular diseases. *Journal of Biomedicine & Biotechnology* **2011** 790132. (doi:10.1155/2011/790132)
- Barrett-Connor E, Khaw KT & Yen SS 1990 Endogenous sex hormone levels in older adult men with diabetes mellitus. *American Journal of Epidemiology* **132** 895–901.
- Basaria S, Coviello AD, Travison TG, Storer TW, Farwell WR, Jette AM, Eder R, Tennstedt S, Ulloor J, Zhang A *et al.* 2010 Adverse events associated with testosterone administration. *New England Journal of Medicine* **363** 109–122. (doi:10.1056/NEJMoa1000485)
- Baumgartner C, Lewis GD, Netzer M, Pfeifer B & Gerszten RE 2010 A new data mining approach for profiling and categorizing kinetic patterns of metabolic biomarkers after myocardial injury. *Bioinformatics* **26** 1745–1751. (doi:10.1093/bioinformatics/btq254)
- Bean KA & Henion JD 1997 Direct determination of anabolic steroid conjugates in human urine by combined high-performance liquid chromatography and tandem mass spectrometry. *Journal of Chromatography. B, Biomedical Sciences and Applications* **690** 65–75. (doi:10.1016/S0378-4347(96)00403-3)
- Beckwith-Hall BM, Nicholson JK, Nicholls AW, Foxall PJ, Lindon JC, Connor SC, Abdi M, Connolly J & Holmes E 1998 Nuclear magnetic resonance spectroscopic and principal components analysis investigations into biochemical effects of three model hepatotoxins. *Chemical Research in Toxicology* **11** 260–272. (doi:10.1021/cx9700679)
- Benton HP, Want E, Keun HC, Amberg A, Plumb RS, Goldfain-Blanc F, Walther B, Reily MD, Lindon JC, Holmes E *et al.* 2012 Intra- and interlaboratory reproducibility of ultra performance liquid chromatography-time-of-flight mass spectrometry for urinary metabolic profiling. *Analytical Chemistry* **84** 2424–2432. (doi:10.1021/ac203200x)
- Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS & Montori VM 2010 Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* **95** 2536–2559. (doi:10.1210/jc.2009-2354)
- Biomarkers Definitions Working Group 2001 Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical Pharmacology and Therapeutics* **69** 89–95. (doi:10.1067/mcp.2001.113989)
- Bollard ME, Holmes E, Lindon JC, Mitchell SC, Branstetter D, Zhang W & Nicholson JK 2001 Investigations into biochemical changes due to diurnal variation and estrus cycle in female rats using high-resolution (1)H NMR spectroscopy of urine and pattern recognition. *Analytical Biochemistry* **295** 194–202. (doi:10.1006/abio.2001.5211)
- Bollard ME, Stanley EG, Lindon JC, Nicholson JK & Holmes E 2005 NMR-based metabolomic approaches for evaluating physiological influences on biofluid composition. *NMR in Biomedicine* **18** 143–162. (doi:10.1002/nbm.935)
- Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligan E, Mosedale DE *et al.* 2002 Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabolomics. *Nature Medicine* **8** 1439–1444. (doi:10.1038/nm1202-802)
- Brockmoller SE, Bucher E, Muller BM, Budczies J, Hilvo M, Griffin JL, Oresic M, Kallioniemi O, Iljin K, Loibl S *et al.* 2012 Integration of metabolomics and expression of glycerol-3-phosphate acyltransferase (GPAM) in breast cancer-link to patient survival, hormone receptor status, and metabolic profiling. *Journal of Proteome Research* **11** 850–860. (doi:10.1021/pr200685r)
- Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HYK, Chen R, Miriami E, Karczewski KJ, Hariharan M, Dewey FE *et al.* 2012 Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148** 1293–1307. (doi:10.1016/j.cell.2012.02.009)
- Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, Provost JP, Le Net JL, Baker D, Walley RJ *et al.* 2006 Pharmacometabonomic phenotyping and personalized drug treatment. *Nature* **440** 1073–1077. (doi:10.1038/nature04648)

- Clayton TA, Baker D, Lindon JC, Everett JR & Nicholson JK 2009 Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *PNAS* **106** 14728–14733. (doi:10.1073/pnas.0904489106)
- Coen M, Lenz EM, Nicholson JK, Wilson ID, Pognan F & Lindon JC 2003 An integrated metabonomic investigation of acetaminophen toxicity in the mouse using NMR spectroscopy. *Chemical Research in Toxicology* **16** 295–303. (doi:10.1021/tx0256127)
- Comas I & Gagneux S 2011 A role for systems epidemiology in tuberculosis research. *Trends in Microbiology* **19** 492–500. (doi:10.1016/j.tim.2011.07.002)
- Dorgan JF, Fears TR, McMahon RP, Aronson Friedman L, Patterson BH & Greenhut SF 2002 Measurement of steroid sex hormones in serum: a comparison of radioimmunoassay and mass spectrometry. *Steroids* **67** 151–158. (doi:10.1016/S0039-128X(01)00147-7)
- Dumas ME, Wilder SP, Bihoreau MT, Barton RH, Fearnside JF, Argoud K, D'Amato L, Wallis RH, Blancher C, Keun HC, Baunsgaard D, Scott J, Sidelmann UG, Nicholson JK & Gauguier D 2007 Direct quantitative trait locus mapping of mammalian metabolic phenotypes in diabetic and normoglycemic rat models. *Nature Genetics* **39** 666–672. (doi:10.1038/ng0206)
- Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R & Griffin JL 2011 Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chemical Society Reviews* **40** 387–426. (doi:10.1039/b906712b)
- Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ & McKinlay JB 2002 Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *Journal of Clinical Endocrinology and Metabolism* **87** 589–598. (doi:10.1210/jc.87.2.589)
- Fernandez-Balsells MM, Murad MH, Lane M, Lampropoulos JF, Albuquerque F, Mullan RJ, Agrwal N, Elamin MB, Gallegos-Orozco JF, Wang AT *et al.* 2010 Clinical review 1: adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. *Journal of Clinical Endocrinology and Metabolism* **95** 2560–2575. (doi:10.1210/jc.2009-2575)
- Gavaghan CL, Holmes E, Lenz E, Wilson ID & Nicholson JK 2000 An NMR-based metabonomic approach to investigate the biochemical consequences of genetic strain differences: application to the C57BL10J and Alpk:ApfCD mouse. *FEBS Letters* **484** 169–174. (doi:10.1016/S0014-5793(00)02147-5)
- Gavaghan CL, Nicholson JK, Connor SC, Wilson ID, Wright B & Holmes E 2001 Directly coupled high-performance liquid chromatography and nuclear magnetic resonance spectroscopic with chemometric studies on metabolic variation in Sprague-Dawley rats. *Analytical Biochemistry* **291** 245–252. (doi:10.1006/abio.2000.5034)
- Gavaghan CL, Wilson ID & Nicholson JK 2002 Physiological variation in metabolic phenotyping and functional genomic studies: use of orthogonal signal correction and PLS-DA. *FEBS Letters* **530** 191–196. (doi:10.1016/S0014-5793(02)03476-2)
- Gould DC, Petty R & Jacobs HS 2000 For and against: the male menopause – does it exist? *BMJ* **320** 858–861. (doi:10.1136/bmj.320.7238.858)
- Griffin JL, Atherton H, Shockor J & Atzori L 2011 Metabolomics as a tool for cardiac research. *Nature Reviews. Cardiology* **8** 630–643. (doi:10.1038/nrcardio.2011.138)
- Haring R & Wallaschofski H 2012 Diving through the “-omics”: the case for deep phenotyping and systems epidemiology. *OMICS* **16** 231–234. (doi:10.1089/omi.2011.0108)
- Haring R, Volzke H, Felix SB, Schipf S, Dorr M, Rosskopf D, Nauck M, Schoff C & Wallaschofski H 2009 Prediction of metabolic syndrome by low serum testosterone levels in men: results from the study of health in Pomerania. *Diabetes* **58** 2027–2031. (doi:10.2337/db09-0031)
- Haring R, Ittermann T, Volzke H, Krebs A, Zygumt M, Felix SB, Grabe HJ, Nauck M & Wallaschofski H 2010 Prevalence, incidence and risk factors of testosterone deficiency in a population-based cohort of men: results from the study of health in Pomerania. *Aging Male* **13** 247–257. (doi:10.3109/13685538.2010.487553)
- Haring R, Baumeister S, Volzke H, Dorr M, Kocher T, Nauck M & Wallaschofski H 2011a Prospective inverse associations of sex hormone concentrations in men with biomarkers of inflammation and oxidative stress. *Journal of Andrology*.
- Haring R, Baumeister SE, Volzke H, Dorr M, Felix SB, Kroemer HK, Nauck M & Wallaschofski H 2011b Prospective association of low total testosterone concentrations with an adverse lipid profile and increased incident dyslipidemia. *European Journal of Cardiovascular Prevention and Rehabilitation* **18** 86–96. (doi:10.1097/HJR.0b013e32833c1a8d)
- Haring R, Ernst F, Schurmann C, Homuth G, Volker U, Volzke H, Nauck M & Wallaschofski H 2011c The androgen receptor CAG repeat polymorphism as a risk factor of low serum testosterone and its cardiometabolic effects in men. *International Journal of Andrology*. In press. (doi:10.1111/j.1365-2605.2011.01220.x)
- Haring R, Spielhagen C & Nauck M 2011d Challenges in the measurement of serum testosterone concentrations as a biomarker of men's health. *Journal of Laboratory Medicine* **35** 1–5.
- Harman SM, Metter EJ, Tobin JD, Pearson J & Blackman MR 2001 Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *Journal of Clinical Endocrinology and Metabolism* **86** 724–731. (doi:10.1210/jc.86.2.724)
- Heijne WH, Lamers RJ, van Bladeren PJ, Groten JP, van Nesselrooij JH & van Ommen B 2005 Profiles of metabolites and gene expression in rats with chemically induced hepatic necrosis. *Toxicologic Pathology* **33** 425–433. (doi:10.1080/01926230509058146)
- Heinzmann SS, Brown IJ, Chan Q, Bictash M, Dumas ME, Kochhar S, Stamler J, Holmes E, Elliott P & Nicholson JK 2010 Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption. *American Journal of Clinical Nutrition* **92** 436–443. (doi:10.3945/ajcn.2010.29672)
- Heinzmann SS, Merrifield CA, Rezzi S, Kochhar S, Lindon JC, Holmes E & Nicholson JK 2012 Stability and robustness of human metabolic phenotypes in response to sequential food challenges. *Journal of Proteome Research* **11** 643–655. (doi:10.1021/pr2005764)
- Holmes E & Nicholson JK 2007 Human metabolic phenotyping and metabolome wide association studies. *Ernst Schering Foundation Symposium Proceedings* 227–249. (doi:10.1007/2789_2008_096)
- Holmes E, Bonner FW, Sweatman BC, Lindon JC, Beddell CR, Rahr E & Nicholson JK 1992 Nuclear magnetic resonance spectroscopy and pattern recognition analysis of the biochemical processes associated with the progression of and recovery from nephrotoxic lesions in the rat induced by mercury(II) chloride and 2-bromoethanamine. *Molecular Pharmacology* **42** 922–930.
- Holmes E, Nicholls AW, Lindon JC, Ramos S, Spraul M, Neidig P, Connor SC, Connelly J, Damment SJ, Haselden J *et al.* 1998 Development of a model for classification of toxin-induced lesions using 1H NMR spectroscopy of urine combined with pattern recognition. *NMR in Biomedicine* **11** 235–244. (doi:10.1002/(SICI)1099-1492(199806/08)11:4/5<235::AID-NBM507>3.0.CO;2-V)
- Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, Ebbels T, De Iorio M, Brown IJ, Veselkov KA *et al.* 2008a Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature* **453** 396–400. (doi:10.1038/nature06882)
- Holmes E, Wilson ID & Nicholson JK 2008b Metabolic phenotyping in health and disease. *Cell* **134** 714–717. (doi:10.1016/j.cell.2008.08.026)
- Hsing AW, Stanczyk FZ, Belanger A, Schroeder P, Chang L, Falk RT & Fears TR 2007 Reproducibility of serum sex steroid assays in men by RIA and mass spectrometry. *Cancer Epidemiology, Biomarkers & Prevention* **16** 1004–1008. (doi:10.1158/1055-9965.EPI-06-0792)
- Hu FB 2011 Metabolic profiling of diabetes: from black-box epidemiology to systems epidemiology. *Clinical Chemistry* **57** 1224–1226. (doi:10.1373/clinchem.2011.167056)
- Isidori AM & Lenzi A 2007 Testosterone replacement therapy: what we know is not yet enough. *Mayo Clinic Proceedings* **82** 11–13.
- Isidori AM, Giannetta E, Gianfrilli D, Greco EA, Bonifacio V, Aversa A, Isidori A, Fabbri A & Lenzi A 2005 Effects of testosterone on sexual function in men: results of a meta-analysis. *Clinical Endocrinology* **63** 381–394. (doi:10.1111/j.1365-2265.2005.02350.x)

- Kaplan SA, Meehan AG & Shah A 2006 The age related decrease in testosterone is significantly exacerbated in obese men with the metabolic syndrome. What are the implications for the relatively high incidence of erectile dysfunction observed in these men? *Journal of Urology* **176** 1524–1527 (discussion 1527–1528). (doi:10.1016/j.juro.2006.06.003)
- Kau AL, Ahern PP, Griffin NW, Goodman AL & Gordon JI 2011 Human nutrition, the gut microbiome and the immune system. *Nature* **474** 327–336. (doi:10.1038/nature10213)
- Kaufman JM & Vermeulen A 2005 The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocrine Reviews* **26** 833–876. (doi:10.1210/er.2004-0013)
- Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, Roberts C, Cooper GJ, Kell DB & Baker PN 2010 Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. *Hypertension* **56** 741–749. (doi:10.1161/HYPERTENSIONAHA.110.157297)
- Kettunen J, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikäinen LP, Kangas AJ, Soininen P, Wurtz P, Silander K *et al.* 2012 Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nature Genetics* **44** 269–276. (doi:10.1038/ng.1073)
- Kinross JM, Alkhamisi N, Barton RH, Silk DB, Yap IK, Darzi AW, Holmes E & Nicholson JK 2011 Global metabolic phenotyping in an experimental laparotomy model of surgical trauma. *Journal of Proteome Research* **10** 277–287. (doi:10.1021/pr1003278)
- Kochhar S, Jacobs DM, Ramadan Z, Berruex F, Fuerholz A & Fay LB 2006 Probing gender-specific metabolism differences in humans by nuclear magnetic resonance-based metabolomics. *Analytical Biochemistry* **352** 274–281. (doi:10.1016/j.ab.2006.02.033)
- Kupelian V, Page ST, Araujo AB, Travison TG, Bremner WJ & McKinlay JB 2006 Low sex hormone-binding globulin, total testosterone, and symptomatic androgen deficiency are associated with development of the metabolic syndrome in nonobese men. *Journal of Clinical Endocrinology and Metabolism* **91** 843–850. (doi:10.1210/jc.2005-1326)
- Laaksonen DE, Niskanen L, Punnonen K, Nyyssonen K, Tuomainen TP, Salonen R, Rauramaa R & Salonen JT 2003 Sex hormones, inflammation and the metabolic syndrome: a population-based study. *European Journal of Endocrinology* **149** 601–608. (doi:10.1530/eje.0.1490601)
- Laaksonen DE, Niskanen L, Punnonen K, Nyyssonen K, Tuomainen TP, Valkonen VP, Salonen R & Salonen JT 2004 Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care* **27** 1036–1041. (doi:10.2337/diacare.27.5.1036)
- Lamers RJ, van Nesselrooij JH, Kraus VB, Jordan JM, Renner JB, Dragomir AD, Luta G, van der Greef J & DeGroot J 2005 Identification of an urinary metabolite profile associated with osteoarthritis. *Osteoarthritis and Cartilage* **13** 762–768. (doi:10.1016/j.joca.2005.04.005)
- Lenfant C 2003 Shattuck lecture – clinical research to clinical practice – lost in translation? *New England Journal of Medicine* **349** 868–874. (doi:10.1056/NEJMs035507)
- Lenz EM, Bright J, Wilson ID, Morgan SR & Nash AF 2003 A 1H NMR-based metabolomic study of urine and plasma samples obtained from healthy human subjects. *Journal of Pharmaceutical and Biomedical Analysis* **33** 1103–1115. (doi:10.1016/S0731-7085(03)00410-2)
- Lenz EM, Bright J, Knight R, Westwood FR, Davies D, Major H & Wilson ID 2005 Metabonomics with 1H-NMR spectroscopy and liquid chromatography–mass spectrometry applied to the investigation of metabolic changes caused by gentamicin-induced nephrotoxicity in the rat. *Biomarkers* **10** 173–187. (doi:10.1080/13547500500094034)
- Lewis GD, Asnani A & Gerszten RE 2008a Application of metabolomics to cardiovascular biomarker and pathway discovery. *Journal of the American College of Cardiology* **52** 117–123. (doi:10.1016/j.jacc.2008.03.043)
- Lewis GD, Wei R, Liu E, Yang E, Shi X, Martinovic M, Farrell L, Asnani A, Cyrille M, Ramanathan A *et al.* 2008b Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. *Journal of Clinical Investigation* **118** 3503–3512. (doi:10.1172/JCI35111)
- Lindon JC, Holmes E & Nicholson JK 2006 Metabonomics techniques and applications to pharmaceutical research & development. *Pharmaceutical Research* **23** 1075–1088. (doi:10.1007/s11095-006-0025-z)
- Lindon JC & Nicholson JK 2008 Spectroscopic and statistical techniques for information recovery in metabolomics and metabolomics. *Annual Review of Analytical Chemistry* **1** 45–69. (doi:10.1146/annurev.anchem.1.031207.113026)
- Lindon JC, Nicholson JK, Holmes E, Antti H, Bollard ME, Keun H, Beckonert O, Ebbels TM, Reilly MD, Robertson D *et al.* 2003 Contemporary issues in toxicology the role of metabolomics in toxicology and its evaluation by the COMET project. *Toxicology and Applied Pharmacology* **187** 137–146.
- Lund E & Dumeaux V 2008 Systems epidemiology in cancer. *Cancer Epidemiology, Biomarkers & Prevention* **17** 2954–2957. (doi:10.1158/1055-9965.EPI-08-0519)
- Macellan WR, Wang Y & Lusis AJ 2012 Systems-based approaches to cardiovascular disease. *Nature Reviews. Cardiology* **9** 172–184. (doi:10.1038/nrcardio.2011.208)
- Madsen R, Lundstedt T & Trygg J 2010 Chemometrics in metabolomics – a review in human disease diagnosis. *Analytica Chimica Acta* **659** 23–33. (doi:10.1016/j.aca.2009.11.042)
- Maggio M & Basaria S 2009 Welcoming low testosterone as a cardiovascular risk factor. *International Journal of Impotence Research* **21** 261–264. (doi:10.1038/ijir.2009.25)
- Makinen VP, Soininen P, Forsblom C, Parkkonen M, Ingman P, Kaski K, Groop PH & Ala-Korpela M 2008 1H NMR metabolomics approach to the disease continuum of diabetic complications and premature death. *Molecular Systems Biology* **4** 167. (doi:10.1038/msb4100205)
- Mamas M, Dunn WB, Neysey L & Goodacre R 2011 The role of metabolites and metabolomics in clinically applicable biomarkers of disease. *Archives of Toxicology* **85** 5–17. (doi:10.1007/s00204-010-0609-6)
- Mareck U, Geyer H, Opfermann G, Thevis M & Schanzer W 2008 Factors influencing the steroid profile in doping control analysis. *Journal of Mass Spectrometry* **43** 877–891. (doi:10.1002/jms.1457)
- Mayr M, Yusuf S, Weir G, Chung YL, Mayr U, Yin X, Ladrone C, Madhu B, Roberts N, De Souza A *et al.* 2008 Combined metabolomic and proteomic analysis of human atrial fibrillation. *Journal of the American College of Cardiology* **51** 585–594. (doi:10.1016/j.jacc.2007.09.055)
- McLachlan RI 2010 Certainly more guidelines than rules. *Journal of Clinical Endocrinology and Metabolism* **95** 2610–2613. (doi:10.1210/jc.2010-0838)
- Merrifield CA, Lewis M, Claus SP, Beckonert OP, Dumas ME, Duncker S, Kochhar S, Rezzi S, Lindon JC, Bailey M *et al.* 2011 A metabolic system-wide characterisation of the pig: a model for human physiology. *Molecular BioSystems* **7** 2577–2588. (doi:10.1039/c1mb05023k)
- Mittelstrass K, Ried JS, Yu Z, Krumsiek J, Gieger C, Prehn C, Roemisch-Margl W, Polonikov A, Peters A, Theis FJ *et al.* 2011 Discovery of sexual dimorphisms in metabolic and genetic biomarkers. *PLoS Genetics* **7** e1002215. (doi:10.1371/journal.pgen.1002215)
- Moolenaar SH, Engelke UF & Wevers RA 2003 Proton nuclear magnetic resonance spectroscopy of body fluids in the field of inborn errors of metabolism. *Annals of Clinical Biochemistry* **40** 16–24. (doi:10.1258/000456303321016132)
- Mooradian AD, Morley JE & Korenman SG 1987 Biological actions of androgens. *Endocrine Reviews* **8** 1–28. (doi:10.1210/edrv-8-1-1)
- Morales A, Bella AJ, Chun S, Lee J, Assimakopoulos P, Bebb R, Gottesman I, Alarie P, Dugre H & Elliott S 2010 A practical guide to diagnosis, management and treatment of testosterone deficiency for Canadian physicians. *Canadian Urological Association Journal* **4** 269–275.
- Morley JE & Perry HM III 2003 Androgen treatment of male hypogonadism in older males. *Journal of Steroid Biochemistry and Molecular Biology* **85** 367–373. (doi:10.1016/S0960-0760(03)00203-6)
- Morley JE, Kaiser FE, Perry HM III, Patrick P, Morley PM, Stauber PM, Vellas B, Baumgartner RN & Garry PJ 1997 Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. *Metabolism* **46** 410–413. (doi:10.1016/S0026-0495(97)90057-3)
- Nicholson JK & Lindon JC 2008 Systems biology: metabolomics. *Nature* **455** 1054–1056. (doi:10.1038/4551054a)

- Nicholson JK & Wilson ID 2003 Opinion: understanding 'global' systems biology: metabolomics and the continuum of metabolism. *Nature Reviews. Drug Discovery* **2** 668–676. (doi:10.1038/nrd1157)
- Nicholson JK, Linton 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, Holmes E & Elliott P 2008 The metabolome-wide association study: a new look at human disease risk factors. *Journal of Proteome Research* **7** 3637–3638. (doi:10.1021/pr8005099)
- Nicholson G, Rantalainen M, Maher AD, Li JV, Malmolin D, Ahmadi KR, Faber JH, Hallgrimsdottir IB, Barrett A, Toft H *et al.* 2011 Human metabolic profiles are stably controlled by genetic and environmental variation. *Molecular Systems Biology* **7** 525. (doi:10.1038/msb.2011.57)
- Nieschlag E & Behre H 2004 Clinical uses of testosterone in hypogonadism and other conditions. In *Testosterone: Action, Deficiency, Substitution*, pp 375–404. Eds E Nieschlag & H Behre. Cambridge: University Press.
- Nordstrom A & Lewensohn R 2010 Metabolomics: moving to the clinic. *Journal of Neuroimmune Pharmacology* **5** 4–17. (doi:10.1007/s11481-009-9156-4)
- O'Connell MD, Roberts SA, Srinivas-Shankar U, Tajar A, Connolly MJ, Adams JE, Oldham JA & Wu FC 2011 Do the effects of testosterone on muscle strength, physical function, body composition, and quality of life persist six months after treatment in intermediate-frail and frail elderly men? *Journal of Clinical Endocrinology and Metabolism* **96** 454–458. (doi:10.1210/jc.2010-1167)
- O'Donnell CJ & Nabel EG 2011 Genomics of cardiovascular disease. *New England Journal of Medicine* **365** 2098–2109. (doi:10.1056/NEJMr1105239)
- Ohlsson C, Wallaschowski H, Lunetta KL, Stolk L, Perry JR, Koster A, Petersen AK, Eriksson J, Lehtimäki T, Huhtaniemi IT *et al.* 2011 Genetic determinants of serum testosterone concentrations in men. *PLoS Genetics* **7** e1002313. (doi:10.1371/journal.pgen.1002313)
- O'Sullivan A, Gibney MJ & Brennan L 2011 Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. *American Journal of Clinical Nutrition* **93** 314–321. (doi:10.3945/ajcn.110.000950)
- Perheentupa A & Huhtaniemi I 2007 Does the andropause exist? *Nature Clinical Practice. Endocrinology & Metabolism* **3** 670–671. (doi:10.1038/ncpendmet0623)
- Psihogios NG, Gazi IF, Elisaf MS, Seferiadis KI & Bairaktari ET 2008 Gender-related and age-related urinalysis of healthy subjects by NMR-based metabolomics. *NMR in Biomedicine* **21** 195–207. (doi:10.1002/nbm.1176)
- Putluri N, Shojia A, Vasu VT, Nalluri S, Vared SK, Putluri V, Vivekanandan-Giri A, Byun J, Pennathur S, Sana TR *et al.* 2011 Metabolomic profiling reveals a role for androgen in activating amino acid metabolism and methylation in prostate cancer cells. *PLoS ONE* **6** e21417. (doi:10.1371/journal.pone.0021417)
- Rantalainen M, Cloarec O, Beckonert O, Wilson ID, Jackson D, Tonge R, Rowlinson R, Rayner S, Nickson J, Wilkison RW, Mills JD, Trygg J, Nicholson JK & Holmes E 2006 Statistically integrated metabolomic-proteomic studies on human prostate cancer xenograft model in mice. *Journal of Proteome Research* **5** 2642–2655. (doi:10.1021/pr060124w)
- Rezzi S, Ramadan Z, Fay LB & Kochhar S 2007 Nutritional metabolomics: applications and perspectives. *Journal of Proteome Research* **6** 513–525. (doi:10.1021/pr060522z)
- Roddam AW, Allen NE, Appleby P & Key TJ 2008 Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *Journal of the National Cancer Institute* **100** 170–183. (doi:10.1093/jnci/djm323)
- Rommerts FFG 2004 Testosterone: an overview of biosynthesis, transport, metabolism and non-genomic actions. In *Testosterone: Action, Deficiency, Substitution*, pp 1–38. Eds E Nieschlag & H Behre. Cambridge: University Press.
- Ruige JB, Mahmoud AM, De Bacquer D & Kaufman JM 2010 Endogenous testosterone and cardiovascular disease in healthy men: a meta-analysis. *Heart* **97** 870–875. (doi:10.1136/hrt.2010.210757)
- Saad F, Aversa A, Isidori AM, Zafalon L, Zitzmann M & Gooren L 2011 Onset of effects of testosterone treatment and time span until maximum effects are achieved. *European Journal of Endocrinology* **165** 675–685. (doi:10.1530/EJE-11-0221)
- Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, Wiegand R, Berriz GF, Roth FP & Gerszten RE 2005 Metabolomic identification of novel biomarkers of myocardial ischemia. *Circulation* **112** 3868–3875. (doi:10.1161/CIRCULATIONAHA.105.569137)
- Sadovsky R, Dhindsa S & Margo K 2007 Testosterone deficiency: which patients should you screen and treat? *Journal of Family Practice* **56** S1–S20 (quiz S21–23).
- Schiff P, Haring R, Friedrich N, Nauck M, Lau K, Alte D, Stang A, Volzke H & Wallaschowski H 2011 Low total testosterone is associated with increased risk of incident type 2 diabetes mellitus in men: results from the Study of Health in Pomerania (SHIP). *Aging Male* **14** 168–175. (doi:10.3109/13685538.2010.524955)
- Seidman SN 2006 Normative hypogonadism and depression: does 'andropause' exist? *International Journal of Impotence Research* **18** 415–422. (doi:10.1038/sj.ijir.3901443)
- Shah SH, Bain JR, Muehlbauer MJ, Stevens RD, Crosslin DR, Haynes C, Dungan J, Newby LK, Hauser ER, Ginsburg GS *et al.* 2010 Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. *Circulation. Cardiovascular Genetics* **3** 207–214. (doi:10.1161/CIRCGENETICS.109.852814)
- 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)
- Shulaev V 2006 Metabolomics technology and bioinformatics. *Briefings in Bioinformatics* **7** 128–139. (doi:10.1093/bib/bbl012)
- Slupsky CM, Rankin KN, Wagner J, Fu H, Chang D, Weljie AM, Saude EJ, Lix B, Adamko DJ, Shah S *et al.* 2007 Investigations of the effects of gender, diurnal variation, and age in human urinary metabolomic profiles. *Analytical Chemistry* **79** 6995–7004. (doi:10.1021/ac0708588)
- Snyder PJ 2008 Decreasing testosterone with increasing age: more factors, more questions. *Journal of Clinical Endocrinology and Metabolism* **93** 2477–2478. (doi:10.1210/jc.2008-0922)
- Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y *et al.* 2009 Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* **457** 910–914. (doi:10.1038/nature07762)
- Stanczyk FZ & Clarke NJ 2010 Advantages and challenges of mass spectrometry assays for steroid hormones. *Journal of Steroid Biochemistry and Molecular Biology* **121** 491–495. (doi:10.1016/j.jsbmb.2010.05.001)
- Strahm E, Kohler I, Rudaz S, Martel S, Carrupt PA, Veuthey JL, Saugy M & Saudan C 2008 Isolation and quantification by high-performance liquid chromatography-ion-trap mass spectrometry of androgen sulfoconjugates in human urine. *Journal of Chromatography. A* **1196–1197** 153–160. (doi:10.1016/j.chroma.2008.04.066)
- Suhre K, Shin SY, Petersen AK, Mohny RP, Meredith D, Wagele B, Altmaier E, Deloukas P, Erdmann J, Grundberg E *et al.* 2011 Human metabolic individuality in biomedical and pharmaceutical research. *Nature* **477** 54–60. (doi:10.1038/nature10354)
- Svartberg J, von Muhlen D, Sundsfjord J & Jorde R 2004 Waist circumference and testosterone levels in community dwelling men. The Tromso study. *European Journal of Epidemiology* **19** 657–663. (doi:10.1023/B:EJEP.0000036809.30558.8f)
- Taieb J, Mathian B, Millot F, Patricot MC, Mathieu E, Queyrel N, Lacroix I, Somma-Delpero C & Boudou P 2003 Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. *Clinical Chemistry* **49** 1381–1395. (doi:10.1373/49.8.1381)
- Thienpont LM, Van Uytanghe K, Blincko S, Ramsay CS, Xie H, Doss RC, Keevil BG, Owen LJ, Rockwood AL, Kushnir MM *et al.* 2008 State-of-the-art of serum testosterone measurement by isotope dilution-liquid chromatography-tandem mass spectrometry. *Clinical Chemistry* **54** 1290–1297. (doi:10.1373/clinchem.2008.105841)

- Torkler S, Wallaschofski H, Baumeister SE, Volzke H, Dorr M, Felix S, Rettig R, Nauck M & Haring R 2011 Inverse association between total testosterone concentrations, incident hypertension and blood pressure. *Aging Male* **14** 176–182. (doi:10.3109/13685538.2010.529194)
- Traish AM, Miner MM, Morgentaler A & Zitzmann M 2011 Testosterone deficiency. *American Journal of Medicine* **124** 578–587. (doi:10.1016/j.amjmed.2010.12.027)
- Travison TG, Araujo AB, Kupelian V, O'Donnell AB & McKinlay JB 2007 The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *Journal of Clinical Endocrinology and Metabolism* **92** 549–555. (doi:10.1210/jc.2006-1859)
- Vasan RS 2006 Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation* **113** 2335–2362. (doi:10.1161/CIRCULATIONAHA.104.482570)
- Vermeulen A, Kaufman JM & Giagulli VA 1996 Influence of some biological indexes on sex hormone-binding globulin and androgen levels in aging or obese males. *Journal of Clinical Endocrinology and Metabolism* **81** 1821–1826. (doi:10.1210/jc.81.5.1821)
- Vesper HW, Bhasin S, Wang C, Tai SS, Dodge LA, Singh RJ, Nelson J, Ohorodnik S, Clarke NJ, Salameh WA *et al.* 2009 Interlaboratory comparison study of serum total testosterone [corrected] measurements performed by mass spectrometry methods. *Steroids* **74** 498–503. (doi:10.1016/j.steroids.2009.01.004)
- Vikan T, Schirmer H, Njolstad I & Svartberg J 2010 Low testosterone and sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. *European Journal of Endocrinology* **162** 747–754. (doi:10.1530/EJE-09-0943)
- Wang C, Catlin DH, Demers LM, Starcevic B & Swerdloff RS 2004 Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography–tandem mass spectrometry. *Journal of Clinical Endocrinology and Metabolism* **89** 534–543. (doi:10.1210/jc.2003-031287)
- Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, Kaufman JM, Legros JJ, Lunenfeld B, Morales A *et al.* 2009a Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA, and ASA recommendations. *Journal of Andrology* **30** 1–9. (doi:10.2164/jandrol.108.006486)
- Wang Z, Tang WH, Cho L, Brennan DM & Hazen SL 2009b Targeted metabolomic evaluation of arginine methylation and cardiovascular risks: potential mechanisms beyond nitric oxide synthase inhibition. *Arteriosclerosis, Thrombosis, and Vascular Biology* **29** 1383–1391. (doi:10.1161/ATVBAHA.109.185645)
- Wang HP, Liang YJ, Zhang Q, Long DX, Li W, Li L, Yang L, Yan XZ & Wu YJ 2011a Changes in metabolic profiles of urine from rats following chronic exposure to anticholinesterase pesticides. *Pesticide Biochemistry and Physiology* **101** 232–239. (doi:10.1016/j.pestbp.2011.09.011)
- Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C *et al.* 2011b Metabolite profiles and the risk of developing diabetes. *Nature Medicine* **17** 448–453. (doi:10.1038/nm.2307)
- Wheeler MJ & Barnes SC 2008 Measurement of testosterone in the diagnosis of hypogonadism in the ageing male. *Clinical Endocrinology* **69** 515–525. (doi:10.1111/j.1365-2265.2008.03325.x)
- Winnike JH, Li Z, Wright FA, Macdonald JM, O'Connell TM & Watkins PB 2010 Use of pharmacometabonomics for early prediction of acetaminophen-induced hepatotoxicity in humans. *Clinical Pharmacology and Therapeutics* **88** 45–51. (doi:10.1038/clpt.2009.240)
- Wopereis S, Rubingh CM, van Erk MJ, Verheij ER, van Vliet T, Cnubben NH, Smilde AK, van der Greef J, van Ommen B & Hendriks HF 2009 Metabolic profiling of the response to an oral glucose tolerance test detects subtle metabolic changes. *PLoS ONE* **4** e4525. (doi:10.1371/journal.pone.0004525)
- Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A *et al.* 2008 Hypothalamic–pituitary–testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. *Journal of Clinical Endocrinology and Metabolism* **93** 2737–2745. (doi:10.1210/jc.2007-1972)
- Wu FC, Tajar A, Beynon JM, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva FF, Forti G *et al.* 2010 Identification of late-onset hypogonadism in middle-aged and elderly men. *New England Journal of Medicine* **363** 123–135. (doi:10.1056/NEJMoa0911101)
- Yap IK, Angley M, Veselkov KA, Holmes E, Lindon JC & Nicholson JK 2010 Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. *Journal of Proteome Research* **9** 2996–3004. (doi:10.1021/pr901188e)
- Zmuda JM, Cauley JA, Kriska A, Glynn NW, Gutai JP & Kuller LH 1997 Longitudinal relation between endogenous testosterone and cardiovascular disease risk factors in middle-aged men. A 13-year follow-up of former Multiple Risk Factor Intervention Trial participants. *American Journal of Epidemiology* **146** 609–617. (doi:10.1093/oxfordjournals.aje.a009326)

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