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Toward Systems-Level Metabolic Analysis in Endocrine Disorders and Cancer

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Metabolism is a dynamic network of biochemical reactions that support systemic homeostasis amidst changing nutritional, environmental, and physical activity factors. The circulatory system facilitates metabolite exchange among organs, while the endocrine system finely tunes metabolism through hormone release. Endocrine disorders like obesity, diabetes, and Cushing's syndrome disrupt this balance, contributing to systemic inflammation and global health burdens. They accompany metabolic changes on multiple levels from molecular interactions to individual organs to the whole body. Understanding how metabolic fluxes relate to endocrine disorders illuminates the underlying dysregulation. Cancer is increasingly considered a systemic disorder because it not only affects cells in localized tumors but also the whole body, especially in metastasis. In tumorigenesis, cancer-specific mutations and nutrient availability in the tumor microenvironment reprogram cellular metabolism to meet increased energy and biosynthesis needs. Cancer cachexia results in metabolic changes to other organs like muscle, adipose tissue, and liver. This review explores the interplay between the endocrine system and systems-level metabolism in health and disease. We highlight metabolic fluxes in conditions like obesity, diabetes, Cushing's syndrome, and cancers. Recent advances in metabolomics, fluxomics, and systems biology promise new insights into dynamic metabolism, offering potential biomarkers, therapeutic targets, and personalized medicine.

Keywords: Metabolism; Endocrinology; Diabetes mellitus; Obesity; Thyroid neoplasms; Pheochromocytoma; Paraganglioma; Cushing syndrome; Aging

INTRODUCTION

Metabolism plays a pivotal role in supporting systemic homeostasis in humans. It adapts to various factors like nutritional levels, surrounding temperatures, and physical exercise. A conduit for metabolism is the circulatory system, which enables transfer of metabolites between organs, resulting in dynamic shifts in the inter-organ metabolic cycle [1,2]. Meanwhile, the endocrine system acts as a master conductor, releasing hormones like insulin, thyroid hormones, sex hormones, and cortisol, to intricately regulate the metabolism of the whole body.

Endocrine disorders, characterized by abnormal hormone secretion or action, encompass a spectrum of conditions such as diabetes, thyroid dysfunction, adrenal disorders, obesity, and meta-

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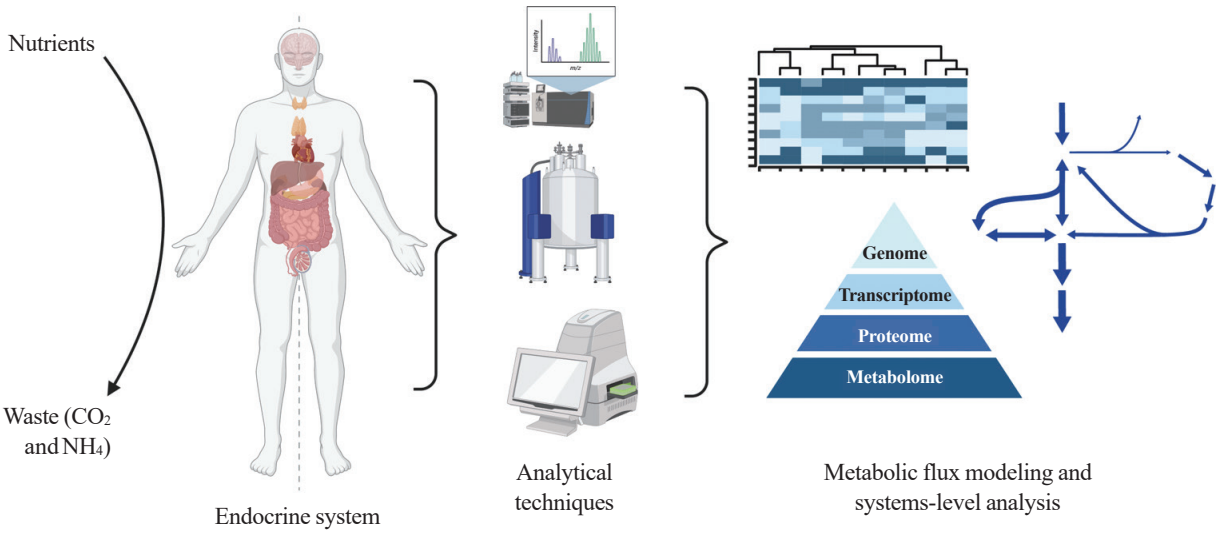

Fig. 1. Systems-level metabolic analysis in the context of endocrine disorders and cancer. By integrating advanced analytical techniques such as liquid chromatography-mass spectrometry, magnetic resonance spectroscopy, and flux analyzers and omics methods, we gain a deeper understanding of the complex metabolic alterations underlying endocrine disorders and cancer, paving the way for more effective diagnostic tools and targeted therapies. Created with BioRender.com.

bolic syndrome, which collectively contribute to systemic inflammation and a global disease burden. These diseases not only impact individual organ systems but also trigger a cascade of molecular events that reverberate throughout the entire organism, resulting in systemic disturbances. Understanding the relationship between metabolism and endocrine disorders has emerged as a promising avenue for deciphering the underlying metabolic dysregulation in organ-organ communications.

Cancer is increasingly considered as a systemic disorder. Cancer metastasis is a complex process that involves manifold biological interactions and requires the metabolic reprogramming of cancer cells. Cancer cells reprogram metabolic pathway utilization in order to meet increased bioenergetic and biosynthetic demands and to resist oxidative stress for growth and survival [3]. Cancer driver mutations coupled with microenvironmental nutrient availability control metabolic pathways [3,4]. Moreover, metabolites, when aberrantly accumulated, can promote tumor progression and metastasis (e.g., 2-hydroxyglutarate [2HG], fumarate, succinate) [5]. The development of innovative technologies over the last few decades has revealed the heterogeneity and plasticity of tumors and allowed us to uncover metabolic pathways involved in supporting tumor growth.

The metabolism research community has witnessed remarkable advancements in quantitative and high-throughput measurement tools to gain systems-level insights into various physiological and pathological processes. Metabolic fluxes, defined as the rates at which metabolites flow through metabolic pathways, quantitatively describe cellular functions, energy homeostasis, and overall physiological balance. In endocrine diseases, dysregulation of metabolic fluxes such as disrupted feedback mechanisms and impaired metabolic pathways go hand in hand with altered hormone signaling and pathogenesis. These aberrant metabolic profiles may serve as biomarkers and point to potential therapeutic targets.

Here we highlight investigations into the interplay between the endocrine system and systems-level metabolism. We delve into obesity, diabetes, Cushing's syndrome, and cancers to illustrate metabolic reprogramming in disease initiation, progression, and therapeutic response. Recent advances in metabolomics, fluxomics, and systems biology promise quantitative investigations into such systemic disorders. Systems-level understanding of metabolism in health and disease will unlock novel biomarkers, therapeutic targets, and personalized medicine (Fig. 1).

INTEGRATIVE HEALTH AND SYSTEMIC DISORDERS

Metabolism supports the growth and development of all organisms and requires coordinated regulation of many pathways. In mammals, metabolism contributes to systemic homeostasis. The advent of integrative health has brought our attention to holistic understanding of the systems of the human body and made a

profound impact on how we approach diets and medicine for overall health and well-being. Obesity is a type of systemic inflammation accompanied by altered metabolism. Several studies have conducted flux analysis using several types of diets. One such focused on fructose diets in a rat model, which is thought to influence the pathologic condition of obesity [6]. However, organ-organ interactions within obesity are not fully understood because of the complex nature of obesity-related pathophysiology. Recently, metabolomic, proteomic, and transcriptomic data have been used to predict metabolic fluxes; evaluating the livers of fasting leptin-deficient (*ob/ob*) mice revealed increased gluconeogenic flux in obesity [7].

Arteriovenous metabolite gradient measurements have been used in obesity to study organ-specific fluxes of metabolites. One study using stable isotope tracing revealed that weight loss from calorie restriction increased glycerol and nonesterified fatty acids released by adipose tissue [8]. Whole-body metabolism of branched-chain amino acids (BCAAs) has also been studied in the context of insulin resistance using high-fat diet-induced (HFD) and leptin receptor-deficient (*db/db*) obese mice as models. Isotope tracing by liquid chromatography-mass spectrometry (LC-MS) upon ^{13}C valine infusion in *db/db* mice and ^{13}C isoleucine infusion in HFD mice revealed a reduced BCAA oxidation state in liver and adipose tissues with a shift towards muscle in insulin-resistant mice [9].

Insulin is an essential hormone that regulates the metabolism of carbohydrates, lipids, and proteins through canonical insulin signaling. A prominent example of systemic disorder is diabetes which impairs systemic glucose homeostasis. Type 1 diabetes mellitus (T1DM) is a chronic immune disorder triggered by the destruction of insulin-producing pancreatic beta cells [10]. Type 2 diabetes mellitus (T2DM) involves insulin resistance, impaired signal transduction in response to insulin stimulation, and impaired insulin secretion.

Glycolysis and gluconeogenesis are functionally opposite metabolic pathways that together support systemic glucose homeostasis. In T1DM, the absence of insulin leads to uncontrolled gluconeogenesis and excessive glucose production by the liver. In T2DM, insulin resistance in peripheral tissues disrupts glycolytic flux, impairing glucose uptake and utilization. Additionally, dysregulated gluconeogenesis persists even in the presence of hyperglycemia, further exacerbating hyperglycemic states [11]. BCAA metabolism has also played a role with increased levels exacerbating the risk of T1DM development and progression [12,13]. HFDs and high levels of valine, leucine, and isoleucine are associated with insulin resistance in T1DM [14]. T2DM is characterized by increased levels of BCAAs, aromatic amino acids, and glutamate-to-glutamine ratio [15]. To link BCAA catabolism to diabetes, 3-hydroxyisobutyrate has been shown to regulate the trans-endothelial flux of fatty acids [16]. A recent study in mice, however, showed that BCAA catabolism does not affect insulin resistance even though it lowers fasting plasma BCAA levels [17]. Intervention studies based on metabolic fluxes are warranted to clarify mechanisms and explore the extent to which each of these factors leads to pathologic conditions of diabetes.

To gain mechanistic insights, mathematical models of insulin action and diabetes progression have been developed. The glucose-insulin model (GIM) integrates a system of 1,117 ordinary differential equations and several thousand parameters to model the first steps of glycolytic pathways, allowing for cell proliferation estimates in humans using labeled glucose [18,19]. Beyond glycolysis, whole-body organ-resolved metabolic reconstruction has recently been combined with GIM to analyze metabolic dysregulation with constraints tied to diet, gene expression, and other physiological parameters [20]. This model provides mechanistic insights associated with changes in metabolic fluxes due to exogenous insulin [21]. Other models in conjunction with constraint-based analysis have been used to predict metabolic flux profiles, showing reduced mitochondrial fatty acid oxidation and oxidative phosphorylation in the pancreatic beta cells of diabetic patients [22].

In T2DM, a study using a rat model identified changes in the hepatic metabolic profile. Proton (^{1}H) nuclear magnetic resonance spectroscopy (NMR) showed rewiring of choline-betaine-methionine and amino acid metabolism as well as a decrease in glycolytic activity [23]. In humans, ^{13}C magnetic resonance imaging was used for noninvasive measurement of cardiac pyruvate dehydrogenase fluxes in T2DM patients [24]. Stable isotope tracing with (U-$^{13}C_6$) glucose and gas chromatography-mass spectrometry has been performed to map metabolism in insulin-secreting beta cells. They found that the pentose phosphate pathway (PPP) was upregulated using glucose-6-phosphate (G6P) derived from glycogen [25]. ^{13}C-based metabolic flux analysis (MFA) was used to probe the role of glucose-6-phosphatase catalytic subunit 2 (G6PC2), which converts G6P to glucose. In pancreatic beta cells, G6PC2 was shown to modulate glycolysis and the citric acid cycle, posing as a therapeutic target for enhancing insulin secretion [26]. Another study probed gluconeogenic flux due to dysregulated glucagon signaling found in T2DM. Glucagon was shown to stimulate mitochondrial anaplerotic flux from glutamine, identifying hepatic glutaminase as a new target to treat hy-

perglycemia [27]. *In vivo* studies in mice using (U-$^{13}C_6$) fructose showed that high systemic glycerate levels cause glucose intolerance and damage pancreatic beta cells [28]. Maternal/fetal metabolic relationship probed in a recent study revealed that fetal nutrient sourcing was dependent on maternal glycemic state. LC-MS and *in vivo* ^{13}C-glucose tracing showed sorbitol accumulation in fetal tissues and decreased neurotransmitter levels in the fetal brain isolated from dams with maternal hyperglycemia [29].

Cushing's syndrome is a rare endocrine disorder that arises from chronic exposure to excessive glucocorticoids [30]. It may be caused by adrenal adenomas, pituitary adenomas, and ectopic sources of adrenocorticotropic hormone (ACTH) production. The hormonal dysregulation in Cushing's syndrome affects endocrine pathways and disrupts systemic metabolism [31]. Glucocorticoids, primarily cortisol in humans, influence glucose metabolism by promoting gluconeogenesis in the liver, increasing glycogenesis, and modulating insulin sensitivity [32]. Excess glucocorticoid levels disrupt these regulatory mechanisms, leading to increased gluconeogenesis, insulin resistance, and impaired glucose tolerance. Steroid hormone-related metabolites have been identified as early biomarkers for different subtypes of the disease helping to predict progression of Cushing's syndrome [33,34]; however, metabolic alterations have yet to be fully elucidated. *In situ* metabolomics of cortisol-producing adrenal adenomas identified the correlation of cortisol and serotonin and tumor size with abundance of fatty acids [35]. ^{1}H-NMR-based metabolomic studies on pituitary adenomas from patients with Cushing's syndrome revealed metabolic heterogeneity when compared to gonadotropic pituitary adenomas. LC-MS has been used to subtype adrenal or pituitary Cushing's syndrome by measuring dehydroepiandrosterone sulfate levels, especially when falsely elevated ACTH is suspected [36]. Cushing's syndrome patients had lower levels of scyllo-inositol, glycine, and phosphoethanolamine and higher levels of aspartate [37]. Metabolic markers can help differentiate pituitary adenomas of various subtypes for noninvasive diagnosis of Cushing's syndrome.

Systems-level metabolic alterations in obesity, diabetes, and other endocrine diseases are yet to be fully understood. The incomplete knowledge is due to the complex nature of multi-organ systems, hormone-associated pathophysiology, and the heterogeneity of the effects across humans. To overcome this shortfall, comprehensive and quantitative measurements of metabolic fluxes using integrative omics tools in humans and model systems are needed.

METABOLISM IN THYROID AND OTHER CANCERS

Cancer cells exhibit metabolic reprogramming that supports their heightened energetic and biosynthetic demands. Thyroid cancer represents one of the most common endocrine malignancies, characterized by abnormal growth and proliferation of thyroid follicular cells [38]. Over the past decades, substantial progress has been made in elucidating the metabolic alterations that underlie the development and progression of thyroid cancer [39-41].

Metabolic insights in thyroid cancer hold clinical implications for diagnosis, prognosis, and therapeutic interventions. Inhibiting glycolysis, fatty acid metabolism, or key metabolic enzymes involved in nutrient utilization have emerged as potential strategies to selectively target thyroid cancer cells [41]. Studies have shown that patients with both diabetes and thyroid cancer have smaller tumor sizes when treated with metformin, a drug used for T2DM [42,43]. A recent study investigated metabolic reprogramming in papillary thyroid cancer *in vitro* and *in vivo* when treated with metformin. Using an extracellular flux analyzer, metformin was shown to decrease mitochondrial respiration, respiration capacity, aerobic glycolysis, glycolytic capacity, and adenosine triphosphate production [44]. Metabolomics has also elucidated the mechanisms underlying metabolic alterations in different subtypes of thyroid cancer. For example, kinase inhibitors dasatinib and trametinib were shown to alter metabolic flux in anaplastic and papillary thyroid cancer cells [45]. In anaplastic thyroid cells in mice, targeting glycogen metabolism by inhibiting glycogen phosphorylase resulted in increased glucose flux, impairment of PPP, reactive oxygen species accumulation, and eventual cytotoxicity [46].

One of the most notable metabolic alterations observed in thyroid cancer is increased glycolytic activity, commonly known as the Warburg effect. Overexpression of glycolytic enzymes at the rate-determining steps (i.e., glucose import and phosphorylation, phosphofructokinase, and lactate export) increases glycolytic flux [47]. Thyroid cancer overexpresses hexokinase and has mitochondrial defects, which contribute to heightened glycolysis [48,49]. These defects have been studied in oncocytic thyroid cancer, which is characterized by the accumulation of abnormal mitochondria in the cytoplasm. Recently, a study comparing oncocytic and non-oncocytic thyroid cancer, showed higher glucose uptake and rerouting of glutamine to glutathione for redox homeostasis in the oncocytic cell line to compensate for its defective mitochondrial function [50]. ^{1}H-NMR spectroscopy has been used to show that oxidative stress alters the metabolic pro-

file in thyroid cancer, with increases in lactate and aromatic amino acids and a decrease in citrate [51].

A recent study elucidated the role of one-carbon metabolism and the serine/glycine metabolic pathway in undifferentiated thyroid cancer using metabolomics, bulk transcriptomics, and single-cell RNA sequencing of cells derived from human tissues [52]. High expression of mitochondrial serine hydroxymethyltransferase (SHMT2) is associated with low thyroid differentiation scores and poor clinical features in thyroid cancer. Inhibition of SHMT2 reduced tumorigenesis in rodent models. Another study reported that genetic inhibition of SHMT2 led to significant inhibition of cell proliferation by depletion of the purine pool [53]. Quantitation of systems-level metabolic fluxes remains an open challenge for the thyroid cancer research community.

Pheochromocytoma and paraganglioma are rare neuroendocrine tumors that are characterized by excessive catecholamine production [54]. Genetics research has led to discoveries of metabolic alterations associated with these cancers, particularly from mutations in genes in the hypoxia signaling pathways including hypoxia-inducible factor 2α, prolyl hydroxylase domain-containing protein 1/2, succinate dehydrogenase, fumarase, or malate dehydrogenase 2 [55]. A mutation in these genes results in accumulation of succinate, fumarate, and/or 2HG leading to tumorigenesis [56]. New technologies have been used to quantify metabolic changes and discover biomarkers; succinate accumulation, in particular, has been a useful marker in characterizing both tumor type and therapeutic response [57-59]. NMR was used to uncover that high succinate-low glutamate tumors also had high levels of methionine [60]. Furthermore, targeted metabolomics by LC-MS was used to quantify lipid and amino acid metabolic changes in catecholamine-producing tumors from human blood plasma [61]. Recently, serum metabolome profiling of adrenal steroids based on LC-MS and machine learning algorithms has suggested a promising one-step diagnostic approach for the classification of adrenal tumor subtypes [62]. Further study of metabolic pathways involved in adrenal tumors may open up new therapeutic strategies.

Tumors arise from mutations within proto-oncogenes and tumor suppressor genes. These genetic mutations directly regulate the expression and activity of metabolic enzymes, and the abnormal metabolism of cancer cells also directly affects tumor signal transduction pathways and cellular reactions. Based on this concept, the next-generation anticancer therapeutics examined in many studies and clinical trials target cancer-specific or mutation-specific metabolic phenotypes. Tumors carrying somatic mutations in BRAF and RET genes (constituting the BRAF-like subtype) exhibit a marked extracellular signal-regulated kinase (ERK)-induced transcriptional signature, and tumors carrying either mutations in RAS genes or Pax-8–peroxisome-proliferator-activated receptor-γ (PPAR-γ) fusion protein (PPFP) display a more pronounced induction of phosphoinositide 3-kinase (PI3K) pathway [63]. In the last decade, alteration of distinct oncogenes has been associated with different tumor cell capacity to undergo metabolic reprogramming, which has emerged as a strong driving force that impacts drug responsiveness and tumor aggressiveness.

RAS mutation promotes the Warburg effect and activates anabolic pathways [64,65]. Increased efforts have gone into elucidating the molecular underpinnings of altered metabolism in cancer. Oncogenic KRAS promotes glycolytic flux by upregulating several key glycolytic enzymes such as hexokinase 1 and 2, enolase 1 (ENO1), and lactate dehydrogenase-A (LDHA) [66-68]. KRAS also induces the hexosamine biosynthesis pathway that provides precursors for lipid and protein biosynthesis and the nonoxidative PPP to support increased nucleic acid biosynthesis [69,70]. RAS-driven cancer cells also alter glutaminolysis and rewire the tricarboxylic acid (TCA) cycle, which generates cellular energy and regulates stress granule (SG) formation. KRAS-mutant cancers upregulate glutaminase and NF-E2-related factor 2 (NRF2)-regulated antioxidant genes to drive SG formation, an indicator of chemoresistance [70,71]. This connection of the KRAS-NRF2 axis and glutaminolysis suggests a potential approach to counteract mutant KRAS-mediated drug resistance. Given that the process of SG accumulation is incompletely understood, elucidating the precise underlying mechanisms and metabolic links in RAS-driven cancer will determine the feasibility of SG-based therapeutic strategies.

A common mutation found in thyroid cancer is $BRAF^{V600E}$ [72-74]. $BRAF^{V600E}$ mutations occur in multiple cancers, such as lung cancer, renal cancer, colorectal cancer, and multiple myeloma. Overexpression of $BRAF^{V600E}$ showed decreased mitochondrial respiration but increased aerobic glycolysis [75]. Data supporting the use of BRAF/mitogen-activated extracellular signal-regulated kinase (MEK) inhibitors that target $BRAF^{V600E}$ mutations in the mitogen-activated protein kinase pathway have begun to emerge; however, most responses targeting $BRAF^{V600E}$ have been often short-lived and drug resistance is inevitable [76]. Thus, exploring the metabolic reprogramming in BRAF mutant cancer is necessary to overcome these issues. In melanoma, activated BRAF promotes metabolic reprogramming by suppression of oxidative phosphorylation through the mitochondrial master regulator PPAR-γ coactivator 1α (PGC1α) [77]. Additionally, a study on

slow-cycling, chemotherapy-resistant *BRAF*^{V600E}-mutated melanoma showed that oxidative phosphorylation enzymes were upregulated, and consequently, their inhibition resulted in cell death [78]. Treatment with an oxidative phosphorylation inhibitor also improved survival and decreased the development of brain metastases in BRAF/MEK inhibitor-resistant mice [79]. In a mouse model of *BRAF*^{V600E}-driven lung cancer, Atg7 deletion initially provided an advantage in tumor growth due to the induction of oxidative stress. However, at later stages of tumorigenesis, loss of autophagy led to metabolic crisis resulting in defective mitochondria, reduced tumor growth and the conversion of adenomas and adenocarcinomas to oncocytomas [80]. In colorectal cancer, *BRAF*^{V600E}-mutated tumors expressed high levels of glycolytic enzyme ENO2 and knockdown of ENO2 led to the inhibition of proliferation and migration of *BRAF*^{V600E}-mutated cancer cells [81]. Inhibition of ENO2 resulted in enhanced sensitivity to vemurafenib, a selective inhibitor of *BRAF*^{V600E}. Thus, targeting mutation-dependent metabolic dysregulation can counteract drug resistance, providing a unique opportunity for identifying novel therapeutic strategies.

Metabolic reprogramming in the tumor microenvironment that houses cancerous and immune cells has become an important consideration for cancer treatment. Failure of T cells to protect against cancer is thought to result from lack of antigen recognition, and tumor cells and tumor-infiltrating lymphocytes are known to compete for glucose within the tumor niche [82]. Immune checkpoint blockade antibodies directly altered by tumors have been shown to dampen glycolysis by inhibiting mammalian target of rapamycin (mTOR) activity and decreasing expression of glycolysis enzymes. Moreover, activated cytotoxic CD8+ T cells require metabolic shift from oxidative phosphorylation to aerobic glycolysis to maintain effector function [83], and serine metabolism is suggested as an indispensable part of T cell growth and function [84]. A combination of bioenergetic profiling and ^{13}C-glucose tracing has been used to study the metabolism of CD8+ T cells responding to infection [85]. Physiologically activated CD8+ T cells displayed greater rates of oxidative metabolism, higher bioenergetic capacity, differential use of pyruvate, and prominent flow of ^{13}C-glucose carbon to anabolic pathways, including nucleotide and serine biosynthesis. Quantitative elucidation of metabolic reprogramming during immune responses in cancer patients is an important future objective. Mapping metabolic fluxes in cells that constitute tumor microenvironment and across tissues that are involved in cancer-associated systemic inflammation will render cancers increasingly surmountable.

CONCLUSIONS

The role of insulin in controlling metabolic fluxes has been extensively studied. However, flux control in response to glucocorticoids and thyroid hormones is less well understood. A comprehensive understanding of the metabolic fluxes underlying endocrine diseases is crucial for the development of targeted therapeutic interventions. By elucidating the metabolic alterations in diseases at a systems level, we can identify potential biomarkers for early detection, prognosis, and monitoring of disease progression. Additionally, studying the intricate metabolic networks and pathways involved in endocrine disorders can unveil novel therapeutic targets, enabling the development of personalized treatment strategies that address the underlying metabolic dysregulation.

Endocrinological disorders frequently have a genetic basis, with mutations in specific genes contributing to their development. Advances in genomics have enabled the identification of numerous genetic variants associated with these disorders. However, the relationship between these genetic variations and the resulting metabolic phenotypes is often intricate and multifaceted. Also, while omics technologies can unveil the molecular aberrations responsible for disruptions in hormone production, secretion, and signaling, the translation of such findings into observable metabolic phenotypes can be challenging due to the intricate nature of hormone regulation. The interplay between genetics, molecular mechanisms, and metabolic outcomes is further complicated by individual variability and environmental influences.

Aging is a complex biological process characterized by the gradual deterioration of various physiological functions and an increased susceptibility to diseases. The endocrine system plays a critical role in regulating various metabolic processes, and its dysregulation can contribute to age-related metabolic changes. Age-related changes in insulin sensitivity and secretion can disrupt glucose homeostasis [86,87]. Thyroid function tends to decrease with age, leading to a decrease in metabolic rate and potential weight gain and changed metabolic pathways [88,89]. Moreover, dysregulation of cortisol secretion, often seen in chronic stress, can contribute to metabolic disturbances and accelerated aging [90]. Integrative health approaches also play a pivotal role in addressing the challenges of aging by promoting a comprehensive understanding of physical, mental, and emotional well-being, which can be essential for maintaining health as individuals grow older.

Advances in *in vivo* MFA using stable isotope tracers have facilitated assessment of differences in metabolic pathway utiliza-

tion in tissues and quantification of systemic turnover rates of circulating metabolites and their contribution to multiple organs [91-94]. To bridge the gap between omics data and metabolic phenotypes in endocrinological disorders, interdisciplinary approaches are crucial [95]. Integrating omics data with clinical observations, longitudinal studies, and functional assays can provide a more holistic understanding of the disease mechanisms and their metabolic consequences. Additionally, computational modeling and systems biology approaches can help unravel the complex relationships between genetic variations, molecular pathways, and metabolic outcomes. Integrating metabolic network analysis and pathway enrichment tools provides a valuable resource to conduct systems-level analyses, enabling the exploration of metabolic alterations, pathway dysregulations, and the dynamic rewiring of metabolic networks (Table 1) [96-114]. As technology and knowledge advance, researchers are working towards unraveling the intricate connections between genetics, molecular biology, and the clinical realities of endocrinological disorders, ultimately leading to improved diagnostics, targeted therapies, and personalized medicine.

Table 1. Metabolic Networks and Pathway Analysis Tools

	Tool	Applications in endocrine disorders and cancer
Metabolic network reconstruction	Metabolic reconstructions (e.g., Recon3D [96])	Provides a detailed metabolic network model for studying metabolic alterations Supports integration of multi-omics data
	Human Metabolome Database (HMDB [97])	A valuable resource for annotating metabolomics data and linking metabolites to pathways
	KEGG (Kyoto Encyclopedia of Genes and Genomes [98])	Provides a wide range of metabolic pathway maps and gene annotations
	HumanCyc [99]	Provides a comprehensive collection of metabolic pathways and enzymatic reactions in humans
	Biochemical Genetic and Genomic (BiGG) models [100]	Offers a repository of curated metabolic models for various organisms
Metabolic network analysis tools	Constraint-Based Reconstruction and Analysis (COBRA) toolbox [101]	Allows constraint-based modeling and flux analysis of metabolic networks
	Pathway tools [102]	Software for pathway/genome databases and systems biology research that supports the construction and visualization of metabolic pathways
	Isotopomer Network Compartmental Analysis (INCA 2.0) [103]	Software that performs steady-state metabolic flux analysis and isotopically non-stationary metabolic flux analysis
	Metran [104]	Software for performing ^{13}C-metabolic flux analysis, tracer experiment design, and statistical analysis
	13CFLUX2 [105]	Facilitates the measurement and modeling of carbon fluxes in metabolic pathways *in vivo*
	mfapy [106]	Offers a user-friendly interface for performing flux balance analysis and metabolic modeling, focusing on data analysis procedures
Pathway enrichment analysis	MetaboAnalyst [107]	Facilitates pathway enrichment analysis using metabolomics data
	Database for Annotation, Visualization, and Integrated Discovery (DAVID) [108]	Useful for gene enrichment analysis to identify overrepresented pathways and functions
	Enrichr [109]	Performs gene set enrichment analysis (GSEA) to identify enriched pathways and processes
	Metabolomics Pathway Analysis (MetPA) tool [110]	Focuses on the interpretation of metabolomics data in the context of metabolic pathways
Network visualization	Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) [111]	Provides protein-protein interaction networks, helping visualize connections between genes and proteins
	OmicsNet [112]	Integrates multi-omics data (e.g., genomics, proteomics, metabolomics) and visualizes molecular interactions
	Cytoscape [113]	Helps visualize metabolic networks and omics data in a network context
	MetExplore [114]	Facilitates the visualization and interpretation of metabolomics data in the context of genome-scale reconstructed metabolic networks

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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