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Review article

Metabolomics applied to the pancreatic islet

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ABSTRACT

Metabolomics, the characterization of the set of small molecules in a biological system, is advancing research in multiple areas of islet biology. Measuring a breadth of metabolites simultaneously provides a broad perspective on metabolic changes as the islets respond dynamically to metabolic fuels, hormones, or environmental stressors. As a result, metabolomics has the potential to provide new mechanistic insights into islet physiology and pathophysiology. Here we summarize advances in our understanding of islet physiology and the etiologies of type-1 and type-2 diabetes gained from metabolomics studies.

1. Introduction

Type 2 diabetes (T2D) mellitus is the most common metabolic disease worldwide, affecting 21 million people in the US alone, and both its incidence and prevalence are on the rise globally. Type 1 diabetes (T1D) is less common, with approximately 3 million patients afflicted in the US [1]. Both T1D and T2D are diseases of insulin deficiency, but with different etiologies. T2D is often associated with excess caloric intake and obesity, which contribute to development of peripheral insulin resistance. Over time, pancreatic β-cells fail to compensate for the increased insulin demand due to loss of key β-cell functions such as glucose-stimulated insulin secretion (GSIS), in concert with a gradual depletion of β-cell mass [2]. In contrast, T1D, which often manifests during childhood, is a result of selective autoimmune destruction of the pancreatic β-cells, leading to insulin deficiency [3]. The mechanisms linking glucose metabolism and insulin secretion in the β-cell are still incompletely understood, as are the mechanisms of β-cell dysfunction and death.

A clearer understanding of β-cell function is essential for ultimate success in creation of surrogate cells for insulin replacement therapy in T1D and for developing better drugs for treatment of T2D. Metabolomics represents an emergent tool for understanding of β-cell biology, and offers a systems level view of metabolism as the cells respond dynamically to glucose stimulation in the healthy state, as well as global changes that occur as β-cells become dysfunctional in the diseased state. Since pancreatic islets are intrinsically difficult and expensive to isolate, and are typically available in limited quantities, pancreatic β-cell lines have been developed as alternative systems for study (reviewed in [4]). The most utilized cell lines for metabolomics studies are the Min6, 832/13 and INS-1E cell lines. Recent studies have compared unbiased GC–MS metabolic profiles from 832/13 cells and primary rat islets [5] as well as from a recently developed human cell line EndoC-βH1 and human islets [6] with the finding that the profiles were quite similar [5,6]. These studies suggest that while not perfect, β-cell lines can serve as reasonable surrogate systems for studies of biochemical features of primary pancreatic β-cells.

A thorough discussion of metabolomics applied to diabetes in general [7,8] or of all current mechanistic models of islet function [9] and dysfunction [2,10] is beyond the scope of this review, and

Abbreviations: 6PGDH, 6-phosphogluconate dehydrogenase; K_{ATP}, ATP-sensitive K^+ channel; ER, endoplasmic reticulum; G6PDH, glucose-6-phosphate dehydrogenase; GC, gas chromatography; GHB, γ-hydroxybutyric acid; GSIS, glucose-stimulated insulin secretion; ICDc, cytosolic NADP-dependent isocitrate dehydrogenase; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; PPP, pentose phosphate pathway; PUFA, polyunsaturated fatty acid; T1D, type 1 diabetes; T2D, type 2 diabetes; TCA, tricarboxylic acid cycle; TOF, time-of-flight mass spectrometry; ZMP, 5-aminoimidazole-4-carboxamide ribonucleotide.

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we therefore refer the reader to excellent work elsewhere. Instead, we choose to focus the current review on new insights in islet biology derived from application of static and dynamic metabolic profiling methods, and the implications of this new information for translational applications.

2. General principles and methods

Metabolomics refers to the measurement of the set of small molecules in a biological system, referred to as the metabolome. The primary value of metabolomics is that it measures analytes that integrate gene expression and environmental regulation on a short time scale to identify emergent properties of a system. The global perspective provided by such analyses can be used to generate and test hypotheses. The variety of chemistries represented by the metabolome provides unique processing and analytical challenges. A generalized typical workflow is illustrated in Fig. 1. These methods typically, but not always, involve a cell lysis and metabolite extraction step to quench metabolism and solubilize the metabolome. The extract is then usually purified and/or derivatized, and the solvent is exchanged to improve compatibility with analytical techniques. Methods may then include optional fractionation steps including capillary electrophoresis, gas or liquid chromatography prior to detection of the analytes by mass spectrometry or nuclear magnetic resonance [11,12]. The resulting data is then processed as needed for the particular technique, and data features are selected, aligned, and scaled. Multivariate statistical tools are often applied in these studies to reduce data dimensionality, identify the metabolites mainly responsible for the differences between experimental groups, and to build classification models. Compound identification may happen during experimental design when choosing instrument settings (targeted analysis), after data processing by searching the data for a known set of compounds (targeted data analysis), or after statistical analysis by matching data of interest to metabolite databases (untargeted analysis). Once identified, compounds can be mapped to known metabolic pathways to aid hypothesis generation and biological interpretation [13–15]. Of the 35 studies referenced in this review, 24 used a mass spectrometry method, 5 used an NMR method, and 6 used a combination of both. Metabolomics methods developed specifically for use in β-cell lines include an extraction protocol determined by statistical design prior to GC–MS analysis [16], and an extraction procedure developed for adherent cells compatible with LC–MS [17]. Cell numbers required for each analysis vary by method. For the studies included here, a typical GC–MS analysis uses 1.2–3.4 million cells [5,18], LC–MS 3.4–35 million [19,20], NMR 1.5–180 million [21–23], and lipidomics 9–94 million cells [24–26]. For context, 300–400 medium-to-large sized islets can be isolated from a single rat, with each islet comprised of 1000–1500 cells, yielding around 400,000 cells per animal. Some of the following studies include data collected from 200 to 500 primary islets [5,27], but this is the exception and not the rule. Further advances in instrument sensitivity and analyte detection are needed to fully maximize the potential of metabolomics methods applied to islet biology. Two emergent technologies that have been reported to this end: a 2D capillary LC–MS strategy to improve metabolite coverage and facilitate analysis of small tissue samples [28], and direct MADLI-TOF analysis of single islets [29].

3. Healthy islet function

The main function of pancreatic islet β-cells is to synthesize and secrete insulin at appropriate rates to control blood glucose within a narrow range. It has been appreciated for many years that the effect of glucose to stimulate insulin secretion is not mediated by a cell surface glucose receptor but rather requires metabolism of the sugar to generate coupling factors that trigger insulin exocytosis [30–32]. The contributions of glycogen synthesis and the pentose phosphate pathway (PPP) to total glucose utilization are thought to be low in β-cells, and >90% of glucose molecules entering β-cells are estimated to engage in glycolysis and subsequent mitochondrial metabolism [33,34]. In agreement with this, unbiased metabolomics analysis of 832/13 cells has demonstrated a significant glucose-induced increment of all glycolytic intermediates measured (glucose-6-phosphate, fructose 1,6-bisphosphate, dihydroxyactone phosphate, 2/3-phosphoglycerate, phosphoenolpyruvate and pyruvate) [18,20]. Furthermore, targeted as well as unbiased metabolomics analyses have revealed a robust increase in TCA intermediates ((iso)citrate, aconitate, α-ketoglutarate, succinate, fumarate and malate) in response to 45–120 min of glucose stimulation [18–20,35,36]. Glucose-mediated changes in metabolite levels reported throughout Section 3are summarized in Table 1.

Insulin secretion from primary β-cells is biphasic. The first phase starts within minutes of glucose stimulation, peaks around 10 min, and is followed by a rapid decline of insulin output. The second and quantitatively more important phase of insulin secretion is characterized by a gradually increasing rate of release until a plateau is reached after an additional 25–30 min [32]. One particular model of glucose stimulus/secretion coupling has gained wide acceptance. In this model, glycolytic and mitochondrial metabolism of glucose lead to increases in cytosolic ATP:ADP ratio, resulting in closure of ATP-sensitive K^+ (K_{ATP}) channels, plasma membrane depolarization, and activation of voltage-dependent Ca^{2+} channels. The resulting increase in cellular calcium promotes exocytosis of insulin-containing granules [37]. However, significant GSIS also occurs under conditions where closure of K_{ATP} channels is prevented, demonstrating an important role for K_{ATP} channel-independent pathways for fuel-regulated insulin granule exocytosis [38,39]. The general consensus is that the K_{ATP} channel-dependent pathway is particularly important in first phase insulin secretion, and although ATP may participate in the second phase of insulin secretion, it is evident that other factors are involved as well. Suggested stimulus/secretion coupling factors for the second phase include NADPH [40,41], GTP [42,43], glycerolipid/free fatty acid cycle and monoacylglycerol [44,45] and glutamate [46] (Fig. 2).

3.1. Metabolite changes during the first phase of GSIS

Several labs have explored metabolic changes during the first phase of insulin secretion using metabolomics and have detected significant increases in glycolytic and TCA intermediates within 2–15 min of glucose stimulation in 832/13 cells. The degree and timing of induction varies slightly among the studies [20,47,48]. Furthermore, Lorenz et al. [20] observed a drop in AMP and ADP, resulting in a distinct increment in ATP/ADP ratio within the first 5 min of glucose stimulation which is in agreement with an important role of K_{ATP} channels in triggering the first phase of insulin secretion.

Using untargeted GC–MS in 832/13 cells, first phase insulin secretion was also found to be negatively associated with aspartate, lysine and proline and positively with ribose-5-phosphate, 6-phosphoglucono-1,5-lactone, gulonic acid γ-lactone, ribitol, sedoheptulose, N-acetylglucosamine, lactose, sorbitol, fructose, alanine, and 3-aminoisobutyrate [48]. In another GC–MS based study, first phase insulin secretion was found to be negatively correlated with aspartate, (iso)leucine, hydroxyproline, proline and valine, and positively correlated with ribose-5-phosphate, lactate, glycerol-3-phosphate, alanine, cysteine, glutamate, and glycine [47]. Lorenz et al. [20] found an inverse relationship between