Biomarkers and Inflammatory Pathways in Diabetes Mellitus Progression

Shristi Roy

Woodward Academy

ABSTRACT

With the rapidly increasing prevalence of diabetes in the global population, an early diagnosis of both type 1 diabetes mellitus and type 2 diabetes mellitus is critical to preventing long-term complications. Two potential components of unlocking a consistent early diagnosis of diabetes are understanding biological inflammatory pathways and identifying more sensitive biomarkers of diabetes. Novel biomarkers, such as interleukin, assist in monitoring the destruction of pancreatic beta cells, while inflammatory pathways can help explain the underpinnings of the insulin resistance found in both types of diabetes. In addition, research into 5-methoxytryptamine and 20-Hydroxy-leukotriene B4 would prove useful as a result of their heightened levels in patients with normal glucose levels.

Abbreviations

NHBs: Non-Hispanic Blacks
NHWs: Non-Hispanic Whites
T1D: Type 1 Diabetes
T2D: Type 2 Diabetes
Tregs: regulatory T cells
TNF-alpha: Tumor necrosis factor-alpha
IL-1β: Interleukin-1 beta:
OGTT: Oral glucose tolerance test
FPG: Fasting plasma glucose
HbA1C: Hemoglobin A1C
DM: Diabetes Mellitus
IL-18: Interleukin-18
IL-1Ra: Interleukin 1 receptor antagonist
NGR: Normal glucose restoration
UPLC-QTOF-MS: Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry
pre-DM: Pre-diabetes
PCA: Principal Component Analysis
5-MT: 5-methoxytryptamine
ROS: Reactive Oxygen Species
CDP-choline: Cytidine diphosphate-choline
PEMT: Phosphatidylethanolamine N-methyltransferase
PE: Phosphatidylethanolamine
ACP: Acyl Carrier Protein
LIP2: octanoyl-transferase
LIP1: lipoyl synthase
PPARs: peroxisome proliferator-activated receptors
Introduction

Diabetes is an increasingly prominent disorder in the human population, especially in the United States. In the status quo, 25.8 million US adults were diagnosed with diabetes mellitus, and the prevalence is highest in the North American and Caribbean regions at 10.7%. Globally, the percentage of the population with diabetes is around 9.3%, and it is estimated to rise to about 10.2% of the population by 2030. Concerning racial demographics, Native Americans suffer significantly with a prevalence rate of around 33%, while Alaskan Natives boast the lowest prevalence rate of 5.5%; in addition, Hispanic Americans and Non-Hispanic Blacks (NHBs) have higher prevalence rates (11.8% and 12.6% respectively), while Asian-Americans and Non-Hispanic Whites (NHWs) have relatively lower rates at 8.4% and 7.1% respectively. However, there are significant disparities in the broad racial groups aforementioned. For example, South American and Cuban men and women display far lower prevalence rates than their Mexican and Dominican peers. A similar phenomenon is observed in the Asian-American demographic, as Asian Indians have a prevalence rate of 14.2%, far outnumbering other Asian subgroups. Furthermore, diabetes leads to death in NHBs, Native Americans, and Hispanic Americans far more often than NHWs. Along with race, there are also disparities between male and female diabetes victims. Although diabetes, particularly type 2, is more common in males, as there were 14 million times more men ailed with diabetes in 2013, women develop a greater risk of cardiovascular disease, strokes, and myocardial infarction. Additionally, a higher frequency of women above the age of 45 is overweight or obese, which is a major factor in the onset of diabetes. However, both men and women are impacted by income inequality, as it is directly related to diabetes prevalence and diabetes mortality rates. This is because those in the lower/middle class are less likely to receive adequate care for their ailments and diabetic symptoms, which are further exacerbated by rising insulin prices, further elucidating the importance of an early diagnosis for minorities and those in poverty.

As the prevalence of both Type 1 diabetes (T1D) and Type 2 diabetes (T2D) increases at a rapid rate, a prompt diagnosis of pre-diabetes is increasingly critical to addressing the development of full-blown diabetes. In addition, about 50% of those with diabetes and 90% of adults with prediabetes roam undiagnosed, making research into innovative methods of screening for diabetes, particularly appealing to combat the symptoms of diabetes; hyperglycemia transpires only after over 90% of beta cells are dismantled, however, the prevalence rate continues to soar at an exponential rate. Those with undiagnosed diabetes are likely to also suffer from expeditious hypertension and lipoprotein cholesterol. In addition, those unaware of their diabetes diagnosis are more likely to be obese and harbor other cardiovascular risk factors, such as smoking. Undiagnosed diabetes may eventually lead to fatal organ failure and cardiovascular disease.

Type 1 vs. Type 2

Patients diagnosed with diabetes suffer from one of two variants, Type 1 or Type 2. Type 1 diabetes is classified as an autoimmune disease that fosters a lack of insulin due to the T-cell-mediated destruction of pancreatic beta cells by the immune system. Though there are proven speculations of genetic reasoning for T1D, the majority of T1D victims are not related to someone with T1D, further complicating the early detection of the disease. However, the mechanics of the disease are far more complicated. Inflammation in the pancreatic beta cells and pathological alterations of other Langerhans cells also are significant in the development of T1D. As insulitis occurs, both central and peripheral immune tolerance mechanisms, such as regulatory T cells (Tregs), and CD4+ and CD8+ T-cells, malfunction. These cells attack critical beta-cell autoantigens and peptide epitopes, therefore furthering the onset of diabetes. Along with the aforementioned T-cell subtypes, CD20+, or immune B cells, dendritic cells, and natural killer cells (NK cells) assist in the destruction of pancreatic beta cells. However, macrophages are one of the most prominent immune instigators of diabetes, as they can release cytokines, such as interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-alpha), and reactive oxygen species. Interleukins are a group of cytokines that are mediated by white blood cells, and IL-1β, in particular, stimulates the levels of inflammatory cells at a site, illustrating the potential of further
research into the aforementioned biomarker. Interferon-gamma should also be investigated, due to its role in the activation of macrophages. Naturally, the debate over the specific workings of diabetes also sparked the discussion of the primary trigger for diabetes. Some believe that patients suffer from a viral infection of their beta cells, leading to inflammation, especially because enteroviral major capsid protein VP1 and RNA have been found in beta cell pancreatic islets. Although T1D is characterized by the loss of beta-cell, the vast majority of people with T1D do not lose all their beta cells. However, insulin production is still severely hampered. The most popular treatment is insulin, whether taken by injection or by pump. Some opt for ultra-rapid inhaled insulin; however, others steer clear due to its inflexibility concerning dosage. Pramlintide and metformin are most often prescribed to those with T1D, although they have limited treatment success. Despite the knowledge gained recently, many facets of T1D are still only vaguely understood.

Type 2 diabetes (T2D) is far more common, consisting of 90-95% of cases. While T1D is usually diagnosed in childhood and has a hereditary aspect, T2D is often self-induced through aging, weight gain, and a sedentary lifestyle, although some have a genetic predisposition or an ethnic disadvantage. Type 2 diabetes involves insulin resistance, whereas T1D is the blatant destruction of the pancreatic beta cell. Type 2 diabetes mellitus begins with an increase in insulin secretion, as the beginning of insulin resistance does not allow full absorption of pancreatic insulin. This phenomenon can precede diagnosis by up to 15 years, especially because, oftentimes, this compensatory insulin secretion still keeps insulin levels in the normal range. Later, hyperglycemia develops due to the inability of insulin secretion from the beta cells. Excess adipose tissue leads to increased free fatty acid release, which is correlated with insulin resistance, and, therefore, type 2 diabetes.

**Biomarkers for the Detection of Diabetes**

Biomarkers assist in the diagnosis of diabetes and pre-diabetes, however, there is always a persistent need for more sensitive and more accurate predictors. The most common biomarkers, oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), and hemoglobin A1c (HbA1c), are more often employed as a tool for diagnosis rather than a preventative predictive measure. Further bolstering the argument for research into novel biomarkers, H1bAc is not always a reliable test, as it can be falsely elevated in those with iron deficiency and asplenia. It also does not account for ethnicity, which is problematic considering diabetes impacts minorities more severely. Some biomarkers of interest are interleukin (specifically IL-1beta), as it plays a significant role in endogenous insulin production and is linked to IKK-beta, a key component of inflammatory response that is shown to be activated in diabetes; 20-Hydroxy-leukotriene E4 due to its role in normal glucose resistance restoration; and 5-methoxytryptamine due to its significance in the alternative melatonin synthetic pathway. In addition, lysoPCs are worthwhile research topics, as they are significant in normal glucose level restoration, development of DM, and T2DM.

Interleukin, a type of cytokine excreted by leukocytes (and various other body cells), has recently been found to have a significant role in the onset of diabetes. Along with Fetuin-A, and tumor necrosis factor-alpha (TNF-alpha), levels of interleukin (IL) -6 have repeatedly been found to be elevated in patients suffering from insulin resistance, T1DM, and T2DM. Interleukin is especially elevated in diabetes because of its inflammatory nature. During insulitis, the inflammation of the beta cell pancreatic islets, many immune tolerance mechanisms fail or malfunction, triggering the emergence of T-cells and macrophages. These macrophages often secrete cytokines, such as interleukin-beta. Although insulin resistance is a key indicator of T2DM, new evidence has found that low-grade inflammation of the beta cells also plays a significant role in its onset. Inflammasomes, particularly the NLRP3 inflammasome which triggers the creation of IL-1β and Interleukin-18 (IL-18), elucidate an important connection between diabetes and the immune system. Higher levels of IL-1beta, a proinflammatory cytokine that moderates insulin secretion and beta-cell apoptosis, have been found in mice with overt T2DM, as it has been associated with beta-cell damage. Throughout the development of T2DM, beta-cell macrophages secrete IL-1β and IL-1 receptor antagonist (IL-1Ra), the regulator of IL-beta levels, and maintaining a balance between the two cytokines is crucial to the mediation of T2DM. Elevated levels of IL-1β have been found in rats to result in beta-cell dysfunction and, therefore, low blood pressure, dilation.
of blood vessels, and, eventually, significant inflammatory events, making IL-1β a prudent biomarker for T2DM detection\textsuperscript{12,13}.

20-Hydroxy-leukotriene B4, a human metabolite, has the potential to be associated with inflammatory and immune functions that may increase the risk of Type 2 Diabetes, cardiovascular disease, and stroke via metabolic syndrome\textsuperscript{14}. In a study where men and postmenopausal women suffering from metabolic syndrome were matched with controls and instructed to undergo weight reduction or weight maintenance, researchers concluded that subjects with metabolic syndrome have lower stimulated leukotriene B4 levels\textsuperscript{15,16}. This corroborates other evidence that 20-hydroxy-leukotriene is associated with normal glucose restoration (NGR). Researchers are using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), a highly accurate tool able to identify numerous biochemical markers in a single sample to gain insight into the metabolic systems that respond to disease, inflammation, or other pathophysiological stimuli. Throughout a longitudinal cohort study where 108 participants suffering from pre-diabetes (pre-DM) were tracked over 10 years, 20 subjects developed full-blown DM, 20 reverted to NGR, and the remainder maintained pre-DM levels of fasting glucose, 2-h glucose, and HbA1C. Based on principal component analysis (PCA) plots of the results, it was evident that the metabolic profiles of the subjects with NGR, DM, and pre-DM differed significantly\textsuperscript{17}. With a fold change value of 1.28, 20-hydroxy-leukotriene was proven to have a relatively higher concentration in NGR subjects, displaying its merit as a possible biomarker to track the progression or regression of diabetes\textsuperscript{18}.

5-methoxytryptamine (5-MT), an indolamine metabolite related to serotonin and melatonin, has also been found to be directly related to NGR restoration, making it a critical novel biomarker to investigate in diabetes research. In a study utilizing UPLC-QTOF-MS, 5-methoxytryptamine was found to be elevated in patients that reverted from pre-diabetes to NGR over 10 years, with a fold change value of 3.56. 5-methoxytryptamine is a particularly relevant biomarker due to its critical role in the alternative synthetic pathway\textsuperscript{19}. First, a methyl group is added to serotonin to create 5-MT, and, then, 5-MT is N-acetylated into melatonin, which is also known as N-acetyl-5-methoxytryptamine. Many studies have found that melatonin limits oxidative damage to lipids and that the absence of the N-acetyl group improves the protective qualities of melatonin\textsuperscript{20}. Higher levels of 5-MT are beneficial against reactive oxygen species (ROS), which degrades pancreatic beta cell function and promotes insulin resistance, further spurring the progression of type 2 diabetes\textsuperscript{21}. Melatonin is also found to balance insulin secretion by simultaneously promoting the phospholipase C/IP3 pathway while hindering the cyclic adenosine monophosphate and cyclic guanosine monophosphate pathways\textsuperscript{22}. In addition, many studies have corroborated that faulty methoxytryptamine production is associated with the onset of DM, effectively garnering enough pertinence to monitor as a biomarker during the development of T2DM\textsuperscript{23,24}.

### Inflammatory Pathways

Frequently, biomarkers stand out due to the activation of a particular inflammatory pathway. Regarding the development from pre-diabetes to normal glucose restoration, there are three prominent metabolic pathways: glycerophospholipid metabolism, lipoate biosynthesis/incorporation II, and melatonin degradation\textsuperscript{25}. However, the first requires particular attention, as diabetes is oftentimes associated with obesity and, therefore, the metabolic disorders of lipids and phospholipids. The glycerophospholipid metabolism pathway helps promote metabolites, such as phosphatidylcholine, phosphatidylethanolamine, and phosphoinositide, that maintain the stability of cell membranes from alterations. Phospholipids usually have a hydrophilic head and two hydrophobic tails and can be divided into two groups depending on their backbone: glycerophospholipids and sphingolipids. Glycerophospholipids, or glycero-based phospholipids, have several variations due to changes in their phosphate groups. For example, adding choline forms phosphatidylcholine while ethanolamine forms phosphatidylethanolamine. The glycerophospholipid metabolism pathway generally starts in the endoplasmic reticulum (ER). To synthesize glycerophospholipids, diacylglycerol or cytidine diphosphate-diacylglycerol is synthesized from phosphatic acid to derive diacylglycerol units. While dihydroxyacetone phosphate created through glycolysis is converted to glycerol-3-phosphate by glycerol-phosphate dehydrogenase,
glycerol is also converted to glycerol-3-phosphate by glycerol kinase. Then, 1-Acylglycerol-3, derived from the glycerol-3-phosphate, is converted to phosphatidic acid by lysophosphatidic acid acyltransferase. Diacylglycerol and cytidine diphosphate-diacylglycerol produced from phosphatic acid that is catalyzed by phosphatic acid phosphatase or CDP-diacylglycerol synthetase are incorporated into phosphatidylcholine and phosphatidylethanolamine. Synthesized by the cytidine diphosphate-choline pathway (CDP-choline), phosphocholine is converted to CDP-choline. Lastly, 1,2-diacylglycerol choline-phosphotransferase catalyzes the generation of phosphatidylcholine from CDP-choline and diacylglycerol. Phosphatidylethanolamine is generated through a similar process via the CDP-ethanolamine pathway. Phosphatidylethanolamine can also be converted to phosphatidylcholine by phosphatidylethanolamine N-methyltransferase (PEMT). The synthesis of choline via PEMT is known to be a critical factor in insulin sensitivity and Type 2 diabetes. High PEMT activity can lead to excess choline, leading to obesity and diabetes due to increased blood lipids. PEMT-deficient mice were found to be more likely to gain weight and become insulin-resistant, as the excess choline hampered glucose metabolism and increased fat transport out of the liver, raising blood lipid levels.

In particular, phosphocholine metabolites, especially LysoPCs, contribute to diabetes-induced inflammation. Arachidonic acid, which originates from the splitting of PCs with a C20:4 fatty acyl group (which creates LysoPCs), leads to the increased production of inflammatory signaling molecules. Phosphatidylethanolamine (PE), the second most abundant phospholipid in the human body, plays important roles in apoptosis, autophagy, and membrane fusion. In a study examining lipidomics analysis on the liver of mice with T2DM, PE levels were found to be lower in mice afflicted with T2DM. Phospholipid irregularities are associated with diabetes and obesity, and monitoring these pathways in the beginning stages of diabetes could be beneficial to the treatment and diagnosis of patients.

Lipoate biosynthesis/incorporation II is the process that details the generation of lipoate, an organosulfur compound that is a critical cofactor to many enzyme complexes involved in oxidative metabolism, such as pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, branched-chain 2-oxoacid dehydrogenase, and the glycine cleavage system. In brief, octanoyl-ACP, a substrate of a two-step reaction catalyzed by octanoyl-transferase (LIP2) and lipoyl synthase (LIP1), generates lipoic acid as a result of β-ketoacyl-(ACP) synthase activity during de novo fatty acids synthesis. First, the octanoyl moiety is shifted from octanoyl-ACP by LIP2 to their respective apolipoproteins, proteins that bind lipids to form lipoproteins, where an amide bond is formed between the proteins and the octanoyl moiety. Then, two sulfur atoms are added to the octanoyl chain in the second reaction by LIP1, creating lipoic acid. Lipoic acid (LA) diminishes reactive oxygen species, a reactive chemical derived from diatomic oxygen that can incite insulin resistance by decreasing expression of the GLUT4 transporter in the cellular membrane and therefore induce the progression of T2DM. In addition, LA regulates peroxisome proliferator-activated receptors (PPARs)-regulated genes and activates both PPAR-alpha, which moderates carnitine palmitoyltransferase 1A and acetyl-CoA synthase, and PPAR-gamma, which elevates the expression of fatty acid translocase/CD36, lipoprotein lipase, and adipocyte fatty acid binding protein. The aforementioned enzymes play significant roles in glucose and lipid metabolism. Finally, LA limits nuclear factor kappa B (NF-κB), which mediates proinflammatory cytokines, such as IL-1, IL-6, and TNF-alpha. LA also diminishes IKK-beta, a protein encoded in the IKBKB gene, which is required for NF-κB activation. Irregular NF-κB activity is related to inflammatory and autoimmune diseases as it plays an integral role in cellular responses to stress, cytokines, free radicals (like ROS), ultraviolet irradiation, and more. In mice lacking IKK-beta in myeloid cells, global insulin sensitivity was preserved, and insulin resistance was not as prevalent when put on a high-fat diet, as the NF-κB activation that would spark inflammation is not present, therefore linking inflammation and insulin resistance. The various effects of lipoic acid on diabetes, insulin resistance, and inflammation make further research into the lipoate biosynthesis pathway prudent.

**Conclusion**

Several novel biomarkers, such as interleukin, LysoPCs, 5-methoxytryptamine, and 20-hydroxy-leukotriene, can assist in the early and prompt diagnosis of diabetes within the pre-DM stage. A swift diagnosis can help patients employ better lifestyle choices sooner in the case of type 2 diabetes, while those suffering from type 1 diabetes will be able to...
prevent their symptoms and further diabetic complications more efficiently. The rapid increase in diabetes diagnoses calls for further research into new biomarkers that can not only elucidate the presence of elevated glucose levels but also follow the progression of pre-diabetes into either normal glucose levels or diabetes. In addition, monitoring inflammation and specific pathways, such as the glycerophospholipid metabolism, lipoate biosynthesis/incorporation II, and melatonin degradation pathways, in diabetes patients may also prove useful for a quick diabetes diagnosis.

Acknowledgements

I would like to thank Alejandro Riveras from UCLA for assisting me in formatting this review paper.

References

25. Nakatsuka, A., Matsuyama, M., Yamaguchi, S., et al. (2016). Insufficiency of phosphatidylethanolamine N-methyltransferase is risk for lean non-alcoholic steatohepatitis. Scientific Reports, 6(1), 21721. DOI: 10.1038/srep21721
32. Homo sapiens lipoate biosynthesis and incorporation. (n.d.). HumanCyc
34. MetaCyc lipoate biosynthesis and incorporation I. (n.d.). MetaCyc