REVIEW

Empowering thyroid hormone research in human subjects using OMICs technologies

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Abstract

OMICs subsume different physiological layers including the genome, transcriptome, proteome and metabolome. Recent advances in analytical techniques allow for the exhaustive determination of biomolecules in all OMICs levels from less invasive human specimens such as blood and urine. Investigating OMICs in deeply characterized population-based or experimental studies has led to seminal improvement of our understanding of genetic determinants of thyroid function, identified putative thyroid hormone target genes and thyroid hormone-induced shifts in the plasma protein and metabolite content. Consequently, plasma biomolecules have been suggested as surrogates of tissue-specific action of thyroid hormones. This review provides a brief introduction to OMICs in thyroid research with a particular focus on metabolomics studies in humans elucidating the important role of thyroid hormones for whole body metabolism in adults.

Key Words

- ▶ free thyroxine
- ▶ thyrotropin
- ▶ metabolomics
- ▶ proteomics
- ▶ machine learning

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Introduction

Thyroid hormone physiology and mode of action

Thyroxine (T₄) and 3,3',5-triiodo-L-thyronine (T₃), the main secretion products of the thyroid gland, are critical for proper development and function of almost all tissues (Yen 2001). Serum concentrations of T₄ and T₃ are controlled by a classical endocrine feedback loop. Thyrotropin (TSH)-releasing hormone is the hypothalamic stimulus for the pituitary, which subsequently releases TSH stimulating the thyroid gland (the hypothalamic–pituitary–thyroid (HPT) axis). An increase in circulating thyroid hormones (THs) in turn causes suppression of synthesis and release of both TRH and TSH, which in turn suppress TH secretion from the thyroid. Due to their highly hydrophobic nature, only a minor part of TH, considered the bio-active pool, circulates not bound to proteins (e.g. <0.05% of T₄; free T₄ (FT₄)) (Yen 2001, Visser 2013).

One of the most important findings in TH research during the last decades was the identification of specific transporters, in particular, monocarboxylate transporter 8 (MCT8), mediating the cellular uptake of TH (Visser 2013). Although T₄ is more abundant in blood, T₃ is considered the major metabolic hormone due to its higher affinity for nuclear receptors and is intracellularly generated from T₄ by deiodination. Besides activation, further deiodination, yielding 3,3'-T₂ or 3,5-T₂, is predominantly considered a mode of inactivation. However, at least for 3,5-T₂ independent effects complementing those of T₃ have been reported (for review see Moreno *et al.* 2017). Briefly, treatment with 3,5-T₂ attenuated or even prevented adverse consequences of a high-fat diet, e.g. insulin resistance (de Lange *et al.* 2011, Moreno *et al.* 2011) or hepatic steatosis (Lombardi *et al.* 2009, Mollica *et al.* 2009, Grasselli *et al.* 2012, 2014) in rats.

Note, that the absence of thyrotoxic side effects of 3,5-T₂ treatment could not be replicated in other animal models (Jonas *et al.* 2015, Lietzow *et al.* 2016). In summary, apart from secretion from the thyroid gland tissue-specific action of T₃ is achieved by specific expression patterns of the deiodinase-encoding genes (Bianco 2011).

Human metabolism is crucially dependent on adequate TH action, among others being integral for lipid and glucose metabolism or thermoregulation. TH mediates their effects by different modes of action and with reference to Flamant and colleagues can be grouped as follows (Flamant *et al.* 2017): (1) Binding to two different nuclear TH receptors (TRs) encoded by the genes *THRA* and *THRB* both being bound to a TH-responsive element (TRE) on a DNA strand. (2) Binding to TRs without consecutive binding to a TRE but tethering to other transcription factors. (3) Binding to TRs without subsequent DNA binding but initiation of intracellular signal cascades involving PI3 kinases and (4) TR-independent effects, e.g. *via* binding to plasma membrane receptors like $\alpha\beta$ 3 integrins.

Thyroid disorders

Thyroid disorders are among the most common diseases of the endocrine system. Apart from clinical symptoms, biochemical tests, i.e. determination of TSH and FT₄ in blood represent the most widely used screening tool for a suspected thyroid disorder and monitoring of treatment (Jonklaas *et al.* 2014, Ross *et al.* 2016). Hypothyroidism, a state of inadequately low thyroid function, is diagnosed by an elevation of TSH in combination with low serum concentrations of FT₄. Hyperthyroidism, on the other hand, is characterized by low TSH and high FT₄ serum concentrations. Clinical symptoms, such as hyperactivity or anxiety in case of hyperthyroidism as well as cold intolerance, fatigue or bradycardia in case of hypothyroidism, are often too unspecific to contribute to the diagnosis of thyroid disorders. This holds true in particular for subclinical alterations, e.g. suppressed/high TSH and unremarkable FT₄ (Franklyn & Boelaert 2012). Even the biochemical diagnosis is hampered by a number of drawbacks. Serum TSH has a broad reference range in the general population, which is in stark contrast to the tight individual set point of TH regulation (Andersen *et al.* 2002). A number of studies associated adverse clinical outcomes, especially with respect to the cardiovascular system and bone metabolism, with alterations of TSH or FT₄ still within the reference range (for review see Biondi & Cooper 2008). Interestingly, over-treatment of

hypothyroidism is the most common exogenous cause of (subclinical) hyperthyroidism (Biondi & Cooper 2008). This emphasizes the need for more adequate instruments in diagnosis and treatment of thyroid disorders. Additionally, a number of rare clinical conditions aggravate the interpretation of thyroid function tests (Gurnell *et al.* 2011). Mutations in *THRB*, e.g. cause loss of function of the TR β (resistance to THs β (RTH β)) and, hence, impede the negative feedback regulation of THs on the HPT axis, in turn resulting in elevated concentrations of both TSH and FT₄ (Gurnell *et al.* 2011).

OMICs techniques as translational and explorative tools

Most of the recent advances in TH research are based on cell culture, tissue or animal models or stem from studies limited to specific hypotheses in humans. Approaches for the comprehensive, hypothesis-free characterization of metabolic effects of classical and non-classical TH in human are urgently needed. OMICs technologies hold great promise in this regard. Advances in technology now allow for the exhaustive determination of biomolecules in a variety of physiological levels, comprising but not limited to the genome, the transcriptome, the proteome or the metabolome (Fig. 1).

A main advantage of OMICs techniques is their unbiased approach. Comprehensive analyses are frequently performed with genome- or metabolome-wide coverage (GWAS and MWAS, respectively). They pave the way for new discoveries including genetic determinants of TH action and regulation, identification of TH target genes or more in-depth characterization of molecular alterations related to TH effects. Findings from OMICs studies are of clinical value as they aid in constructing the needed diagnostic tools for uncertain thyroid disorders, e.g. in case of monogenetic causes (RTH β) or to monitor adequate hormone replacement therapy by keeping track of peripheral adaptations to the altered thyroid state.

Genomics

Apart from few Mendelian disorders, the genomic architecture of thyroid function has a polygenic basis. Many single-nucleotide variants (SNVs) have been associated with thyroid function. The interested reader is referred to an excellent review by Medici and colleagues for details (Medici *et al.* 2015). In the following, only the

Figure 1

Simplified sketch of the different OMICs layers and their connections. Starting with the genome, transcription results in mRNA molecules that can be translated by the protein biosynthesis apparatus. Synthesized protein enzymes may undergo additional post-translational modifications and are then able to catalyze metabolic reactions. This ability of enzymes determines the dynamics of the metabolome. Alterations in metabolite levels, e.g. resulting from disturbed energy metabolism, may cause or reflect disease. Note that interactions are possible not only within (dashed lines), but also between different layers (solid lines).

most important findings about the polygenic basis of thyroid disorders are summarized.

In total, 37 genomic loci have been identified which either increase the risk for thyroid disorders or affect parameters of thyroid function. Such loci harbor genes encoding enzymes directly involved in THs metabolism, e.g. *DIO1* or transport, e.g. *MCT8*, transcription factors, e.g. *FOXE1*, and growth factors crucial for thyroid development, as well as proteins related to signaling along the HPT axis, e.g. *TSHR*. Based on SNVs at the *DIO1* and *DIO2* loci, several studies on clinically relevant phenotypes including bone and neurocognitive disorders were performed. Notably, these studies showed associations independent of circulating TH (Medici et al. 2015).

GWAS investigating hypothyroidism pinpointed several genes previously implicated in autoimmune disorders, e.g. the *HLA* class I region, *PTPN22*, *SH2B3* or *VAV3*. Similar holds true for GWAS results on hyperthyroidism including *Tg*, *GPR174-ITM2A*, the *C1QTNF6-RAC2* locus, *SLAMF6* or the 6q27 and 14q32.2 loci. These results were confirmed by TPO antibody measurements (Medici et al. 2014). Given the frequent autoimmune origin of both disorders, these results are supportive of the current state of knowledge but did not lead to seminal new discoveries. Nevertheless, due to their hypothesis-free character, GWAS also allow for unexpected, novel findings. Among the loci associated with hyperthyroidism, a variant within the

ABO locus has been identified, whose exact role in thyroid autoimmunity or hyperthyroidism is yet to be elucidated. One of the most strongly associated loci is *PDE8B* encoding a phosphodiesterase involved in the degradation of cAMP, which is crucial for the TSH-induced signal transduction in thyrocytes. A number of loci could not be related to pre-existing knowledge of thyroid function, e.g. *AADAT*, *MAF* or *MBIP*, and further experimental efforts or integration of other OMICs layers are required to uncover their role.

Despite the effect sizes of SNVs being very small, explaining typically less than one percent of the variation in the phenotype of interest, the cumulative effect of many associated SNVs reaches up to 20% explained variance (Taylor et al. 2015). This reflects the polygenic basis of thyroid disorders and may explain the huge inter-individual variation of TSH and FT_4 throughout the general population compared to tight changes within each individual.

In summary, GWAS empower researchers to pinpoint the most promising candidate genetic variants to be investigated in follow-up studies.

Transcriptomics

Despite the strong hurdles to obtain human tissue biopsies, non-targeted gene expression studies revealed a strong

tissue-depending gene expression pattern following TH treatment using array-based techniques in muscle biopsies (Clement *et al.* 2002, Visser *et al.* 2009), adipocyte explants (Viguerie *et al.* 2002) or fibroblasts (Moeller *et al.* 2005), and more recently, whole blood samples from athyroid patients analyzed by next-generation sequencing (Massolt *et al.* 2017) (Table 1).

Clement *et al.* (2002) were the first to analyze gene expression in skeletal muscles from healthy human males treated with T₃ for 14 days. In line with the known role of TH to induce gene transcription, 381 genes were upregulated and only two were downregulated. Out of these, a significant amount is critical for proper function of skeletal muscle. The enrichment of genes belonging

Table 1 Transcriptomics studies using human specimens.

Author (year)	Model	Number of genes	Functional categories of genes	Remarks
Clement <i>et al.</i> (2002)	Muscles biopsies from five healthy men treated 14 days with 75 µg T ₃	393 (381 upregulated; 2 downregulated)	Transcriptional control (up) Protein synthesis and catabolism (ubiquitin/proteasome pathway) (up) Glycogen synthesis and glucose utilization (up) Signal transduction (up) Mitochondrial energy metabolism (respiratory chain) (up) Cytoskeleton (up)	Highlighted the important role of TH in the regulation of genes central for skeletal muscle cells
Viguerie <i>et al.</i> (2002)	Human subcutaneous adipose tissue explants were treated with T ₃	19 (13 upregulated; 6 downregulated)	Signal transduction (down) Lipogenesis (down) Inflammatory response (up)	Upregulation of β2-adrenergic receptors mediates the lipolytic effects of TH through enhanced catecholamine sensitivity
Moeller <i>et al.</i> (2005)	T ₃ treatment of cultured fibroblasts from two euthyroid subjects and two RTHβ patients	96 (91 upregulated; 5 downregulated)	Performed no enrichment analyses	Identified several novel target genes, e.g. <i>HIF1A</i> and <i>BTEB1</i> (up) or <i>FGF7</i> (down); downregulated genes remained responsive even in RTHβ cells
Visser <i>et al.</i> (2009)	Muscle biopsies from ten athyroid patients on and off (4 weeks) L-T ₄ treatment	607 (349 upregulated; 258 downregulated)	Monocarboxylic acid transport (up) Mitochondrial transport (up) Energy reserve metabolism (up) Organic acid transport (up) Carbohydrate metabolism (up) Lipid metabolism (up) Phosphate metabolism (up) Glycogen synthesis (up) Regulation of cell growth* (down)	Identified microRNA pair miR-206/miR-133b as putative downstream mediators of suppressive TH-action; *downregulated genes included among others those inhibiting cell growth
Visser <i>et al.</i> (2010)	Fibroblasts from MCT8-deficient patients and controls	1617 (526 upregulated; 1091 downregulated)	Cell adhesion Actin cytoskeleton organization Nervous system development	Only subtle effects of T ₃ treatment on native gene expression differences between patient and controls; related findings to <i>MCT8</i> co-expressed genes in human brain tissues (spatial and temporal) and identified <i>THRA2</i> as important link to patient results
Massolt <i>et al.</i> (2017)	Whole blood samples from eight athyroid patients on and off L-T ₄ treatment (4 weeks)	486 (370 upregulated; 116 downregulated)	Translational elongation (down) Hemostasis (including platelets) (up)	Identified a downregulated cluster of snoRNAs

BTEB1, basic transcription element-binding protein 1; FGF7, fibroblast growth factor 7; *HIF1A*, hypoxia-inducible factor 1-alpha; L-T₄, levothyroxine; MCT8, monocarboxylate transporter 8; RTHβ, resistance to thyroid hormone β; T₃, triiodothyronine; TH, thyroid hormones; *THRA2*, thyroid hormone receptor α 2 (alternative splicing variant).

to the ubiquitin/proteasome pathway responsible for the non-lysosomal degradation of intracellular proteins supports the pronounced proteolysis observed under thyrotoxic conditions (Riis *et al.* 2008). Further, T_3 treatment-induced transcription of genes controlling skeletal muscle metabolism, i.e. being central for glycogen synthesis (e.g. glycogen-branching enzyme), the respiratory apparatus (e.g. NADH:ubiquinone oxidoreductase) or the tricarboxylic acid (TCA) cycle (pyruvate dehydrogenase) and several transcription factors such as transcription elongation factor B. Increased abundances of several genes belonging to adhesions complexes were interpreted as T_3 -induced remodeling of skeletal muscle cells.

Complementary to Clement and coworkers, Visser *et al.* (2009) identified 607 differentially expressed genes (DEGs) in muscle biopsies from athyroid patients on and off levothyroxine ($L-T_4$) treatment. Notably, only 7% of the genes were overlapping with the study by Clement *et al.* Despite using the same tissue (vastus lateralis), the most likely explanations for the missing overlap arises from different array technologies used, covering ~24K probes (Clement *et al.* 2002) vs ~55K probes (Visser *et al.* 2009), and the different conditions of the participants (experimental thyrotoxicosis (Clement *et al.* 2002) vs withdrawal of $L-T_4$ in athyroid patients). A significant amount of the identified DEGs (40%) was downregulated under euthyroid conditions, emphasizing the important role of negative TH regulation of transcription. Many DEGs were again involved in energy and fuel metabolism while 43 were reported in the context of TH action for the first time. It is worth noting that even a number of TH targets known from animal models, e.g. *ME1*-encoding malic enzyme, showed no differential expression. In other words, the authors highlighted the discordance between *in vitro* or animal studies and the human situation. A completely novel finding was a strong downregulation of the noncoding microRNA pair miR-206/miR-133b. MicroRNAs are important post-transcriptional regulators of gene expression (He & Hannon 2004). They mostly exert negative regulation and indeed a significant overlap between predicted miR-133b targets and downregulated DEGs was found. In a later study (Massolt *et al.* 2017), the authors investigated whole blood gene expression. Although they found only little overlap with their previous work (attributed to the specific role of TH in hemostasis), they identified a novel cluster of snoRNAs that were downregulated in response to THs. Hence, non-protein-coding RNAs

may provide a new layer of regulation of metabolic TH actions in target tissues.

Tissue-specific gene expression patterns following TH treatment were further observed in human adipocyte explants (Viguerie *et al.* 2002), cultured fibroblast (Moeller *et al.* 2005) and circulating whole blood (Massolt *et al.* 2017). Briefly, T_3 treatment of human adipocyte explants changed the expression of 19 genes (13 upregulated and 6 downregulated) (Viguerie *et al.* 2002). Importantly, the study confirmed the indirect effect of TH on lipolysis through upregulation of β_2 -adrenergic receptors and hence increased sensitivity to catecholamines. The lipolytic character of the T_3 -treated adipocytes was further emphasized by downregulation of the sterol regulatory element-binding protein-1c, a central transcription factor for lipogenic enzymes. Moeller *et al.* (2005) investigated a $TR\beta$ -dependent effect of T_3 treatment in human skin fibroblasts. None of the 91 positively regulated genes in fibroblasts from healthy donors was differentially expressed in T_3 -treated fibroblasts derived from RTH β patients. In contrast, the suppressive effect of T_3 treatment, e.g. on *FGF7* and *ADH1B*, remained unaffected in RTH β -derived fibroblasts.

Transcriptional action of TH requires their cellular uptake by, among others, MCT8 (Visser 2013). Consequently, gene expression profiles in fibroblast obtained from MCT8-deficient patients strongly differed from control samples (Table 1) in particular strongly exceeding the effect of T_3 treatment (Visser *et al.* 2010). The authors compared DEGs from their own study with freely accessible whole-genome expression data from human brain tissues searching for MCT8-coexpressing genes. In doing so, they demonstrated how genome-wide expression data from patient-derived cells related to the human brain transcriptome could be employed to improve the understanding of this disease (Visser *et al.* 2010).

In summary, gene expression or transcriptomic studies in humans suggest a pleiotropic panel of genes putatively regulated by TH and the tissue-dependent effects clearly indicate the need to monitor TH replacement therapy on a more detailed level than in current clinical practice. Further, crude comparison of TH-induced gene expression patterns between human and *in vitro* or animal studies with respect to skeletal muscle indicates a discordance emphasizing the desperate need for human studies. The latter are of particular importance as intensively studied tissues in rodent models, e.g. the liver (Feng *et al.* 2000, Flores-Morales *et al.* 2002), lack human equivalents.

Proteomics

The long half-life of proteins and their tight coupling to pathophysiology makes them promising candidates for biomarkers. Consider, e.g. the determination of TSH to monitor adequate secretion of TH. Despite the benefits of plasma proteomics, its application in biomarker research in general and thyroid research in particular is still limited (Geyer *et al.* 2017, Alfadda *et al.* 2018). The major challenge in plasma proteomics is the great dynamic range covering about ten orders of magnitude from highly abundant albumin (~0.6 mol/L) to low-abundant TSH (~5 pmol/L) (Hortin & Sviridov 2010). Recent advances in mass spectrometry (MS) have made it possible to profile 100s to 1000s of proteins at once (see Geyer *et al.* 2017 for a comprehensive review).

One study used non-targeted MS-based plasma proteomics analyses in a human model of thyrotoxicosis ($N=16$) to follow the shifts in the plasma proteome during a long-term (8 weeks) L-T₄ treatment (Engelmann *et al.* 2015, Pietzner *et al.* 2017). Remarkably, no subjective clinical symptoms were reported by the volunteers (Gottlich *et al.* 2015). However, we identified three protein signatures namely decreased abundance of apolipoproteins (APOD, APOB and APOC-III) as well as increased abundances of proteins related to the coagulation cascade and the complement system.

Briefly, the drop in apolipoproteins can be explained by a TH-dependent increase in the expression of the genes encoding the LDL and HDL receptors since the former are components either of LDL (APOB; Dominiczak & Caslake 2011) or HDL (APOD and APOC-III; Dominiczak & Caslake 2011) particles.

Concordant with a hypercoagulable state frequently observed in hyperthyroid patients (for review see Stuijver *et al.* 2012), there was an increased abundance of coagulation factors V, II, XI and XIII already after 4 weeks of L-T₄ treatment (Engelmann *et al.* 2015). This prothrombotic state seemed to rely on signaling through TR β (Elbers *et al.* 2016), as there was no increase in coagulation factors in RTH β patients.

Previous studies reported contradictory behavior of the complement system in response to TH concentration changes (Yu *et al.* 2006, Zhang *et al.* 2008, Jafarzadeh *et al.* 2010, Potlukova *et al.* 2010, Hooper *et al.* 2012). For the first time, detailed coherent data about the increased abundance of multiple proteins related to the complement system has been presented (Pietzner *et al.* 2017). However, a comparable study (Hooper *et al.* 2012) observed no alterations in serum C3 levels (and coagulation factors)

after 2 weeks of L-T₄ application. Moreover, endogenous hypothyroidism has been characterized by increased abundance of complement proteins using a plasma proteomic approach (Alfadda *et al.* 2018). Hence, this seems to be a mid- to long-term effect with a U-shaped association with respect to the thyroid state.

Metabolomics

THs affect diverse aspects of human metabolism and metabolomics techniques are ideally suited to map and connect these effects in a hypothesis-free manner. Figure 2 provides a sketch summarizing important findings from metabolomics studies with respect to TH effects.

A very heterogeneous set of small molecules is summarized under the term metabolome. The metabolome includes highly abundant species like carbohydrates, lipids or amino acids but also less abundant molecules like organic acids. They originate either from endogenous metabolism, exogenously, e.g. from nutrition, medication or life style (e.g. smoking) as well as from microbiota. They fulfill diverse functions, ranging from signal transduction to energy fuels (Psychogios *et al.* 2011, Bouatra *et al.* 2013). In the context of OMICs technologies, metabolomics can be regarded closest to the phenotype of interest reflecting complex interactions between genetics, environment, lifestyle or even medical treatment in so-called 'metabotypes' (Holmes *et al.* 2008b). In other words, metabotypes could be seen as intermediate or subclinical phenotypes enabling identification of subjects at high risk for metabolic diseases with still undetectable shifts in classical clinical parameters. Consider, e.g. the accumulation of branched-chain amino acids in plasma of incident type 2 diabetes patients (Wang *et al.* 2011). Metabolomics is not only suited to improve diagnostics. The identification of novel (patho)physiological mechanisms, like the role of formate in the progression of hypertension (Holmes *et al.* 2008a), and the extension of the knowledge about the metabolic implication of endogenous modulators like TH provide key aspects of basic medical research.

Methodological considerations

The complex structure of the metabolome poses a tremendous challenge. Several spectroscopic techniques are employed to provide a comprehensive readout of the metabolome. The two most important being ¹H-NMR spectroscopy and MS mostly coupled with either

Figure 2

Simplified sketch summarizing the most prominent findings from metabolomics studies in the context of TH research. Metabolites marked in bold were significantly associated in one of the studies listed in 'Metabolomics' section. Enzymes that are transcriptionally regulated by thyroid hormones are depicted in yellow. Line types indicated either a positive or inverse relation with serum thyroxine. AA, amino acid; ADRB2, β_2 -adrenergic receptor; Apo, apolipoprotein; CPT-1/2, carnitine palmitoyltransferase I/II; DDH, dimethyl arginine dimethyl arginine hydrolase; GCL, glutamate cysteine ligase; GGT, γ -glutamyl transpeptidase; HSL, hormone-sensitive lipase; LDLR, LDL receptor; NRF2, nuclear factor (erythroid-derived 2)-like 2; ROS, reactive oxygen species; SRB1, scavenger receptor class B member 1; TH, thyroid hormone; UCP3, uncoupling protein 3.

liquid (LC) or gas chromatography (GC) (Bictash *et al.* 2010, Dunn *et al.* 2011). Briefly, ^1H -NMR spectroscopy distinguishes by high reproducibility and minimal sample preparation, whereas MS distinguishes by higher sensitivity, capturing a broader set of metabolites at the cost of being non-quantitative.

It is worth noting, that both techniques are rather complementary than redundant (Psychogios *et al.* 2011, Bouatra *et al.* 2013) and integration of metabolite data

from both platforms is of particular interest. However, a number of factors put a strong constrain on the applicability of multi-platform approaches, including high costs, availability of devices or the sample volume/material obtained. Hence, up to now, only one recent study (Lange *et al.* 2018) took advantage of such a comprehensive profiling with respect to thyroid research and the vast majority of studies employ either NMR or MS.

Population-based approaches

Most of the metabolomics studies up to now were conducted as population-based studies seeking for linear relationships with either TSH or FT₄ and metabolite levels (Table 2). They confirmed the pleiotropic impact of TH on not only lipid and amino acid metabolism but also nutritional behavior. Importantly, FT₄ was the predominant marker and TSH concentrations were not able to mirror the biochemical changes seen with FT₄. Compared to tight clinical settings, such approaches allow for coping with bystander effects, i.e. confounding of other variables.

The first comprehensive profiling of TH-associated changes in the metabolome in an epidemiological setting investigated the associations between serum concentrations of TSH and FT₄ with 151 serum metabolites determined by LC-MS in 1463 euthyroid individuals of the KORA F4 study (Jourdan *et al.* 2014). A FT₄-associated molecular signature was exclusively composed of lipid species, including an accumulation of acylcarnitines (ACs) of various chain lengths and a depletion of phosphatidylcholines with increasing serum FT₄. Notably, there were no associations with TSH. AC species are formed as part of the β -oxidation to enable the entrance of fatty acids into the mitochondria *via* conjugation with carnitine (Fig. 2). The latter step is facilitated by carnitine-palmitoyl transferases I alpha (CPT-1 α) a well-known transcriptional target of TH. Hence, increased plasma ACs species might indicate a higher rate of mitochondrial respiration.

The restriction to serum samples clearly limits the potential of gaining holistic insights in TH action on human metabolism. To address this issue, we previously analyzed ¹H-NMR spectral data of 3327 urine samples from the baseline evaluation of the Study of Health in Pomerania (SHIP) (Pietzner *et al.* 2015a). Besides serum TSH and FT₄ concentrations also the ratio log(TSH)/FT₄ was included into the analysis to account for an individual set point of the HPT axis. The results revealed different metabolic profiles for TSH and FT₄, which was partially resolved by the results of the analyses based on the log(TSH)/FT₄ ratio. These results highlighted the need for a refined assessment of individual thyroid function. Briefly, serum TSH was inversely associated with urinary excretion of tyrosine and several metabolite ratios including hippurate, whereas serum FT₄ displayed inverse associations to ethanolamine, citrate or formate as well as several metabolite ratios including methanol and amino acids (including alanine, glycine or histidine). A link to

Jourdan *et al.* (Jourdan *et al.* 2014) has been provided by the strong inverse association between FT₄ and ethanolamine pointing toward a role of TH in phospholipid metabolism. Note that all of these findings are derived from a cross-sectional study design not permitting insights in causal relations.

We screened similar urine metabolomics data for a molecular signature preceding shifts in TSH and FT₄ among two large population-based studies from Denmark (Friedrich *et al.* 2017). Apart from otherwise confirmative results, urine levels of alanine were strongly positively associated with a shift in FT₄ concentrations during the observation period. Subjects with low urine alanine levels at baseline showed a decline of serum FT₄ in the follow-up examination. Those with high baseline levels showed stable or a slight increase of serum FT₄ at follow-up. We interpreted this association as surrogate for a metabolic demand or overload (e.g. alanine utilization in the liver), which is subsequently counteracted at least in part by adaptations in FT₄ levels. We further leveraged the metabolomics coverage of exogenous influences to highlight a dependency between urine trigonelline and FT₄, a known surrogate marker of coffee consumption (Friedrich *et al.* 2017). Another metabolite of interest was dimethylamine (also associated to TSH), which is either produced by gut microbiota or as degradation product of asymmetric dimethylarginine, a potent vasodilator (Fig. 2).

Through combination of NMR and MS techniques, we were able to shed more light onto the tight connection between a TH-induced decrease in plasma phosphatidylcholines (Jourdan *et al.* 2014) and a mutual decrease in lipoprotein subfractions (Lange *et al.* 2018). Briefly, analyses based on 952 participants revealed a lipid-rich fingerprint of FT₄ once more virtually without any TSH-related associations. The pronounced positive association between FT₄ and plasma free fatty acids (FAs) reflects the well-known lipolytic effect of TH on adipocytes in white adipose tissue (Fig. 2). Notably, sex-specific findings were attributable to different lifestyle factors, i.e. alcohol consumption or the higher variance of TH measures among women, i.e. more frequent presence of an adverse thyroid state.

Apart from the comprehensive characterization of classical TH effects, urine metabolomics was particularly useful to exploit a possible endogenous fate of the putative T₃-metabolite 3,5-T₂ as its endogenous relevance in humans is still a matter of debate (Pietzner *et al.* 2015b). A first population-based study provided only vague evidence in relation 3,5-T₂ to glucose metabolism or the

Table 2 Summary of metabolomics studies in humans done so far.

Author (year)	Type of study	Platform	Metabolites	Suspected functional relations of metabolites	Remarks
Jourdan et al. (2014)	Population-based study (KORA-F4; N = 1463); linear relations with TSH and FT ₄	LC-MS/MS (serum samples; N = 151 metabolites)	FT ₄ : 67 (25 positively)	Diacyl and ether phosphatidylcholines (inversely) Lyso-phosphatidylcholines (inversely) Acylcarnitines (positively) Sphingomyelins (positively)	Performed analysis in strictly euthyroid participants; observed no associations with TSH
Pietzner et al. (2015a)	Population-based study (SHIP; N = 3327); linear relations with TSH, FT ₄ and log(TSH)/FT ₄	¹ H-NMR spectroscopy (urine samples; N = 56 metabolites)	TSH: tyrosine inversely FT ₄ : 3 (all inversely) log(TSH)/FT ₄ : 2 (all inversely)	Phenylalanine and tyrosine metabolism (inversely with TSH) TCA cycle (upregulated) Gluconeogenesis from amino acids (upregulated) Alcohol dehydrogenase activity (downregulated)	Strongly different metabolic patterns of TSH and FT ₄ , partially joined by the log(TSH)/FT ₄ ratio
Friedrich et al. (2017)	Population-based study (Inter99 (N = 5620) and Health06/08 (N = 3788)); prediction of changes in TSH/FT ₄ and hypothyroidism	¹ H-NMR spectroscopy (urine samples; N = 17 metabolites and 500 spectral bins)	TSH: 4 (all inversely) FT ₄ : 5 (1 positively)	Gluconeogenesis from amino acids (upregulated) Dimethylamine production (downregulated)	Alanine concentrations were predictive for changes in FT ₄ to follow-up examinations
Lange et al. (2018)	Population-based study (SHIP-TREND; N = 952); linear relations with TSH, FT ₃ and FT ₄	¹ H-NMR spectroscopy/ LC-MS/MS (plasma (N = 613) and urine (N = 587) samples) + lipoprotein subfractions (N = 117)	TSH: VLDL-cholesterol and HDL particles (all positively) FT ₄ : 106 plasma (67 positively); 12 urine (9 positively); VLDL, LDL and HDL (all inversely) FT ₃ : 55 plasma (53 positively); 13 urine (5 positively); small dense LDL and HDL (all positively)	Coffee intake (positive with FT ₄) Lipolysis from white adipose tissue (up) Peripheral/hepatic uptake of LDL and HDL particles (up) Bile acid metabolism	Sex-specific findings due to phenotypic confounding; TSH not able to mirror peripheral relevance of adequate TH supply
Pietzner et al. (2015b)	Population-based study (SHIP-TREND; N = 715); linear relations with 3,5-T ₂	¹ H-NMR spectroscopy (urine samples; N = 50 metabolites)	3,5-T ₂ : 4 (all positively)	Oxidation of fatty acids (upregulated) Hepatic detoxification Coffee intake (positively)	Partial translation of results from animal models and distinct metabolic fingerprint of 3,5-T ₂ compared to FT ₄ and TSH
Chng et al. (2016)	Female Graves' disease patients (N = 24) at time of diagnosis and after achievement of euthyroidism	LC-MS/MS (plasma samples; N = 55)	36 (33 higher; 3 lower in Graves' disease)	Acylcarnitines (elevated) Phenylalanine and tyrosine metabolism (elevated) TCA cycle (elevated)	Plasma acylcarnitine levels strongly respond to treatment of hyperthyroidism
Al-Majdoub et al. (2017)	Female Graves' disease patients (N = 10) at time of diagnosis and after achievement of euthyroidism	LC-MS/MS (plasma samples; N = 80)	15 (9 higher; 6 lower in Graves' disease)	Medium-chain acylcarnitines (elevated) Phenylalanine and tyrosine metabolism (elevated)	Only plasma levels of medium-chain acylcarnitines respond to treatment of hyperthyroidism
Pietzner et al. (2017)	Experimental thyrotoxicosis in 16 healthy males (8 weeks)	LC/GC-MS/MS (plasma samples; N = 349)	65 (45 positively with FT ₄)	Lipolysis (upregulated) Fatty acid oxidation (upregulated) Amino acid catabolism (upregulated) Defense against oxidative stress (upregulated)	Signature of metabolites and proteins enabled TSH- and FT ₄ -independent classification of thyrotoxicosis

3,5-T₂, 3,5-diiodothyronine; FT₄, free thyroxine; GC, gas chromatography; ¹H-NMR, proton nuclear magnetic resonance spectroscopy; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; TSH, thyrotropin.

HPT axis but anticipated relations with anthropometric parameters or blood lipids could not be detected (Pietzner *et al.* 2015c). However, application of urine metabolomics among 715 euthyroid subjects enabled us to partially confirm observations from animal models with respect to the oxidation of FAs (acetone as proxy for β -oxidation) and oxidative stress or detoxification of xenobiotics (pyroglutamate as intermediate in the γ -glutamyl cycle) (Pietzner *et al.* 2015b). Importantly, only minor overlap with the classical markers of thyroid function became obvious, comprising trigonelline (FT₄) and hippurate (TSH). We presumed altered peripheral conversion of TH following coffee consumption (Friedrich *et al.* 2017) (with trigonelline and in part hippurate as surrogate markers) and high FT₄ levels might therefore align with high 3,5-T₂ levels. However, the direct relationship between 3,5-T₂, FT₄ and trigonelline represents a matter of future research.

In summary, several population-based approaches started elucidating the metabolic map of classical markers of thyroid function and revealed novel players in the field. This facilitated translating previous results from animal studies to the humans and led to novel findings (e.g. the triangle between FT₄–trigonelline–3,5-T₂). However, there are still many gaps and more comprehensive approaches employing multiple metabolomics platforms, and biofluids hold promise to gain a systematic understanding, e.g. highlighting bidirectional relationships.

Thyroid disorders and models

Until very recently, metabolomics applications in thyroid disorders were limited to hypothyroid rodent models employing different matrices, namely serum (Montoya *et al.* 2013, Wu *et al.* 2013a), urine (Wu *et al.* 2013b) or cerebellum (Constantinou *et al.* 2011). The observations are in good agreement with the ubiquitous impact of TH on metabolic processes. Equivalent studies in humans (Chng *et al.* 2016, Al-Majdoub *et al.* 2017, Pietzner *et al.* 2017) highlighted the tremendous potential of metabolomics to improve our understanding of thyroid disorders, in particular, TH excess and even further the usefulness to complement diagnosis of thyroid disorders.

Graves' disease

The most prominent small-molecule signature characterizing the restoration of euthyroidism after treatment for Graves' disease is a decline in plasma AC levels (Chng *et al.* 2016, Al-Majdoub *et al.* 2017), which is well in line with observations from population-based

studies (Jourdan *et al.* 2014, Lange *et al.* 2018). Yet, the quantitative impact of TH on the blood AC profile remains to be established, since observations in a small cohort (N=12) of hypothyroid and hyperthyroid did not support strong departures in plasma AC levels from healthy controls (Wong *et al.* 2013). However, the studies done by Chng *et al.* (2016) and Al-Majdoub *et al.* (2017) confirmed previous observations with respect to phenylalanine, tyrosine as well as TCA cycle intermediates. In general, successful restoration of euthyroidism is characterized by a less pronounced utilization of FAs for energy demands.

Experimental thyrotoxicosis

The major drawback of patients studies are the concomitantly pathophysiologic conditions of acquired hyperthyroidism like autoimmune disease. Therefore, tightly controlled experiments using generally healthy human volunteers with similar background, e.g. with respect to sex, anthropometric markers, age or medical treatment are needed to dissect the metabolic effects of TH in humans.

Only few studies, using either T₃ or a combination of T₃ and L-T₄, have done this in a hypothesis-driven approach targeting whole-body protein turnover, energy expenditure or insulin secretion (Lovejoy *et al.* 1997, 1999, Riis *et al.* 2008). In contrast to population-based findings, induction of mild hyperthyroidism altered flux of branched-chain amino acids, indicating proteolysis. Strikingly, protein synthesis, estimated from phenylalanine disposal, was increased.

More comprehensive work was done in the already introduced (see 'Proteomics' section) long-term thyrotoxicosis model using a sample of 16 healthy young male volunteers (Pietzner *et al.* 2017). In addition to untargeted MS-based proteomics, an untargeted MS-based metabolome analysis was performed, complemented by a variety of established laboratory assays. The resulting molecular alterations included a number of the known TH effects described earlier, demonstrating suitability of the model (Fig. 2).

One prominent signature indicated exhaustive utilization of FAs for energy generation, and, hence respiration, with a concordant accumulation of reactive oxygen species (ROS) (Fig. 2). A parallel increase in γ -glutamyl amino acids might be a proxy for the latter, as they are integral metabolites in the synthesis of the important anti-oxidant glutathione (Soga *et al.* 2011). The increase in polyunsaturated FAs might further fit into this picture assuming structural remodeling of mitochondrial membranes, replacing saturates with unsaturated membrane lipids (Fig. 2) rendering them more resilient

against lipid oxidation by ROS. Confirmatory evidence has been obtained in animal models (Hoch 1988, Guerrero *et al.* 1999).

Of particular clinical interest are discordant alterations in surrogate markers of kidney function (decrease in serum creatinine while cystatin C level rose) and increased levels of methylated arginine, a severe risk factor for cardiovascular diseases (Willeit *et al.* 2015). Consequently, the evaluation of kidney function from circulating parameters in the context of thyroid disorders is severely hampered. A relationship between thyroid state and the plasma levels of asymmetric dimethylarginine align well with the observations of diminished urinary excretion of dimethylamine noted earlier (Friedrich *et al.* 2017) and the concordant decline in plasma citrulline levels within the study further fits in this context. Hence, the metabolism of asymmetric dimethylarginine is a simple example to reveal novel putatively TH-dependent pathways.

In summary, applying state-of-the-art metabolomics techniques allowed for characterization of the metabolic consequences of thyrotoxicosis in unmatched comprehensiveness. The discrepancy between the subjective well-being and massive molecular alterations indicates that clinical diagnosis of thyroid disorders might frequently miss the onset of disease by several months.

Integration within and across the OMICs layers

Gene–gene as well as protein–protein interactions form well-established biological networks. Metabolic networks, especially in urine or plasma, are far less commonly investigated. However, it could be expected that alterations in metabolic pathways translate into clusters of affected metabolites rather than in single target molecules. Further, given the high dependency among metabolomic data, it is highly unlikely that all metabolites associated with thyroid function originate from distinct (patho)physiological processes. This motivates the more integrative investigation of metabolomics together with other OMICS layers.

A data-driven approach to metabolic networks

Remodeling of metabolic networks is a highly valuable approach that allows for the aggregation of lists of metabolites in biologically meaningful clusters. For example, sex differences comprise more than one-third of the metabolome in serum and Krumsiek and coworkers were able to shrink this difference to several key metabolite

clusters (Krumsiek *et al.* 2015). Importantly, clusters like *tyrosine* comprised metabolites of different sub-pathways. Moreover, purely data-driven approaches allowed for the incorporation of exogenous, unlisted or even unknown compounds (Krumsiek *et al.* 2015).

In detail, Krumsiek *et al.* utilized Gaussian graphical models (GGMs) to reconstruct metabolic networks in a purely data-driven manner (Krumsiek *et al.* 2011, 2015). A GGM consists of partial correlations, i.e. a connection (edge) between metabolites (nodes) is only drawn if the observed correlation is independent of all other metabolites in the network (Fig. 3). In this way, the typical hairball-like structure of simple correlation networks is transformed into sparse networks. Such an approach enabled us in previous work to dissect the multiple associations seen with insulin-like growth factor 1 into a number of manageable pathways or groups of metabolites (Knacke *et al.* 2016). Note that such analysis strategies strongly rely on rich data sources, e.g. population-based studies, as many observations for each metabolite are needed to reconstruct meaningful networks.

Only one of the metabolomics studies published so far (Lange *et al.* 2018), exploring the relationship between bile acid metabolism and TH, utilized such an integrative approach. Nevertheless, the increasing availability of large-scale metabolomics data among population-based cohorts makes it very attractive to use this concept in the context of THs research.

OMICs integration with random forests

A major challenge for the integration of different OMICs layers is the huge diversity in data types, e.g. different range of gene and protein expression. In case of biomarker research, one is not interested in a large list comprising dozens of molecules but rather in a small but very accurate set of molecules for patient stratification, diagnosis and prognosis. Several machine-learning techniques have been developed to address problems like this. While many different approaches exist (Smolinska *et al.* 2012, Xia *et al.* 2013), random forests (RFs) are a widely used technique due to their high prediction accuracy, scale-invariance and their inherent measure of importance of each feature, i.e. molecule, for the prediction (Breiman 2001).

Briefly, an RF is an ensemble of individual decision trees (Breiman 2001). For all practical purposes, a decision tree is a simple tree-shaped model that recursively splits its input data into sub-groups. Each split aims for making the resulting sub-groups as ‘pure’ as possible. That is, one tries to achieve sub-groups in which all samples have the

Figure 3

Metabolic Networks: Sketch of the improvement in interpretability of metabolite networks when using full-order partial correlation (Gaussian graphical models) instead of ordinary correlation. **Random Forest:** Simplified scheme how a random forest is generated from the data and how the final decision is made. **Two-stage cross-validation procedure:** Each outer loop starts with splitting off the validation data from the remaining data that are further divided in a training set and a test set at each start of a training period. The training set is used to build a RF exploiting all features. Predictions are made on the test set and feature importance is measured as the Gini index. Training is repeated on different splits of the data and average importance (Imp.) for all features is computed. Afterward, a new RF restricted to the top *i* features (those with the highest mean Gini index) is built. It is trained on the combination of training and test data and employed to classify the validation data. Variable importance after each run is weighted by the achieved predictive performance using the area under the receiver-operating characteristic curve. RF, random forest.

same label or value of interest. As the impurity of a set can be measured, e.g. by the Gini-impurity (Breiman *et al.* 1984), the importance of features can be derived from the decrease in impurity due to the current split. An RF draws its strength from each tree being trained on a bootstrapped subset of the complete data and each split choosing features only from a randomly sampled subset of all features (Fig. 3). Testing each tree on the part of the data it has not seen during training prevents overfitting, i.e. an artificial

adaptation to the observed data limiting generalizability of the RF. A more detailed description of the procedure can be found in the original literature (Breiman 2001). The final prediction of the RF is obtained by aggregating all predictions from the individual trees (Fig. 3).

While an RF is a very robust machine-learning approach, it would still be necessary to test it on a completely independent unseen dataset to estimate how well it generalizes. Unfortunately, such validation data

are seldom available. To enable a robust feature selection and estimation of generalizability, one can employ a two-stage cross-validation procedure (for cross-validation and bootstrap see, e.g. [Kohavi 1995](#)). The procedure is detailed in [Fig. 3](#). Briefly, an inner loop is used for the feature selection and an outer loop estimates the generalized performance of the RF on data not used in the inner loop. By repeating each loop a sufficient number of times (depending on the data at hand), the performances can be averaged and model stability can be assessed straightforwardly.

Application to a human thyrotoxicosis model

We used the work flow described earlier to derive a TSH- and FT_4 -independent molecular signature discriminating a thyrotoxic from a euthyroid state, i.e. robustly separating between samples collected at baseline and those obtained under $L-T_4$ treatment from the study described earlier ([Pietzner et al. 2017](#)). After several permutations of the data set, a robust classification of thyrotoxic samples with a subsample of 15 metabolites and proteins was achieved. Key biological entities were reflected within the final signature, e.g. β -oxidation and oxidative stress, the complement system and the coagulation cascade. Further, cysteine and its putative degradation intermediate 4-amino-2-hydroxybutyrate were among the strongest predictors providing a link to the very recent discovery of a direct TH effect on cystathionine generation ([Hine et al. 2017](#)). The appearance of threonate replicates animal studies but has an uncertain biological meaning ([Constantinou et al. 2011](#), [Montoya et al. 2013](#)).

A short notion on integrated OMICs

Apart from biomarker research as outlined in the previous section, the integration of OMICs layers for mechanistic insights is of particular interest. One possible path to do so using observational studies in humans could be the use of so-called Mendelian randomization analyses ([Haycock et al. 2016](#)). Briefly, one uses thyroid-specific genetic instruments, e.g. SNVs previously identified to affect THs concentrations (see 'Genomics' section), as exposure and either the metabolite levels or comorbidities as outcome. A significant association implies a causal effect of variation in THs and the outcome of interest. Sequential testing at each entity, e.g. gene expression or protein measurements, might then pinpoint strongly relevant connections within and across OMICs conditioned on variations in THs. Candidate molecules could be identified by simple

per-layer association studies or by experimental studies in cells or animals. Mendelian randomization, when appropriately carried out, is thought to overcome the most important drawbacks of observational studies, i.e. reverse causation and confounding ([Haycock et al. 2016](#)).

Besides such a thyroid-centric approach, the hypothesis-free integration of different OMICs data sets possesses numerous challenges and the interested reader is referred to more comprehensive reviews on this topic ([Alyass et al. 2015](#)). Briefly, data are generated on different platforms with different scales and sources of measurement error complicating statistical analysis. Further, addition of OMICs layers steadily increases the number of variables thereby exceeding the number of observations by far ('curse of dimensionality'). How to cope with this challenge, e.g. by combination with prior biological knowledge as constraint for network construction or as a mapping object might provide fruitful topics for future research ([Robinson & Nielsen 2016](#)).

Even manual curation of different OMICs publications, although work intensive, could provide valuable hypothesis. A TH-dependent suppression of the degradation of asymmetric dimethyl arginine was already named. Furthermore, the increased amount of transcripts specific for platelets following $L-T_4$ treatment ([Massolt et al. 2017](#)) might indicate an activated state and hence activation of the coagulation cascade, which well aligns with numerous observational studies. A more complex example might be given by use of kynurenine as key metabolite ([Fig. 4](#)). The SNV rs11726248 was linked

Figure 4

Outline of the intersection between metabolomics and genomics studies with respect to the thyroid state using the small molecule kynurenine as example. AADAT, kynurenine/alpha-aminoadipate aminotransferase; mGWAS, metabolome-wide genome-wide association study; SH2B3, SH2B adapter protein 3; SNV, single-nucleotide variant; T4, thyroxine; numbers in brackets indicate referenced publications.

to higher FT₄ concentrations (Porcu *et al.* 2013) and AADAT was identified as the most likely candidate gene. AADAT encodes for kynurenine/alpha-aminoacidopate aminotransferase, which catalyzes the transamination of kynurenine to kynurenate. Plasma kynurenine levels in turn are elevated under thyrotoxic conditions (Pietzner *et al.* 2017). The intersection between genetic and metabolomics studies could be further extended to the *SH2B3* locus. More precisely, the SNV rs3184504, a missense variant within *SH2B3*, was shown to increase serum kynurenine levels (Shin *et al.* 2014) as well as to decrease the risk of developing hypothyroidism (Eriksson *et al.* 2012). Although the exact biological mechanism has to be revealed in detail, summarization of already published studies provide a strong motivation to follow-up this hypothesis.

Conclusion

Current analytical techniques facilitate the identification of molecular alterations in less invasive specimens from humans, e.g. blood or urine. Such techniques, in particular metabolomics, are a growing field of research in the thyroid community and hold great promise to transfer experimental results to humans, identify biomarkers to complement and advance diagnosis and treatment of thyroid disorders and to identify novel factors important for adequate thyroid homeostasis. The combination of different OMICs layers along with sophisticated bioinformatics tools paves the way for important advancements in our understanding of the role of the thyroid for human metabolism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

- Al-Majdoub M, Lantz M & Spegel P 2017 Treatment of Swedish patients with Graves' hyperthyroidism is associated with changes in acylcarnitine levels. *Thyroid* **27** 1109–1117. (<https://doi.org/10.1089/thy.2017.0218>)
- Alfadda AA, Benabdelkamel H, Masood A, Jammah AA & Ekhzaimy AA 2018 Differences in the plasma proteome of patients with hypothyroidism before and after thyroid hormone replacement: a proteomic analysis. *International Journal of Molecular Sciences* **19** E88. (<https://doi.org/10.3390/ijms19010088>)
- Alyass A, Turcotte M & Meyre D 2015 From big data analysis to personalized medicine for all: challenges and opportunities. *BMC Medical Genomics* **8** 33. (<https://doi.org/10.1186/s12920-015-0108-y>)
- Andersen S, Pedersen KM, Bruun NH & Laurberg P 2002 Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *Journal of Clinical Endocrinology and Metabolism* **87** 1068–1072. (<https://doi.org/10.1210/jcem.87.3.8165>)
- Bianco AC 2011 Minireview: cracking the metabolic code for thyroid hormone signaling. *Endocrinology* **152** 3306–3311. (<https://doi.org/10.1210/en.2011-1104>)
- Bictash M, Ebbels TM, Chan Q, Loo RL, Yap IKS, Brown IJ, de Iorio M, Daviglus ML, Holmes E, Stamlor J, *et al.* 2010 Opening up the 'Black Box': metabolic phenotyping and metabolome-wide association studies in epidemiology. *Journal of Clinical Epidemiology* **63** 970–979. (<https://doi.org/10.1016/j.jclinepi.2009.10.001>)
- Biondi B & Cooper DS 2008 The clinical significance of subclinical thyroid dysfunction. *Endocrine Reviews* **29** 76–131. (<https://doi.org/10.1210/er.2006-0043>)
- Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorn Dahl TC, Krishnamurthy R, Saleem F, Liu P, *et al.* 2013 The human urine metabolome. *PLoS ONE* **8** e73076. (<https://doi.org/10.1371/journal.pone.0073076>)
- Breiman L 2001 Random forests. *Machine Learning* **45** 5–32. (<https://doi.org/10.1023/A:1010933404324>)
- Breiman L, Friedman J, Olshen RA & Stone CJ 1984 *Classification and Regression Trees*. London, UK: Chapman and Hall, CRC.
- Chng CL, Lim AY, Tan HC, Kovalik JP, Tham KW, Bee YM, Lim W, Acharyya S, Lai OF, Chong ME, *et al.* 2016 Physiological and metabolic changes during the transition from hyperthyroidism to euthyroidism in Graves' disease. *Thyroid* **26** 1422–1430. (<https://doi.org/10.1089/thy.2015.0602>)
- Clement K, Viguier N, Diehn M, Alizadeh A, Barbe P, Thalamas C, Storey JD, Brown PO, Barsh GS & Langin D 2002 In vivo regulation of human skeletal muscle gene expression by thyroid hormone. *Genome Research* **12** 281–291. (<https://doi.org/10.1101/gr.207702>)
- Constantinou C, Chrysanthopoulos PK, Margarity M & Klapa MI 2011 GC-MS metabolomic analysis reveals significant alterations in cerebellar metabolic physiology in a mouse model of adult onset hypothyroidism. *Journal of Proteome Research* **10** 869–879. (<https://doi.org/10.1021/pr100699m>)
- de Lange P, Cioffi F, Senese R, Moreno M, Lombardi A, Silvestri E, De Matteis R, Lionetti L, Mollica MP, Goglia F, *et al.* 2011 Nonthyrotoxic prevention of diet-induced insulin resistance by 3,5-diiodo-L-thyronine in rats. *Diabetes* **60** 2730–2739. (<https://doi.org/10.2337/db11-0207>)
- Dominiczak MH & Caslake MJ 2011 Apolipoproteins: metabolic role and clinical biochemistry applications. *Annals of Clinical Biochemistry* **48** 498–515. (<https://doi.org/10.1258/acb.2011.011111>)
- 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. (<https://doi.org/10.1039/B906712B>)
- Elbers LP, Moran C, Gerdes VE, van Zaane B, Meijers J, Endert E, Lyons G, Chatterjee VK, Bisschop PH & Fliers E 2016 The hypercoagulable

- state in hyperthyroidism is mediated via the thyroid hormone beta receptor pathway. *European Journal of Endocrinology* **174** 755–762. (<https://doi.org/10.1530/EJE-15-1249>)
- Engelmann B, Bischof J, Dirk AL, Friedrich N, Hammer E, Thiele T, Fuhrer D, Homuth G, Brabant G & Volker U 2015 Effect of experimental thyrotoxicosis onto blood coagulation: a proteomics study. *European Thyroid Journal* **4** 119–124. (<https://doi.org/10.1159/000381769>)
- Eriksson N, Tung JY, Kiefer AK, Hinds DA, Francke U, Mountain JL & Do CB 2012 Novel associations for hypothyroidism include known autoimmune risk loci. *PLoS ONE* **7** e34442. (<https://doi.org/10.1371/journal.pone.0034442>)
- Feng X, Jiang Y, Meltzer P & Yen PM 2000 Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. *Molecular Endocrinology* **14** 947–955. (<https://doi.org/10.1210/mend.14.7.0470>)
- Flamant F, Cheng SY, Hollenberg AN, Moeller LC, Samarut J, Wondisford FE, Yen PM & Refetoff S 2017 Thyroid hormone signaling pathways: time for a more precise nomenclature. *Endocrinology* **158** 2052–2057. (<https://doi.org/10.1210/en.2017-00250>)
- Flores-Morales A, Gullberg H, Fernandez L, Stahlberg N, Lee NH, Vennstrom B & Norstedt G 2002 Patterns of liver gene expression governed by TRbeta. *Molecular Endocrinology* **16** 1257–1268.
- Franklyn JA & Boelaert K 2012 Thyrotoxicosis. *Lancet* **379** 1155–1166. ([https://doi.org/10.1016/S0140-6736\(11\)60782-4](https://doi.org/10.1016/S0140-6736(11)60782-4))
- Friedrich N, Pietzner M, Cannet C, Thuesen BH, Hansen T, Wallaschofski H, Grarup N, Skaaby T, Budde K, Pedersen O, et al. 2017 Urinary metabolomics reveals glycemic and coffee associated signatures of thyroid function in two population-based cohorts. *PLoS ONE* **12** e0173078. (<https://doi.org/10.1371/journal.pone.0173078>)
- Geyer PE, Holdt LM, Teupser D & Mann M 2017 Revisiting biomarker discovery by plasma proteomics. *Molecular Systems Biology* **13** 942. (<https://doi.org/10.15252/msb.20156297>)
- Gottlich M, Heldmann M, Gobel A, Dirk AL, Brabant G & Munte TF 2015 Experimentally induced thyrotoxicosis leads to increased connectivity in temporal lobe structures: a resting state fMRI study. *Psychoneuroendocrinology* **56** 100–109. (<https://doi.org/10.1016/j.psyneuen.2015.03.009>)
- Grasselli E, Voci A, Demori I, Canesi L, De Matteis R, Goglia F, Lanni A, Gallo G & Vergani L 2012 3,5-Diiodo-L-thyronine modulates the expression of genes of lipid metabolism in a rat model of fatty liver. *Journal of Endocrinology* **212** 149–158. (<https://doi.org/10.1530/JOE-11-0288>)
- Grasselli E, Voci A, Canesi L, Salis A, Damonte G, Compalati AD, Goglia F, Gallo G & Vergani L 2014 3,5-Diiodo-L-thyronine modifies the lipid droplet composition in a model of hepatosteatosis. *Cellular Physiology and Biochemistry* **33** 344–356. (<https://doi.org/10.1159/000356674>)
- Guerrero A, Pamplona R, Portero-Otin M, Barja G & Lopez-Torres M 1999 Effect of thyroid status on lipid composition and peroxidation in the mouse liver. *Free Radical Biology and Medicine* **26** 73–80. ([https://doi.org/10.1016/S0891-5849\(98\)00173-7](https://doi.org/10.1016/S0891-5849(98)00173-7))
- Gurnell M, Halsall DJ & Chatterjee VK 2011 What should be done when thyroid function tests do not make sense? *Clinical Endocrinology* **74** 673–678. (<https://doi.org/10.1111/j.1365-2265.2011.04023.x>)
- Haycock PC, Burgess S, Wade KH, Bowden J, Relton C & Davey Smith G 2016 Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *American Journal of Clinical Nutrition* **103** 965–978. (<https://doi.org/10.3945/ajcn.115.118216>)
- He L & Hannon GJ 2004 MicroRNAs: small RNAs with a big role in gene regulation. *Nature Review Genetics* **5** 522–531. (<https://doi.org/10.1038/nrg1379>)
- Hine C, Kim HJ, Zhu Y, Harputlugil E, Longchamp A, Matos MS, Ramadoss P, Bauerle K, Brace L, Asara JM, et al. 2017 Hypothalamic-pituitary axis regulates hydrogen sulfide production. *Cell Metabolism* **25** 1320–1333.e1325. (<https://doi.org/10.1016/j.cmet.2017.05.003>)
- Hoch FL 1988 Lipids and thyroid hormones. *Progress in Lipid Research* **27** 199–270. ([https://doi.org/10.1016/0163-7827\(88\)90013-6](https://doi.org/10.1016/0163-7827(88)90013-6))
- Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, Ebbs 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. (<https://doi.org/10.1038/nature06882>)
- Holmes E, Wilson ID & Nicholson JK 2008b Metabolic phenotyping in health and disease. *Cell* **134** 714–717. (<https://doi.org/10.1016/j.cell.2008.08.026>)
- Hooper JM, Stuijver DJ, Orme SM, van Zaane B, Hess K, Gerdes VE, Phoenix F, Rice P, Smith KA, Alzahrani SH, et al. 2012 Thyroid dysfunction and fibrin network structure: a mechanism for increased thrombotic risk in hyperthyroid individuals. *Journal of Clinical Endocrinology and Metabolism* **97** 1463–1473. (<https://doi.org/10.1210/jc.2011-2894>)
- Hortin GL & Sviridov D 2010 The dynamic range problem in the analysis of the plasma proteome. *Journal of Proteomics* **73** 629–636. (<https://doi.org/10.1016/j.jprot.2009.07.001>)
- Jafarzadeh A, Poorgholami M, Izadi N, Nemati M & Rezayati M 2010 Immunological and hematological changes in patients with hyperthyroidism or hypothyroidism. *Clinical and Investigative Medicine* **33** E271–E279. (<https://doi.org/10.25011/cim.v33i5.14352>)
- Jonas W, Lietzow J, Wohlgemuth F, Hoefig CS, Wiedmer P, Schweizer U, Kohrle J & Schurmann A 2015 3,5-Diiodo-L-thyronine (3,5-t2) exerts thyromimetic effects on hypothalamus-pituitary-thyroid axis, body composition, and energy metabolism in male diet-induced obese mice. *Endocrinology* **1** 389–399. (<https://doi.org/10.1210/en.2014-1604>)
- Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, et al. 2014 Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association task force on thyroid hormone replacement. *Thyroid* **24** 1670–1751. (<https://doi.org/10.1089/thy.2014.0028>)
- Jourdan C, Linseisen J, Meisinger C, Petersen A-K, Gieger C, Rawal R, Illig T, Heier M, Peters A, Wallaschofski H, et al. 2014 Associations between thyroid hormones and serum metabolite profiles in an euthyroid population. *Metabolomics* **10** 152–164. (<https://doi.org/10.1007/s11306-013-0563-4>)
- Knacke H, Pietzner M, Do KT, Romisch-Margl W, Kastenmuller G, Volker U, Volzke H, Krumsiek J, Artati A, Wallaschofski H, et al. 2016 Metabolic fingerprints of circulating IGF-I and the IGF-I/IGFBP-3 ratio: a multi-fluid metabolomics study. *Journal of Clinical Endocrinology and Metabolism* **101** jc20162588. (<https://doi.org/10.1210/clinem.2016-2588>)
- Kohavi R 1995 A study of cross-validation and bootstrap for accuracy estimation and model selection. In *IJCAI*, pp 1137–1145. San Francisco, CA, USA: Morgan Kaufmann Publishers, Inc.
- 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. (<https://doi.org/10.1186/1752-0509-5-21>)
- Krumsiek J, Mittelstrass K, Do KT, Stuckler F, Ried J, Adamski J, Peters A, Illig T, Kronenberg F, Friedrich N, et al. 2015 Gender-specific pathway differences in the human serum metabolome. *Metabolomics* **11** 1815–1833. (<https://doi.org/10.1007/s11306-015-0829-0>)
- Lange T, Budde K, Homuth G, Kastenmuller G, Artati A, Krumsiek J, Volzke H, Adamski J, Petersmann A, Volker U, et al. 2018 Comprehensive metabolic profiling reveals a lipid-rich fingerprint of free thyroxine far beyond classical parameters. *Journal of Clinical Endocrinology and Metabolism* **103** 2050–2060.
- Lietzow J, Golchert J, Homuth G, Volker U, Jonas W & Kohrle J 2016 3,5-T2 alters murine genes relevant for xenobiotic, steroid, and thyroid hormone metabolism. *Journal of Molecular Endocrinology* **56** 311–323. (<https://doi.org/10.1530/JME-15-0159>)
- Lombardi A, de Lange P, Silvestri E, Busiello RA, Lanni A, Goglia F & Moreno M 2009 3,5-Diiodo-L-thyronine rapidly enhances

- mitochondrial fatty acid oxidation rate and thermogenesis in rat skeletal muscle: AMP-activated protein kinase involvement. *American Journal of Physiology: Endocrinology and Metabolism* **296** E497–E502. (<https://doi.org/10.1152/ajpendo.90642.2008>)
- Lovejoy JC, Smith SR, Bray GA, DeLany JP, Rood JC, Gouvier D, Windhauser M, Ryan DH, Macchiavelli R & Tulley R 1997 A paradigm of experimentally induced mild hyperthyroidism: effects on nitrogen balance, body composition, and energy expenditure in healthy young men. *Journal of Clinical Endocrinology and Metabolism* **82** 765–770.
- Lovejoy JC, Smith SR, Zachwieja JJ, Bray GA, Windhauser MM, Wickersham PJ, Veldhuis JD, Tulley R & de la Bretonne JA 1999 Low-dose T(3) improves the bed rest model of simulated weightlessness in men and women. *American Journal of Physiology* **277** E370–E379.
- Massolt ET, Meima ME, Swagemakers SMA, Leeuwenburgh S, van den Hout-van Vroonhoven M, Brigante G, Kam BLR, van der Spek PJ, van IWEFJ, Visser TJ, et al. 2017 Thyroid state regulates gene expression in human whole blood. *Journal of Clinical Endocrinology and Metabolism* **103** 169–178. (<https://doi.org/10.1210/jc.2017-01144>)
- Medici M, Porcu E, Pistis G, Teumer A, Brown SJ, Jensen RA, Rawal R, Roef GL, Plantinga TS, Vermeulen SH, et al. 2014 Identification of novel genetic loci associated with thyroid peroxidase antibodies and clinical thyroid disease. *PLoS Genetics* **10** e1004123. (<https://doi.org/10.1371/journal.pgen.1004123>)
- Medici M, Visser WE, Visser TJ & Peeters RP 2015 Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand? *Endocrine Reviews* **36** 214–244. (<https://doi.org/10.1210/er.2014-1081>)
- Moeller LC, Dumitrescu AM, Walker RL, Meltzer PS & Refetoff S 2005 Thyroid hormone responsive genes in cultured human fibroblasts. *Journal of Clinical Endocrinology and Metabolism* **90** 936–943. (<https://doi.org/10.1210/jc.2004-1768>)
- Mollica MP, Lionetti L, Moreno M, Lombardi A, De Lange P, Antonelli A, Lanni A, Cavaliere G, Barletta A & Goglia F 2009 3,5-Diiodo-L-thyronine, by modulating mitochondrial functions, reverses hepatic fat accumulation in rats fed a high-fat diet. *Journal of Hepatology* **51** 363–370. (<https://doi.org/10.1016/j.jhep.2009.03.023>)
- Montoya GA, Strauss V, Fabian E, Kamp H, Mellert W, Walk T, Looser R, Herold M, Krennrich G, Peter E, et al. 2013 Mechanistic analysis of metabolomics patterns in rat plasma during administration of direct thyroid hormone synthesis inhibitors or compounds increasing thyroid hormone clearance. *Toxicology Letters* **225** 240–251. (<https://doi.org/10.1016/j.toxlet.2013.12.010>)
- Moreno M, Silvestri E, De Matteis R, de Lange P, Lombardi A, Glinni D, Senese R, Cioffi F, Salzano AM, Scaloni A, et al. 2011 3,5-Diiodo-L-thyronine prevents high-fat-diet-induced insulin resistance in rat skeletal muscle through metabolic and structural adaptations. *FASEB Journal* **25** 3312–3324. (<https://doi.org/10.1096/fj.11-181982>)
- Moreno M, Giacco A, Di Munno C & Goglia F 2017 Direct and rapid effects of 3,5-diiodo-L-thyronine (T2). *Molecular and Cellular Endocrinology* **458** 121–126. (<https://doi.org/10.1016/j.mce.2017.02.012>)
- Pietzner M, Budde K, Homuth G, Völker U, Völzke H, Nauck M, Wallaschofski H & Friedrich N 2015a Distinct urinary metabolic profiles associated with serum TSH and FT4 concentrations. *Metabolomics* **11** 1316–1326. (<https://doi.org/10.1007/s11306-015-0788-5>)
- Pietzner M, Homuth G, Budde K, Lehmphul I, Volker U, Volzke H, Nauck M, Kohrle J & Friedrich N 2015b Urine metabolomics by H-NMR spectroscopy indicates associations between serum 3,5-T concentrations and intermediary metabolism in euthyroid humans. *European Thyroid Journal* **4** 92–100. (<https://doi.org/10.1159/000381308>)
- Pietzner M, Lehmphul I, Friedrich N, Schurmann C, Ittermann T, Dorr M, Nauck M, Laqua R, Volker U, Brabant G, et al. 2015c Translating pharmacological findings from hypothyroid rodents to euthyroid humans: is there a functional role of endogenous 3,5-T2? *Thyroid* **25** 188–197. (<https://doi.org/10.1089/thy.2014.0262>)
- Pietzner M, Engelmann B, Kacprowski T, Golchert J, Dirk AL, Hammer E, Iwen KA, Nauck M, Wallaschofski H, Fuhrer D, et al. 2017 Plasma proteome and metabolome characterization of an experimental human thyrotoxicosis model. *BMC Medicine* **15** 6. (<https://doi.org/10.1186/s12916-016-0770-8>)
- Porcu E, Medici M, Pistis G, Volpato CB, Wilson SG, Cappola AR, Bos SD, Deelen J, den Heijer M, Freathy RM, et al. 2013 A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. *PLoS Genetics* **9** e1003266. (<https://doi.org/10.1371/journal.pgen.1003266>)
- Potlukova E, Jiskra J, Freiburger T, Limanova Z, Zivorova D, Malickova K, Springer D, Grodecka L, Antosova M, Telicka Z, et al. 2010 The production of mannan-binding lectin is dependent upon thyroid hormones regardless of the genotype: a cohort study of 95 patients with autoimmune thyroid disorders. *Clinical Immunology* **136** 123–129. (<https://doi.org/10.1016/j.clim.2010.02.015>)
- Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, et al. 2011 The human serum metabolome. *PLoS ONE* **6** e16957. (<https://doi.org/10.1371/journal.pone.0016957>)
- Riis AL, Jorgensen JO, Ivarsen P, Frystyk J, Weeke J & Moller N 2008 Increased protein turnover and proteolysis is an early and primary feature of short-term experimental hyperthyroidism in healthy women. *Journal of Clinical Endocrinology and Metabolism* **93** 3999–4005. (<https://doi.org/10.1210/jc.2008-0550>)
- Robinson JL & Nielsen J 2016 Integrative analysis of human omics data using biomolecular networks. *Molecular BioSystems* **12** 2953–2964. (<https://doi.org/10.1039/C6MB00476H>)
- Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, Rivkees SA, Samuels M, Sosa JA, Stan MN, et al. 2016 2016 American Thyroid Association Guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. *Thyroid* **26** 1343–1421. (<https://doi.org/10.1089/thy.2016.0229>)
- Shin S-Y, Fauman EB, Petersen A-K, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang T-P, et al. 2014 An atlas of genetic influences on human blood metabolites. *Nature Genetics* **46** 543–550. (<https://doi.org/10.1038/ng.2982>)
- Smolinska A, Blanchet L, Buydens LMC & Wijnga SS 2012 NMR and pattern recognition methods in metabolomics: from data acquisition to biomarker discovery: a review. *Analytica Chimica Acta* **750** 82–97. (<https://doi.org/10.1016/j.aca.2012.05.049>)
- Soga T, Sugimoto M, Honma M, Mori M, Igarashi K, Kashikura K, Ikeda S, Hirayama A, Yamamoto T, Yoshida H, et al. 2011 Serum metabolomics reveals gamma-glutamyl dipeptides as biomarkers for discrimination among different forms of liver disease. *Journal of Hepatology* **55** 896–905. (<https://doi.org/10.1016/j.jhep.2011.01.031>)
- Stuijver DJ, van Zaane B, Romualdi E, Brandjes DP, Gerdes VE & Squizzato A 2012 The effect of hyperthyroidism on procoagulant, anticoagulant and fibrinolytic factors: a systematic review and meta-analysis. *Thrombosis and Haemostasis* **108** 1077–1088. (<https://doi.org/10.1160/TH12-07-0496>)
- Taylor PN, Porcu E, Chew S, Campbell PJ, Traglia M, Brown SJ, Mullin BH, Shihab HA, Min J, Walter K, et al. 2015 Whole-genome sequence-based analysis of thyroid function. *Nature Communications* **6** 5681. (<https://doi.org/10.1038/ncomms6681>)
- Viguerie N, Millet L, Avizou S, Vidal H, Larrouy D & Langin D 2002 Regulation of human adipocyte gene expression by thyroid hormone. *Journal of Clinical Endocrinology and Metabolism* **87** 630–634. (<https://doi.org/10.1210/jcem.87.2.8200>)
- Visser TJ 2013 Thyroid hormone transporters and resistance. *Endocrine Development* **24** 1–10.
- Visser WE, Heemstra KA, Swagemakers SM, Ozgur Z, Corssmit EP, Burggraaf J, van Ijcken WF, van der Spek PJ, Smit JW & Visser TJ 2009 Physiological thyroid hormone levels regulate numerous skeletal muscle transcripts. *Journal of Clinical Endocrinology and Metabolism* **94** 3487–3496. (<https://doi.org/10.1210/jc.2009-0782>)

- Visser WE, Swagemakers SM, Ozgur Z, Schot R, Verheijen FW, van Ijcken WF, van der Spek PJ & Visser TJ 2010 Transcriptional profiling of fibroblasts from patients with mutations in MCT8 and comparative analysis with the human brain transcriptome. *Human Molecular Genetics* **19** 4189–4200. (<https://doi.org/10.1093/hmg/ddq337>)
- 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. (<https://doi.org/10.1038/nm.2307>)
- Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M, Di Angelantonio E & Chowdhury R 2015 Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies. *Journal of the American Heart Association* **4** e001833. (<https://doi.org/10.1161/JAHA.115.001833>)
- Wong S, Hannah-Shmouni F, Sinclair G, Sirrs S, Dahl M & Mattman A 2013 Acylcarnitine profile in thyroid disease. *Clinical Biochemistry* **46** 180–183. (<https://doi.org/10.1016/j.clinbiochem.2012.10.006>)
- Wu S, Gao Y, Dong X, Tan G, Li W, Lou Z & Chai Y 2013a Serum metabolomics coupled with Ingenuity Pathway Analysis characterizes metabolic perturbations in response to hypothyroidism induced by propylthiouracil in rats. *Journal of Pharmaceutical and Biomedical Analysis* **72** 109–114. (<https://doi.org/10.1016/j.jpba.2012.09.030>)
- Wu S, Tan G, Dong X, Zhu Z, Li W, Lou Z & Chai Y 2013b Metabolic profiling provides a system understanding of hypothyroidism in rats and its application. *PLoS ONE* **8** e55599. (<https://doi.org/10.1371/journal.pone.0055599>)
- Xia J, Broadhurst DI, Wilson M & Wishart DS 2013 Translational biomarker discovery in clinical metabolomics: an introductory tutorial. *Metabolomics* **9** 280–299. (<https://doi.org/10.1007/s11306-012-0482-9>)
- Yen PM 2001 Physiological and molecular basis of thyroid hormone action. *Physiological Reviews* **81** 1097–1142. (<https://doi.org/10.1152/physrev.2001.81.3.1097>)
- Yu H, Yang Y, Zhang M, Lu H, Zhang J, Wang H & Cianflone K 2006 Thyroid status influence on adiponectin, acylation stimulating protein (ASP) and complement C3 in hyperthyroid and hypothyroid subjects. *Nutrition and Metabolism* **3** 13. (<https://doi.org/10.1186/1743-7075-3-13>)
- Zhang B, Jiang Y, Yang Y, Peng F & Hu X 2008 Correlation between serum thyroxine and complements in patients with multiple sclerosis and neuromyelitis optica. *Neuro Endocrinology Letters* **29** 256–260.

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