Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy

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REVIEW

Novel biomarkers for prediabetes, diabetes, and associated complications

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Abstract: The number of individuals with prediabetes is expected to grow substantially and estimated to globally affect 482 million people by 2040. Therefore, effective methods for diagnosing prediabetes will be required to reduce the risk of progressing to diabetes and its complications. The current biomarkers, glycated hemoglobin (HbA1c), fructosamine, and glycated albumin have limitations including moderate sensitivity and specificity and are inaccurate in certain clinical conditions. Therefore, identification of additional biomarkers is being explored recognizing that any single biomarker will also likely have inherent limitations. Therefore, combining several biomarkers may more precisely identify those at high risk for developing prediabetes and subsequent progression to diabetes. This review describes recently identified biomarkers and their potential utility for addressing the burgeoning epidemic of dysglycemic disorders.

Keywords: prediabetes, biomarkers, inflammatory markers, diabetes, diabetes complications

Introduction

Prediabetes is defined as an intermediate state with plasma glucose levels ranging between normoglycemia and diabetes. The Centers for Disease Control estimated that in 2012 about 86 million, or one out of three, adults had prediabetes in the US.[1] However, 90% of these individuals were unaware of their diagnosis. In 2015, the International Diabetes Federation estimated that the worldwide prevalence of impaired glucose tolerance (IGT) in adults was 318 million and expected to reach 482 million by 2040.[2] The annual progression rate to diabetes is 5–10%,[3] with older individuals, those with severe insulin resistance (IR), low insulin secretion, and other diabetes risk factors even more likely to progress.[4] How can we identify patients with prediabetes and what can we do to prevent progression to diabetes?

Lifestyle and pharmacological interventions have been most effective in preventing progression to diabetes and associated complications. Preservation of β-cell function and reduction in IR and diabetes complications such as retinopathy, cardiovascular disease (CVD), and all-cause mortality were observed subsequent to lifestyle modification.[5,6] The Da Qing Diabetes Study in China,[6] the Finnish Diabetes Prevention Study,[7,8] and the U.S. Diabetes Prevention Program[3,9] have shown that changes in dietary habits, weight loss, and increased physical activity reduced the risk of progression to diabetes. Bariatric surgery promotes weight loss and is beneficial in prediabetes.[10]

Identification of risk and diagnosis of prediabetes

Development of prediabetes involves multiple factors including genetics, peripheral IR, defects in insulin secretion, glucotoxicity, lipotoxicity, impaired incretin release,

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amylin accumulation, inflammation, oxidative stress, and decreased β-cell mass leading to β-cell dysfunction.[11–13] Prediabetes is classified as isolated impaired fasting glucose (IFG) or IGT.[14] Glucose and glycated hemoglobin (HbA1c) criteria for diagnosing dysglycemic states are controversial as there are differing thresholds recommended by the American Diabetes Association (ADA) and the World Health Organization.[15,16] We will review several additional biomarkers used to predict the risk of progression to diabetes.

Diagnostic biomarkers and their clinical utility

Hemoglobin A1c

HbA1c is the most commonly used biomarker to diagnose prediabetes and diabetes. HbA1c forms when glucose attaches to the amino-terminal group of the β subunit of hemoglobin.[17] HbA1c reflects chronic glycemia rather than glucose levels at a single time point. Currently, the ADA criteria for diabetes are HbA1c ≥6.5% (48 mmol/mol) and 5.7–6.4% (39–46 mmol/mol) for prediabetes.[14] Increased HbA1c levels are associated with increased morbidity and mortality. In the Norfolk prospective study, higher HbA1c levels were also associated with increased CVD, cancer, and all-cause mortality.[18] Long-term prospective studies, including the Diabetes Control and Complications Trial, the UK Prospective Diabetes Study Group, and the Epidemiology of Diabetes Interventions and Complications study have shown that diabetic complications are directly related to the mean HbA1c, with a level ≥6.5% (48 mmol/mol) associated with retinopathy.[19–21] Additionally, HbA1c was more strongly correlated with retinopathy than fasting plasma glucose (FPG). Thus, HbA1c may be a better predictor of microvascular complications than FPG.[22]

HbA1c has several advantages versus FPG and oral glucose tolerance test (OGTT) including greater convenience as fasting is not required, greater pre-analytical stability, and less day-to-day perturbation during periods of stress and illness.[23] Since HbA1c reflects chronic exposure to glucose, it is particularly useful for lifestyle modification counseling.[23,24] However, there is conflicting evidence regarding the usefulness of HbA1c as it provides moderate sensitivity in diabetes diagnosis when compared to OGTT and FPG (Table 1).[23,24] OGTT is more strongly correlated with IR and secretion than HbA1c,[25] which is expected since the response to a high dose of glucose would more accurately reflect an individual's physiologic response and insulin secretion and actions. For

Table 1 Characteristics of biomarkers for prediabetes, diabetes, and associated complications

Traditional biomarkers					
Biomarker	**Mechanism of action**	**Sensitivity and specificity**	**Advantages**	**Disadvantages**	**Association with dysglycemia**
1. HbA1c	HbA1c forms when glucose attaches to the amino-terminal group of the β subunit of hemoglobin[17]	**Diabetes**[28] When HbA1c≥6.5% compared to FPG FPG≥126 mg/dL (7.0 mmol/L) and 2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) Sensitivity: 0.589 Specificity: 0.960 **Prediabetes**[28] HbA1c ≥5.7, <6.5 for prediabetes Sensitivity: 0.354 Specificity: 0.834	Increased HbA1c levels are associated with increased morbidity and mortality[18] More reliable biomarker of chronic glycemia HbA1c correlates with greater convenience, greater pre-analytical stability, and less day-to-day perturbation during periods of stress and illness[23]	HbA1c has moderate sensitivity in diagnosing diabetes when compared to OGTT and FPG[24–26] No consensus which cut-off points for HbA1c would be most sensitive[26–29] HbA1c threshold for prediabetes does not consider ethnicity, BMI, and age, all of which may significantly alter HbA1c levels[30,31] HbA1c is not always a reliable measure of average circulating glucose levels[40] Changes in the production rate or circulating life span of red blood cells affect HbA1c levels, as well as hemoglobin variants such as HbS, HbC, HbD, and HbE[36,39]	HbA1c is a reflection of chronic glycemia[17]

(*Continued*)

Table 1 (*Continued*)

Traditional biomarkers					
Biomarker	**Mechanism of action**	**Sensitivity and specificity**	**Advantages**	**Disadvantages**	**Association with dysglycemia**
2. FA	FA is a ketoamine created by glycosylation of total serum proteins, primarily albumin[41]	**Diabetes**[38] 2.5 mmol/L for diabetes (FPG >7 mmol/L or HbA1c≥6.5%) Sensitivity: 0.82 Specificity: 0.94 **Prediabetes**[38] Limited data	FA reflects average blood glucose concentration over the previous 1–4 weeks[38] FA is especially beneficial in conditions that affect hemoglobin status or rate of erythrocyte turnover[52] FA is cost-effective, simple, and convenient, as it does not require fasting[42,43]	FA has higher within-subject variation and falsely low levels in conditions leading to rapid albumin turnover[44] Not all studies have found that mean serum FA levels are useful for prediabetes screening[37,38,41,47]	FA increases in states of high glucose concentrations[41]
3. GA	Glycosylation of albumin and measured by the ratio of GA to total albumin	**Diabetes**[50] When GA is ≥15.5% Sensitivity: 0.83 Specificity: 0.83 **Prediabetes**[49] When GA >13.35% for prediabetes Sensitivity: 0.41 Specificity: 0.71 The combination of GA with HbA1c can predict prediabetes with greater sensitivity than HbA1c alone[49]	GA is a superior index of glycemic control than HbA1c in patients with renal failure, hemolytic anemia, and those receiving blood transfusions[41] GA is preferred over FA in clinical conditions that result in protein loss such as nephrotic syndrome, liver disease, and thyroid disease[41] GA may be artificially low in individuals with increased BMI, body fat mass, and visceral adiposity[53]	Inaccurate when there are changes in albumin turnover Falsely lower levels in obesity[52]	Serum GA is associated with prediabetes and diabetes[49]
4. OGTT	Measures fasting and 2-hour plasma glucose levels	**Diabetes** Sensitivity: 0.93[155] **Prediabetes** Sensitivity: 0.811[155]	OGTT is more strongly correlated with IR and secretion than HbA1c[25] OGTT provides important information with regard to risk that HbA1c or FPG cannot[4]	OGTT is variable, invasive, and time consuming It is inconvenient because it requires fasting and shows day-to-day perturbation during periods of stress and illness	Elevated FPG and 2-hour levels are associated with prediabetes and diabetes
5. 1, 5 AG	1,5 AG is a dietary monosaccharide. Plasma concentrations are inversely correlated with plasma glucose[60]	**Diabetes**[47] When 1,5 AG <17 mcg/mL for diabetes defined by optimal HbA1c >6% cutoff Sensitivity: 0.96 Specificity: 0.88 **Prediabetes**[62] Data not available	1,5 AG is a useful biomarker as it reflects glucose levels within the past 10–14 days It is stable, replicable, and less costly compared to other glycemic diagnostic tests[61]	Plasma 1,5 AG levels can change based on dietary habits, sex, and race[64,65] Levels are also affected by renal hemodynamics or treatment with $SGLT_2$ inhibitors[66,67]	Plasma 1,5 AG levels are lowered in subjects with prediabetes and diabetes compared with subjects with normoglycemia

(*Continued*)

Table 1 (*Continued*)

Novel biomarkers		
Biomarker	**Mechanism of action**	**Association with dysglycemia and complications**
6. Adiponectin	Adiponectin is derived from adipose tissues and exhibits insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties. It is an independent predictor of diabetes[68]	Lower levels of adiponectin are associated with increased IR and obesity, while higher levels have been related to lifestyle intervention groups in diabetes prevention trials[68] Adiponectin levels are inversely related to the risk of incident prediabetes, independent of ethnic or sex differences[69] Adiponectin levels were directly correlated with insulin sensitivity and indirectly correlated with insulin secretion[69]
7. FetA	FetA is a hepatic secretory glycoprotein that has been proposed to promote lipid-induced IR through the TLR4-inflammatory signaling pathway, resulting in production of inflammatory cytokines[71]	FetA correlates with increased risk of developing T2DM and associated complications[70]
8. α-HB	α-HB is an organic acid byproduct produced during the synthesis of α-KB, a product of amino acid catabolism (threonine and methionine) and glutathione anabolism (cysteine formation pathway) in hepatic tissue[156]	IR, increased oxidative stress, and lipid oxidation may cause chronic shifts in glutathione synthesis leading to elevated α-HB levels[88] α-HB was found to be significantly associated with IR independent of sex, age, BMI, and collection site[76] IR was associated with reductions in glycine and serine, which are upstream of α-KB
9. L-GPC	L-GPC is a metabolite formed by the enzyme phospholipase A2 in the liver and by lecithin-cholesterol acyltransferase in the circulation. Phospholipase A2 activity is increased during inflammation[76,92,93]	L-GPC is a negative predictor of T2DM progression[76,93]
10. Lp(a)	Lp(a) is a lipoprotein subclass that contributes to atherogenesis[94]	Lp(a) has an inverse relationship with prevalence of prediabetes and T2DM[95]
11. Triglycerides	Esters derived from glycerol and three fatty acids	Associated with β-cell dysfunction and reduced insulin secretion in subjects with prediabetes[94,96]
12. HDL	A major lipoprotein	HDL-C promotes insulin secretion • Low HDL-C concentration may lead to progression from prediabetes to diabetes • Increased proportion of small HDL3 over HDL-C in subjects with prediabetes • Decreased proportion of HDL-LpPLA$_2$ in prediabetes[97]
13. Ceramide	Lipid molecules	Positively associated with prediabetes and T2DM[85,98]
14. Ferritin and transferrin	Ferritin is an intracellular protein that stores and releases iron	Elevated serum ferritin and transferrin saturation have been strongly associated with increased risk of prediabetes and diabetes. Iron has properties that contribute to IR such as production of highly active radical formation, damage to DNA and cell membrane integrity, β-cell oxidative stress leading to decreased insulin secretory capacity, and interference with glucose uptake in skeletal muscles and adipocytes. Moreover, catalytic iron stimulates the formation of reactive oxidative species, hepatic dysfunction, and β-cell apoptosis, all of which contribute to IR[103–106]
15. MBL -associated serine proteases	MBL-associated serine proteases are important enzymes for innate immune responses and activation of the lectin pathway of the complement system[108]	MASP1 has been shown to positively correlate with prediabetes, diabetes, and CVD[109]

(*Continued*)

Table 1 (*Continued*)

Novel biomarkers		
Biomarker	**Mechanism of action**	**Association with dysglycemia and complications**
16. THBS1	THBS1 directs formation of multiprotein complexes that modulate cellular phenotype (e.g., stimulates/inhibits migration of vascular smooth muscle cells or endothelial cells, respectively)[110]	THBS1 positively associated with:[109] • Higher prediabetes prevalence • Increased IR • Increased 2-hour glucose • Adipose inflammation and metabolic dysregulation in obesity and type 2 diabetes
17. GPLD1	GPLD1 has a postulated role in the insulin-mimetic signaling pathway of glycosylphosphatidylinositol compounds, though the exact mechanism is not known GPLD1 cleavage generates second messengers that can act as inulin-mimetic molecules[109]	GPLD1 positively associated with:[109] • Type 2 diabetes and prediabetes (less strongly) • MASP1, another novel prediabetes biomarker • HDLs in serum
18. Acyl-carnitine	Acyl-carnitines interact with NF-Kβ, which promotes inflammation and IR The precise role acyl-carnitines play has not yet been elucidated[118]	Elevated levels of acyl-carnitine found in individuals with prediabetes Associated with inflammation and IR[116,117]
19. miRNA	miRNAs are involved in cell growth, differentiation, proliferation, and death **Specific miRNAs**[124,126] miR-192 regulates the tumor protein p53 miR-193b involved in brown adipocyte differentiation and inflammation reduction miR-15a thought to directly inhibit uncoupling protein-2 gene expression, leading to increased oxygen consumption and reduced ATP generation, thus promoting insulin synthesis Others thought to play a role in β-cell function[100]	Many miRNAs have been found to be elevated in individuals with prediabetes[122] miR-192 and 193b associated with subjects with IFG and IGT; associated with high triglyceride levels and fatty liver index[120] Other miRNAs play a role in insulin production, secretion, and regulation[126]
Inflammatory biomarkers		
Biomarker	**Mechanism of action**	**Association with dysglycemia and complications**
20. CRP	Derived from IL-6-dependent hepatic biosynthesis Primary marker of acute phase response[131]	Associated with type 2 diabetes and IR Found to be associated with prediabetes CRP found more elevated in subjects who had prediabetes and IR than those with prediabetes but insulin sensitive[108]
21. IL-6	IL-6 cytokines exhibit immunoregulatory actions and are involved in glucose homeostasis and metabolism through action on pancreatic β cells,[157] adipocytes, hepatocytes, and skeletal muscles[158]	Associated with type 2 diabetes and IR[131]
22. WBCs	WBC count is a marker of immunity and inflammation[140] NLR is an indicators of subclinical inflammation and microvascular and macrovascular complications in diabetes[142,143]	WBC count has been predictive of: • Worsening insulin action • Secretory function and T2DM • Coronary heart disease • Higher 1-hour post-load glucose level[140]

(*Continued*)

Table 1 (*Continued*)

Inflammatory biomarkers		
Biomarker	**Mechanism of action**	**Association with dysglycemia and complications**
23. Fibrinogen	Fibrinogen actions affect blood viscosity, platelet aggregation, and fibrin formation	Fibrinogen associated with: • Prediabetes and weakly associated with diabetes • Higher 1-hour post-load glucose level atherosclerosis[134]
24. PAI-1	Marker of reduced fibrinolysis Decreased activity leads to defective coagulation[147,148]	Independent predictor of diabetes[147]
25. IL-18	IL-18 increases during hyperglycemia[149]	Associated with increased risk of T2DM Increased IL-18 correlated with progression from prediabetes to diabetes[149]
26. IL-1RA	IL-1RA is an anti-inflammatory marker elevated when IL-1 pathway induced by glucose and free fatty acids during overfeeding[150]	Associated with: • Decreasing insulin sensitivity • Temporary increasing β-cell function • Elevated in prediabetes and diabetes[151]

Abbreviations: HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; BMI, body mass index; FA, fructosamine; GA, glycated albumin; OGTT, oral glucose tolerance test; IR, insulin resistance; 1, 5 AG, 1, 5 anhydroglucitol; FetA, fetuin-A; TLR4, toll-like receptor 4; T2DM, type 2 diabetes mellitus; α-HB, alpha-hydroxybutyrate; α-KB, α-ketobutyrate; L-GPC, L-alpha glycerylphosphorylcholine; Lp(a), lipoprotein(a); HDL-C, high-density lipoprotein cholesterol; HDL-LpPLA$_2$, HDL-associated lipoprotein-associated phospholipase A2; MBL, mannose binding lectin; CVD, cardiovascular disease; THBS1, thrombospondin 1; GPLD1, glycosylphosphatidylinositol-specific phospholipase D1; NF-Kβ, nuclear factor-κB; miRNA, microRNA; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; CRP, C-reactive protein; IL, interleukin; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; PAI-1, plasminogen activator inhibitor-1; IL-1RA, IL-1 receptor antagonist; SGLT$_2$, sodium-glucose co-transporter 2.

this reason, HbA1c and OGTT levels may be discrepant as individuals classified as having prediabetes according to OGTT results may be normoglycemic by HbA1c standards.

Moreover, it is not clear which cut points for HbA1c would be most sensitive. Utilizing ADA criteria, HbA1c may miss individuals with prediabetes despite levels <5.5% (37 mmol/mol).[26] The NHANES and Screening for Impaired Glucose Tolerance studies demonstrated that only 60–70% of subjects had normal glucose tolerance (NGT) when HbA1c levels were <5.7% (39 mmol/mol).[27–29] In addition, the HbA1c threshold for prediabetes does not consider ethnicity, body mass index (BMI), and age, all of which may significantly alter HbA1c levels.[30–33] For example, HbA1c levels are higher among African Americans, Hispanics, and Asian/Pacific Islanders compared to non-Hispanic whites. One study demonstrated that HbA1c is 0.3% higher in black men and 0.4% higher in black women.[34] As a result, standard classification ranges may misdiagnose some individuals from certain ethnic groups with prediabetes, thus overestimating the prevalence of prediabetes.

HbA1c is not always a reliable measurement of average circulating glucose levels. HbA1c has a life span related to the half-life of the red blood cell ranging from 90 to 120 days.[35] Therefore, changes in the production rate or circulating life span of red blood cells will affect HbA1c levels; for example, reduced production leads to a greater percent of older cells, whereas more rapid turnover reduces the average time during which the red cells are exposed to hyperglycemia.[36] Several clinical conditions may result in overestimation or underestimation of HbA1c levels. Conditions in which HbA1c is falsely elevated include iron deficiency anemia, asplenia, folate and vitamin B-12 deficiency, severe hypertriglyceridemia, and uremia.[36] Falsely low HbA1c occurs in hemolytic anemia, blood loss,[37,38] splenomegaly, and end-stage renal disease.[36] Hemoglobin variants, such as HbS, HbC, HbD, and HbE,[39] may also result in overestimation or under-estimation of HbA1c, depending on which method is used.[36] For these reasons, HbA1c alone can be inadequate for diagnosing prediabetes, and more accurate diagnosis may require confirmation with other biomarkers.[40]

Fructosamine

Fructosamine (FA) has been used as an alternate glycemic marker for diabetes screening and may be potentially useful for diagnosing prediabetes. FA is a ketoamine created by glycosylation of total serum proteins, primarily albumin.[41] FA increases in states of high glucose concentrations. Since it reflects average blood glucose concentrations over the previous 1–4 weeks,[38] it can be a useful clinical marker of short-term glycemic fluctuation and glucose control.[38,52] FA is especially useful in conditions that affect hemoglobin reliability, as already described, and has moderate sensitivity and high specificity (Table 1).[52] Other advantages of FA include cost-effectiveness and convenience, as its measurement does not require fasting.[42,43]

Limitations of FA include higher within-subject variability and falsely low levels when conditions leading to rapid

albumin turnover are present such as nephrotic syndrome and liver disease.[44] There are conflicting data regarding its efficacy as a biomarker for prediabetes. Several studies found that FA correlates with hyperglycemia and HbA1c levels in both type 1 diabetes mellitus and type 2 diabetes mellitus (T2DM).[41,45,46] FA may also indicate the risk for developing microvascular complications.[45] However, not all studies have found that mean serum FA levels are useful for prediabetes screening.[37,38,41,47–49]

Thus, FA could be a valuable complementary marker in clinical conditions where HbA1c may be inaccurate. However, as the literature concerning FA is limited, and studies included small or niche patient cohorts, there are insufficient data to conclude its role as an alternate biomarker for microvascular complications.

Glycated albumin

Similar to FA, glycated albumin (GA) has been found to be a superior index of glycemic control than HbA1c in patients with renal failure, hemolytic anemia, and those receiving blood transfusions.[41,45] FA refers to all glycated serum proteins, which includes GA. Therefore, as FA is not corrected for albumin or total protein concentration, FA levels can fluctuate in certain conditions such as liver disease. Alternatively, GA measures the ratio of GA to total albumin.[50] Thus, GA is preferred to FA in clinical conditions that result in protein loss such as nephrotic syndrome, liver, and thyroid disease.[50] While it is unclear whether FA should be corrected for total serum protein concentration, one study described an improvement in the correlation of FA with HbA1c when serum FA was corrected for albumin.[46,51]

Serum GA levels of 15–16% in Asian populations were associated with diabetes.[52,53] Furthermore, GA has moderate sensitivity and specificity for diagnosing prediabetes and diabetes (Table 1). However, combining FPG <100 mg/dL (5.56 mmol/L) with serum GA <15% to exclude diabetes, and FPG ≥126 mg/dL (7.0 mmol/L) or serum GA ≥17% to diagnose diabetes, increased the sensitivity of GA. There are no clear threshold values for FA and GA for prediabetes but one study used a level ≥230 µmol/L for FA and ≥13.35% for GA, both of which correlated with HbA1c of 5.7% (39 mmol/mol) for detecting prediabetes.[49] GA as well as FA is associated with CVD, ischemic stroke, retinopathy, chronic kidney disease, and death in the Atherosclerosis Risk in Communities Study.[51,54] These associations were similar to those of HbA1c,[54] suggesting that FA and GA may be useful alternative biomarkers in clinical conditions where HbA1c is inaccurate.[51] The combination of GA with HbA1c was shown to predict prediabetes with greater sensitivity than HbA1c alone.[49] In addition, GA may be superior to FA for detecting prediabetes, whereas the combination of FA with HbA1c was not superior to HbA1c alone.[47,49,50,55]

There are conditions in which GA may be inaccurate due to changes in albumin turnover. For example, the lower GA levels observed in obesity may be due to increased albumin catabolism and decreased rate of albumin synthesis from obesity-associated inflammation. However, the precise mechanism for the lower GA in obesity is unclear.[52,53,56–58] GA may be artificially low in individuals with increased BMI, body fat mass, and visceral adiposity.[53] The mechanism for alterations in GA levels in these conditions is not well understood.[59]

1,5 Anhydroglucitol

1,5 Anhydroglucitol (1,5 AG), a dietary monosaccharide, has been suggested as a prediabetes marker. As the proximal tubules in the kidney have a greater affinity for glucose than 1,5 AG, high glucose levels prevent 1,5 AG reabsorption resulting in elevated 1,5 AG urinary excretion. Therefore, plasma 1,5 AG concentrations are inversely correlated with plasma glucose levels[60] demonstrated in a study in which the 1,5 AG level was highest in the control group followed by the prediabetes and diabetes groups, respectively.[60] Some studies, but not all, have found an inverse relationship between 1,5 AG and OGTT 2-hour post-glucose levels.[61,62] Studies have also shown an inverse relationship between 1,5 AG and HbA1c as well as FPG levels.[63]

Similar to FA, 1,5 AG may be a useful biomarker as it reflects glucose levels within the preceding 10–14 days.[61] 1,5 AG is stable, reproducible, and less costly when compared to other glycemic diagnostic tests.[61] It may be useful for identifying postprandial glycemic excursions and individuals at risk of complications as 1,5 AG has been associated with retinopathy and microvascular and macrovascular events in diabetes. However, it is unclear if 1,5 AG is superior to HbA1c. Plasma 1,5 AG levels can change based on diet, sex,[64,65] and race. Levels are also affected by renal hemodynamics or treatment with sodium-glucose co-transporter 2 inhibitors.[66,67] There is no consensus on the use of 1,5 AG as a prediabetes screening tool.[65,66]

Adiponectin

Adiponectin, derived from adipose tissue, exhibits insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties and is an independent predictor of diabetes.[68] Lower levels of adiponectin are associated with increased IR and

obesity, while higher levels have been related to lifestyle intervention groups in diabetes prevention trials.[68] The association of adiponectin with diabetes risk appears to be evident at a much earlier stage in the progression to diabetes; more specifically, lower adiponectin levels were observed a decade before diabetes was diagnosed, particularly in men.[68] Additionally, in offspring of parents with T2DM, baseline adiponectin levels are inversely related to the risk of incident prediabetes independent of ethnic or sex differences.[69] Using the hyperinsulinemic euglycemic clamp and intravenous glucose tolerance test, adiponectin levels were directly correlated with insulin sensitivity and indirectly with insulin secretion.[69]

Fetuin-A

Fetuin-A (FetA) is a hepatic secretory glycoprotein that correlates with increased risk of developing T2DM and associated complications.[70] Importantly, unlike adiponectin, the EPIC-Potsdam prospective cohort study found that FetA was independently associated with T2DM after controlling for BMI and waist circumference.[70] FetA has been proposed to promote lipid-induced IR through the toll-like receptor 4 (TLR4)-inflammatory signaling pathway, which results in production of inflammatory cytokines.[71] As chronic inflammation induced by free fatty acids (FFAs) has been thought to result in IR, the FFA-TLR4 signaling pathway has been recognized as a cause of IR. However, FFA may not bind directly to TLR4. Pal et al showed that FetA binds to TLR4, and regulates insulin sensitivity through this interaction.[71] High-fat diet-fed FetA knockdown mice have less TLR4-mediated inflammatory signaling in adipose tissue and IR, whereas intravenous injection of FetA in this model induced inflammatory signaling and IR. FFA-induced inflammatory cytokine expression in adipocytes occurred only in the presence of both FetA and TLR4; removing either prevented FFA-induced IR. FFAs did not produce IR in adipocytes with mutated TLR4 or galactoside-cleaved FetA. FetA, TLR4, interleukin (IL)-6, and tumor necrosis factor (TNF)-α were elevated in obese subjects with diabetes, suggesting an association between lipids, FetA concentrations, and TLR4 expression with IR.

Studies of the association between FetA concentration and CVD have been conflicting. No association,[72] a positive association,[73] or an inverse association have been reported. However one multi-ethnic US study found a positive trend in those with IFG or diabetes.[74] FetA may be associated with higher risk of CVD in those susceptible to the development of IR.[74]

Taken together, these findings suggest that FetA is an endogenous ligand for TLR4 through which lipids induce IR. FetA may therefore serve as a novel therapeutic target for IR.

Metabolites and microRNA

Amino acids

Felig et al found that fasting branched chain and aromatic amino acids correlated with obesity and serum insulin, whereas glucose loading decreased amino acid levels in insulin-sensitive individuals but not in insulin-resistant individuals.[75] This is likely due to insulin-mediated inhibition of proteolysis by skeletal muscle. More recent studies have demonstrated a correlation between amino acids and prediabetes, IR, and obesity.[76] Branched chain amino acids (BCAAs), isoleucine, leucine, valine, tyrosine, as well as aromatic amino acid phenylalanine and glycine have been significantly associated with diabetes risk.[75,77–80] In addition, glutamine, methionine, cysteine, and 2-aminoadipic acid are increased in insulin-resistant states.[81–83] By contrast, glycine levels are decreased in individuals with prediabetes.[84–86] To further support this, a comprehensive systematic review and meta-analysis demonstrated positive associations with BCAAs and aromatic amino acids and inverse associations with glycine and glutamine with risk of T2DM.[85] Alterations in circulating amino acid levels may represent a significant predictive biomarker for IR and T2DM.

α-Hydroxybutyrate

α-Hydroxybutyrate (α-HB) is an organic acid byproduct produced during the synthesis of α-ketobutyrate (α-KB), a product of amino acid catabolism (threonine and methionine) and glutathione anabolism (cysteine formation pathway) in hepatic tissue.[76] The formation of α-KB is catalyzed by lactate dehydrogenase and α-HB. During oxidative stress, the rate of hepatic glutathione synthesis increases, resulting in increased production of α-KB.[85,87] This causes a decrease in the availability of L-cysteine for glutathione synthesis and an elevation in α-HB. Thus, in IR, increased oxidative stress and lipid oxidation may cause chronic shifts in glutathione synthesis leading to elevated α-HB levels.[88,89] This is demonstrated by increased urinary α-HB excretion in IR.[90]

Using α-HB as a biomarker, previous studies were able to distinguish NGT-insulin-sensitive (NGT-IS) subjects from IGT and IFG subjects and NGT-IS subjects from those with NGT-IR. Furthermore, using multiple logistic regression analyses, α-HB was found to be significantly associated with IR, independent of sex, age, BMI, and collection site.[76] Furthermore, IR was associated with reductions in glycine

and serine, which are upstream of α-KB, and an elevation in cysteine. The underlying mechanism may be related to redox imbalance with IR resulting in an increase in α-HB. α-HB may be an effective biomarker for prediabetes.[76,89]

Linoleoyl-glycerophosphocholine

Choline-containing phospholipids and sphingomyelins have been associated with increased risk of T2DM. Linoleoyl-glycerophosphocholine (L-GPC) was investigated in the Relationship Between Insulin Sensitivity and Cardiovascular Disease study.[91] L-GPC is formed by hepatic phospholipase A2 and circulatory lecithin-cholesterol acyltransferase. Phospholipase A2 activity is increased with inflammation. L-GPC may inhibit phospholipase A2 through noncompetitive enzyme inhibition, thereby exhibiting anti-inflammatory properties.[76,92] Thus, L-GPC is a negative predictor of T2DM progression in contrast to α-HB, a positive predictor.[76,93]

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is synthesized by the liver. Elevated levels of LP(a) are an independent risk factor for developing CVD.[94] An inverse relationship between serum Lp(a) and the prevalence of prediabetes and T2DM has been reported, although the mechanism for the relationship between serum Lp(a) and T2DM is not clear.[95] Insulin may play a role in reducing Lp(a) levels.[95]

Triglycerides and high-density lipoprotein

Elevated serum triglyceride (Tg) levels have been associated with β-cell dysfunction and reduced insulin secretion in prediabetes.[94] Mechanistically, hypertriglyceridemia reduces glucose-induced insulin secretion through the glucose-fatty acid cycle, and promotes β-cell apoptosis by stimulating the production of ceramide and nitric oxide.[96] Also, elevated Tg levels can cause lipotoxicity by accumulating within pancreatic β cells.[94,96]

Cholesteryl ester transfer protein mediates the exchange of lipids from Tg-rich lipoproteins with high-density lipoprotein (HDL). Increased Tg levels in insulin-resistant states accelerate this exchange.[97] Then, the Tg in HDL cholesterol (HDL-C) are hydrolyzed by hepatic lipase, resulting in smaller HDL-C particles. ATP-binding cassette transporter A (ABCA1) mediates the efflux of cholesterol to small HDL3 particles.[97] Subjects with prediabetes have significantly increased levels of small HDL3 particles compared with HDL-C levels.[97] The proportion of small HDL3 particles is positively associated with Tg and negatively associated with HDL-C.[97] In contrast to Tg, HDL-C promotes insulin secretion through its interaction with ABCA1. Low HDL-C concentrations may also lead to progression to diabetes from prediabetes. However, it is unclear if HDL-C levels are associated with β-cell dysfunction.[96]

Lipoprotein-associated phospholipase A_2 ($LpPLA_2$) is an enzyme that degrades oxidatively fragmented phospholipids and may play a role in atherogenesis.[97] Individuals with IFG have significantly decreased HDL-associated $LpPLA_2$ (HDL-$LpPLA_2$) activity compared with subjects with normoglycemia. Low-density lipoprotein-associated $LpPLA_2$ may exert pro-inflammatory effects, whereas HDL-$LpPLA_2$ may have an atheroprotective role.[97] Increased levels of small HDL3 particles and decreased activity of the anti-atherogenic HDL-$LpPLA_2$ were found in subjects with IFG.[97] Thus, subclasses of HDL-C may play a role in the pathogenesis of prediabetes.

Ceramide

In addition to Tg, ceramides have a positive association with prediabetes and T2DM.[85,98] Ceramides are lipid molecules that mediate IR.[99,100] Studies have shown that ceramides inhibit insulin action by decreasing phosphorylation and activation of Akt; accumulate in insulin-resistant tissues; and induce inflammation through the nuclear factor-κB (NF-Kβ)–TNF-α axis.[100,101] Furthermore, ceramides correlate with coronary artery disease.[102]

Additional studies are needed to understand the relationships between lipid metabolism, prediabetes, and diabetes.

Ferritin and transferrin

Ferritin is an intracellular protein that stores and releases iron. Elevated serum ferritin and transferrin saturation have been strongly associated with increased risk of prediabetes and diabetes.[103,104] Furthermore, a positive correlation with increased FPG and serum ferritin has been demonstrated.[104] Iron contributes to IR through the production of highly active radical formation, damage to DNA and cell membrane integrity, β-cell oxidative stress resulting in decreased insulin secretory capacity,[105] and interference with glucose uptake in skeletal muscles and adipocytes. Moreover, catalytic iron stimulates the formation of reactive oxidative species, hepatic dysfunction, and β-cell apoptosis,[105,106] all of which contribute to IR. Dietary iron restriction or chelation prevents the development of diabetes and loss of β-cell function.[105] The threshold levels of ferritin that correlate with IR are uncertain as these may vary with sex and age.[107] Additional studies are needed to better understand ferritin and its role in prediabetes.

Mannose binding lectin serine peptidase, thrombospondin 1, and glycosylphosphatidylinositol-specific phospholipase D1

Mannose binding lectin-associated serine proteases are important for innate immune responses and activation of the lectin pathway of the complement system.[108] MASP1, the most abundant serine protease of the complement lectin pathway, has a major role in the complement cascade.[108] MASP1 has been shown to positively correlate with prediabetes, diabetes, as well as CVD. One study demonstrated that the onset of prediabetes and IR occurred earlier in those with increased MASP1 plasma levels.[109] There was a positive association with elevated FPG and 2-hour glucose levels, but this weakened when adjusted for triacylglycerol levels. This suggests that triacylglycerol may mediate part of the association between MASP1 and HOMA-IR.[109]

Thrombospondin 1 (THBS1) and glycosylphosphatidylinositol-specific phospholipase D1 (GPLD1) are positively associated with prediabetes, whereas apolipoprotein A-IV (ApoA-IV) is inversely associated.[109] THBS1 is a glycoprotein and member of the THB family, which has numerous functions such as cellular adhesion and migration regulation, cytoskeletal organization, cell proliferation and apoptosis, and cell-to-cell interactions.[110] This matrix protein has been found to be associated with increased IR, which may be attributable to THB's inflammatory properties, as well as increased 2-hour glucose levels and higher prediabetes prevalence.[109]

GPLD1, mainly produced in the liver, releases glycosylphosphatidylinositol-anchored membrane proteins.[111] It is associated with serum lipoproteins and has been linked with diabetes and prediabetes.[109,111] ApoA-IV, a component of chylomicrons, very low-density lipoprotein and HDL, has been shown to have a significant inverse relationship with prediabetes and diabetes.[109] The exact role of ApoA-IV in vivo is unknown aside from regulating appetite and chylomicron production but it may have antioxidant and anti-inflammatory properties.[112,113]

There are limited studies describing the relationship between THBS1, GPLD1, and ApoA-IV and prediabetes; therefore, more data are required to better understand their respective roles.

Acyl-carnitine

Fatty acid oxidation (FAO) is a major source of cellular energy.[114] L-carnitine plays a significant role in FAO as it transports activated long chain fatty acids (LCFA) from the cytosol into the mitochondria, a process referred to as the carnitine shuttle. Once inside, fatty acids undergo esterification to CoA. Carnitine palmitoyltransferase 1 exchanges the CoA moiety for carnitine resulting in acyl-carnitine production.[115] Recently, serum levels of acyl-carnitines have been shown to be elevated in prediabetes.[116,117] However, the essential role of acyl-carnitine in FAO and its mechanism in IR are uncertain. It has been proposed that impairment of FAO and dysregulated mitochondrial function result in accumulation of intermediary products such as acyl-carnitines. Thus, there is a mismatch of LCFA delivery and the tricarboxylic acid cycle.[118] Furthermore, acyl-carnitines interact with NF-Kβ, which promotes inflammation and IR.[118] However, there are few studies of acyl-carnitines in prediabetes.

MicroRNAs

MicroRNAs (miRNAs) are small, noncoding RNAs involved in post-transcriptional gene expression. These are pertinent to many biological and pathophysiological processes such as growth, development, differentiation, proliferation, and cell death. Recently, miRNAs have been studied in prediabetes,[119–121] several of which were increased including miR-192 and miR-193b. miR-192 regulates tumor protein p53, and miR-193b is important for the differentiation of brown adipocytes and inflammation reduction.[122] Levels of both miRNAs were elevated in those with IFG and IGT. Furthermore, miR-192 and miR-193b have been correlated with Tg levels and the fatty liver index in animal models,[120] which may be significant as a fatty liver can be associated with prediabetes.[123] Moreover, exercise was shown to significantly decrease miR-192 and miR-193b concentrations.[120]

Other miRNAs significantly elevated in T2DM include miR-9, miR-29a, miR-30d, miR-34a, miR-124a2, miR-146a, and miR-375, all thought to play a role in β-cell function. These miRNAs were found to negatively regulate insulin expression, production, or secretion. However, no statistically significant increases were found in subjects with prediabetes, suggesting that there may be reversible pathophysiological processes that occur during prediabetes but not in T2DM.[124]

Additional miRNAs are decreased in prediabetes. Circulating levels of miR-126, abundant in endothelial cells playing a role in endothelial homeostasis and vascular integrity, are decreased in IGT/IFG and T2DM.[125] miR-126 levels are increased with diet and exercise. miRNA-15a levels were also significantly lower in prediabetes, T2DM, and IFG/IGT.[119] miR-15a is thought to regulate and promote insulin biosynthesis by inhibiting endogenous uncoupling protein-2

gene expression and increasing insulin secretion.[126] Therefore, miR-15a has been suggested to play a significant role in β-cell function and insulin synthesis.

Inflammatory markers

Prediabetes and IR are characterized by a marked inflammatory state.[127–129] Biochemical markers of acute-phase reactants and inflammatory cytokines are elevated on the onset of T2DM and may even further increase with disease progression. These markers, such as C-reactive protein (CRP), white blood cell count, and fibrinogen, have been investigated as potential predictors for the development of T2DM such as in the Atherosclerosis Risk in Communities study.[130]

CRP and IL-6

CRP is the most widely studied inflammatory marker in CVD and its clinical use continues to evolve. CRP is primarily derived from IL-6-dependent hepatic biosynthesis and is a primary marker of the acute phase response. Many investigations have demonstrated elevated levels of both IL-6 and CRP among individuals with T2DM and IR. In the Women's Health Study, a nationwide cohort of 27,628 women without diabetes mellitus (DM), CVD, or cancer at baseline, 188 women developed DM over a 4-year follow-up period.[131] Median baseline levels of IL-6 and CRP were significantly higher among cases than controls. In addition, higher levels of IL-6 and CRP were associated with a greater risk of diabetes development. Relative risks of incident T2DM for increasing quartiles of IL-6 were 1.0, 2.5, 4.1, and 7.5, respectively ($p<0.001$ for trend), and for increasing quartiles of CRP were 1.0, 2.2, 8.7, and 15.7, respectively ($p<0.001$ for trend). While adjustment for BMI attenuated these relative risks, the results were still positive. These findings were similar when only including women with a baseline HbA1c of 6.0% (42 mmol/mol) or less and after adjustment for fasting insulin levels. This suggests that these inflammatory markers may be useful in identifying individuals at risk of developing T2DM.

The Insulin Resistance Atherosclerosis Study (IRAS) was a multicenter study of 1,625 individuals followed over 5.2 years.[132] Individuals with prediabetes were defined as those developing diabetes during follow-up. Subjects with prediabetes who were also insulin resistant had elevated CRP levels compared to both insulin-sensitive individuals with prediabetes and non-diabetics. These differences were thought partly due to differences in body weight. Since subjects with prediabetes and IR were not hyperglycemic, subclinical inflammation could not be attributed solely to hyperglycemia. This hypothesis was supported in another study demonstrating that the glycemic index was not associated with CRP and risk of T2DM, suggesting that hyperglycemia per se is not the underlying mechanism linking diabetes and inflammation.[133]

The association between CRP and prediabetes has been confirmed in other studies. The Gutenberg Health Study was a prospective, observational single-center cohort study that included 15,010 adults, 1,425 of whom had prediabetes and 1,299 had diabetes according to HbA1c concentrations.[134] CRP was shown to increase incrementally from normoglycemia to prediabetes (1.4 vs. 2.3 mg/L), whereas only a small increase was observed between subjects with prediabetes and diabetes (2.3 vs. 2.4 mg/L), suggesting that early immune activation plays a role in the onset of diabetes. Genetic variants in the innate immune system and inflammatory cascade also affect CRP and predisposition to T2DM.[128,135]

Other studies have further supported the association between increased levels of CRP and IL-6 and prediabetes.[127,136] In a meta-analysis evaluating IL-6 with the risk for developing T2DM, the overall relative risk was 1.31 (95% confidence interval [CI] 1.17–1.46; $p=0.000$) per 1 log pg/mL increment in IL-6 levels.[137] In another meta-analysis, these authors found that the overall risk of T2DM was 1.26 (95% CI 1.16–1.37; $p=0.000$) per 1 log mg/L increment in CRP levels.[137] There may be differences in CRP as a predictor of diabetes in women compared to men.[137] Interestingly, the meta-analysis showed that IL-6 was more strongly associated with T2DM than CRP, questioning the pathogenesis of subclinical inflammation in diabetes. CRP may therefore have a potential downstream role in this process but may not be the precipitating factor. However, in contrast to the latter findings, another study demonstrated that CRP was elevated in obesity and IR, whereas levels were low in insulin-sensitive or obese individuals.[138]

White blood cell count, fibrinogen, and hematological indices

White blood cell count and fibrinogen are also markers of immunity and inflammation that may have clinical relevance for disease progression and organ-specific complications in diabetes. Leukocytosis may also predict coronary heart disease.[139] Therefore, the early identification of high-risk individuals may prevent the onset and/or progression of CVD. A high white blood cell count has been shown to predict worsening insulin action, insulin secretory function, and T2DM development in Pima Indians.[140] Fibrinogen may contribute to atherosclerosis by affecting blood viscosity, platelet aggregation, and fibrin formation. Fibrinogen also

modulates coagulation activation and fibrinolysis, and may enhance plaque progression. In the Gutenberg Health Study, fibrinogen levels were higher in prediabetes than in diabetes, although the reason for this finding is not clear.[134]

The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are also indicators of subclinical inflammation. In a study of 110 adults, subjects were divided into four groups: NGT, IGT, newly diagnosed T2DM, and known T2DM without complications. NLR values were significantly higher in those with prediabetes, newly diagnosed diabetes, and known diabetes compared to the control group.[141] PLR values were significantly lower in the prediabetes and newly diagnosed diabetes groups but higher in subjects with T2DM. Of note, NLR was higher in obese patients with diabetes than in those without diabetes. NLR has also been associated with both microvascular and macrovascular complications in diabetes.[141–144]

Another investigation demonstrated that 1-hour post-load glucose levels were associated with a significantly higher white blood cell count and fibrinogen.[145] Fiorentino et al showed that individuals with prediabetes had a significant increase in CRP, fibrinogen, and white blood cell count after adjusting for sex, age, smoking, and fasting and 1- and 2-hour post-load glucose levels.[146]

Plasminogen activator inhibitor-1

Tissue plasminogen activator-1 (PAI-1) is a marker of reduced fibrinolysis with decreased activity resulting in coagulation abnormalities.[147] Changes in PAI-1 levels were shown to be an independent predictor of incident diabetes in IRAS.[148]

IL-18

Through oxidative mechanisms, hyperglycemia acutely increases cytokine concentrations including plasma IL-6, as already described, as well as TNF-α and IL-18.[149] In a prospective case-cohort study, subjects with IL-18 in the highest quartile had a 70% increased risk of developing T2DM compared to those in the lowest quartile.[149] IL-18 also increased with progression from prediabetes to diabetes in the Gutenberg study.[134]

IL-1 receptor antagonist

The IL-1 pathway may be induced by glucose and FFAs in the setting of overfeeding and contribute to an inflammatory state.[150] The IL-1 receptor antagonist (IL-1RA), produced by adipocytes, is an anti-inflammatory marker elevated in prediabetes and diabetes, possibly as a reactive response to inflammation.[134] In the Whitehall Study, a case-cohort study of 355 individuals with incident T2DM, an increase in IL-1RA in individuals with prediabetes occurred in parallel with decreasing insulin sensitivity, transiently increasing β-cell function, and 2-hour glucose levels, all of which occurred years before the occurrence of T2DM.[151] Levels of IL-1RA were elevated 13 years before the diagnosis of T2DM. Of note, IL-1RA increased rapidly 6 years before diagnosis even after adjusting for obesity.

Patient-focused perspectives

According to the Centers for Disease Control and Prevention, as many as 90% of individuals with prediabetes are undiagnosed[1] and therefore fail to receive guidance on lifestyle changes to prevent progression to T2DM. While HbA1c and glucose determinations for screening have been used in clinical practice for many years, each has deficiencies. As previously discussed, since HbA1c is insensitive for diagnosing acute and intermittent hyperglycemia and can be affected by various medical conditions and ethnicity, identification of other biomarkers would be of immense value. OGTT, while providing important information with regard to risk of developing T2DM that HbA1c or FPG do not, is associated with considerable variability, requires fasting, and is invasive and time consuming. However, intermediate time points during the OGTT (e.g., 30 or 60 minute post-load values) appear to predict progression to T2DM better than fasting, 2-hour post-load glucose or HbA1c levels, making this approach more favorable with the possibility of shortening the traditional 2-hour test.[152] Additional studies are required to identify the most accurate biomarker(s), recognizing that a single determinant will likely have inherent limitations. Therefore, combining several biomarkers may more precisely predict those at high risk for developing prediabetes and subsequent progression to diabetes.

Conclusion and future perspectives

Categorical or absolute definitions of dysglycemia when applied to a continuous pathophysiologic process may inadvertently underestimate those at risk for progression. Progressively rising glucose levels, even within the so-called "normal range", occur relatively late in the evolution to T2DM when β-cell function may already be reduced.[153] Therefore, a vital need exists to identify more sensitive and precise biomarkers capable of predicting progression to dysglycemic states at the earliest time point when β-cell function is still relatively more optimal and may be more responsive to lifestyle modification. Combining biomarkers in a clinical setting may provide better sensitivity and specificity in predicting prediabetes and

diabetes. Additional comparison studies of biomarkers will be required to ascertain their clinical utility. Furthermore, genetic studies assessing mutations may provide additional insight into associations with metabolic abnormalities.[153,154]

Disclosure

The authors report no conflicts of interest in this work.

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