Section A-Research paper

An Insight about Markers of diabetes mellitus

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Abstract

Background: More than half of individuals with diabetes, mainly T2DM, are undiagnosed; cases are frequently not diagnosed until severe complications appear. However, even early diagnosis is not enough to slow the rise in the incidence of T2DM and its complications. Even in diagnosed patients, the disease's progression may be accelerated by aggravating factors such as the lack of rigorous glycemic monitoring, under-treatment, inadequate treatment adherence, and omission of lifestyle changes. In addition to the inability of biomarkers to reflect glycemic status accurately. Because early diabetes is largely asymptomatic, many patients are at risk of developing life-threatening complications due to hyperglycemia. In low- and medium-income countries, patients present with an even higher risk of complications due to inadequate healthcare. Irisin, a novel myokine produced in response to physical activity, promotes white-to-brown fat transdifferentiation. The name irisin referred to the ancient Greek goddess Iris, the messenger who delivered (bad) news from the gods. In mice, it has been demonstrated that irisin plays a key role in metabolic regulation, energy expenditure and glucose homeostasis. Since its discovery, it has been hypothesized that irisin may have beneficial effects on chronic diseases such as obesity and type 2 diabetes (T2D), as well as protective effects against cardiovascular risk. However, attempts to reproduce in humans the encouraging findings obtained in animal studies have had conflicting results.

Keywords: Markers, Diabetes mellitus, Irisin

Introduction

More than half of individuals with diabetes, mainly T2DM, are undiagnosed; cases are frequently not diagnosed until severe complications appear. However, even early diagnosis is not enough to slow the rise in the incidence of T2DM and its complications. Even in diagnosed patients, the disease's progression may be accelerated by aggravating factors such as the lack of rigorous glycemic monitoring, under-treatment, inadequate treatment adherence, and omission of lifestyle changes ***(1).***

In addition to the inability of biomarkers to reflect glycemic status accurately. Because early diabetes is largely asymptomatic, many patients are at risk of developing life-threatening complications due to hyperglycemia. In low- and medium-income countries, patients present with an even higher risk of complications due to inadequate healthcare ***(1).***

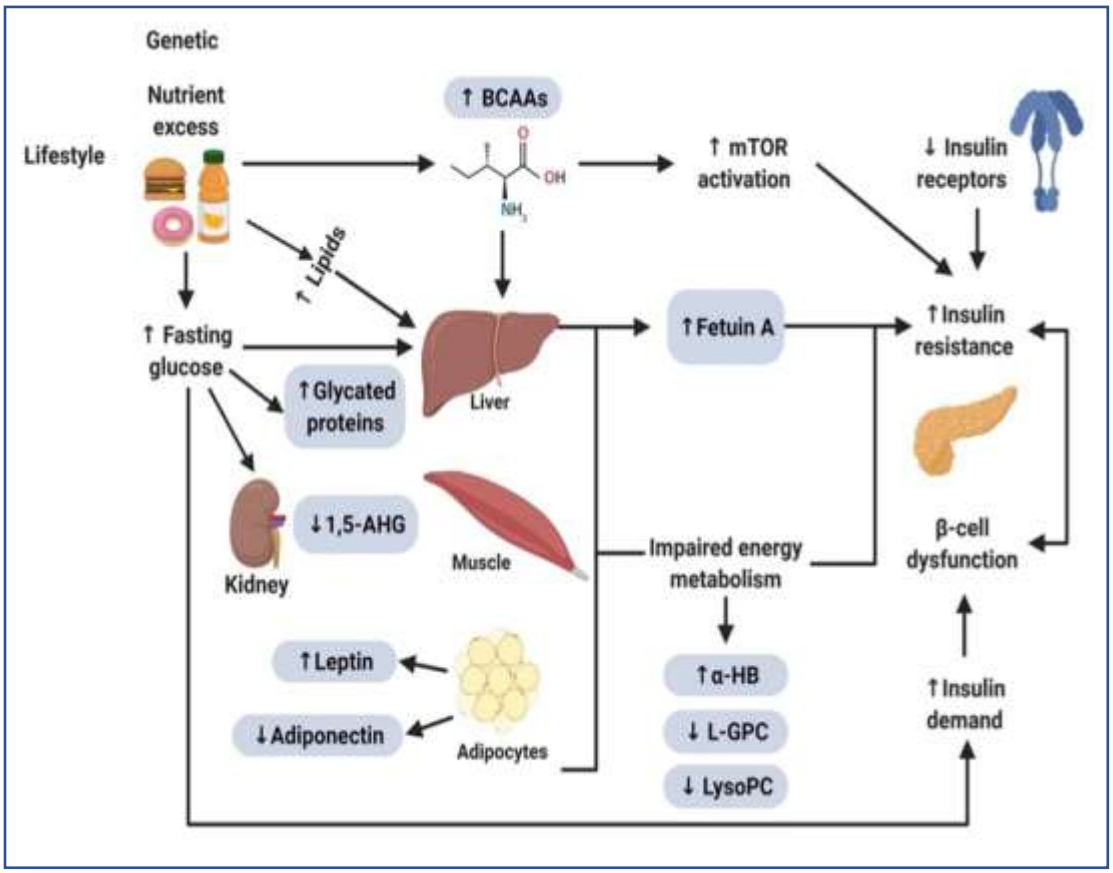

Figure (1): *Model representing the relationship between impaired energy metabolism and biomarkers for the screening and diagnosis of type 2 diabetes mellitus. Abbreviations: 1,5-AHG, 1,5-anhydroglucitol; BCAAs, branched-chain amino acid; α-HB, α-hydroxybutyrate; L-GPC, linoleoylglycerophosphocholine; LysoPC, lysophosphatidylcholine; mTOR, mammalian target of rapamycin* ***(2).***

Currently, there are several challenges in the management of T2DM that need to be addressed. On the technical side, there is a need for novel, more comprehensive strategies for optimal screening, early diagnosis, and adequate management of T2DM ***(3).***

Approaches combining the use of resources for risk assessment, such as Finnish Diabetes Risk Score (FINDRISC), along with more effective biomarkers for screening and progression T2DM, have a higher probability of success in managing the global diabetes epidemic. It will also positively impact the prevention of complications caused by hyperglycemic episodes in individuals diagnosed with diabetes and prediabetes by reducing the under-diagnosis and under-treatment of diabetes ***(4).***

Conventional Markers:

Fasting plasma glucose has been the gold standard diagnostic criterion for T2DM and is still the most widely accepted due to its availability, low cost, and compatibility with automated clinical chemistry analyzers. Among the disadvantages of FPG are that it requires at least 8-h fasting, shows substantial biological and diurnal variability, reflects only a single point in time, and the samples involved present stability issues. Despite this, FPG is still widely used individually and as part of blood chemistry panels ***(3).***

Currently, HbA1c measurement is a crucial part of the international guidelines for the diagnosis of T2DM. HbA1c testing is more clinical-workflow convenient than FPG and OGTT, as it does not require fasting, samples may be obtained at any time, and is a better predictor of long-term complications. Another significant advantage is that blood samples used for HbA1c testing present high stability and low short-term variability—one sample reflects average blood glucose concentrations over three months ***(5).***

HbA1c may also be used as a monitoring test and guide for T2DM treatment. It is essential that the test is performed by a National Glycohemoglobin Standardization Program (NGSP) certified method and standardized to the Diabetes Control and Complications Trial (DCCT) assay to avoid misdiagnosis. ***(1).***

Despite its advantages over FPG and OGTT, HbA1c presents some inconveniences, such as lower clinical sensitivity at the designated diagnostic threshold. Moreover, age, race, ethnicity, and any clinical condition that alters the lifetime of erythrocytes or haemoglobin levels can alter HbA1c independent of glucose concentration. Additionally, the limited availability and expense of HbA1c testing make it infeasible for routine use in some regions of the world ***(5).***

As a marker of early impaired glucose homeostasis, OGTT is a more sensitive method of prediabetes and diabetes diagnosis than FPG and HbA1c. Abnormally high plasma glucose concentration in OGTT is a proven indicator of prediabetes and diabetes. However, OGTT is relatively costly, can be complicated, and have low reproducibility in some settings ***(6).***

The test protocol requires that the patient ingest an oral load of 75g of glucose and undergo multiple blood draws over a two-hour period, which can be inconvenient and invasive for the patient. The need for timed samples creates logistical and analytical constraints. Despite its indication for T2DM screening by the ADA, OGTT is not usually performed on non-pregnant adults ***(3).***

FPG, OGTT, and HbA1c are not always perfectly concordant. This discordance can be partly explained because different physiological stages of glucose metabolism are measured; the same occurs between HbA1c and glucose-based tests. OGTT and HbA1c tests are not routinely performed in middle-to-low-income countries due to time and cost constraints. In these countries, FPG is still a valuable test for the screening, diagnosis, and monitoring of T2DM ***(6).***

On the other hand, HbA1c is a more reliable marker for assessing the presence and severity of the disease. Therefore, it is recommended by the ADA and WHO as an appropriate test for diabetes screening and diagnosis. The final objective in the guidelines for the screening, diagnosis, and monitoring of T2DM should not be to seek a perfect concordance between biomarkers. Rather, their determination should identify individuals with altered glycemic levels at risk of suffering long-term complications ***(3).***

Glucose in Unconventional Samples:

Non-conventional biological fluids such as saliva have been explored to develop non-invasive, cost-effective, and sensitive methods that can be applied to T2DM screening, diagnosis, and monitoring. Despite this, a method for glucose quantification in saliva has not been clinically validated, nor have reference values been established ***(7).***

Glucose concentrations in the saliva of normoglycemic patients may be below the detection limit of the method used, or interfering compounds in the saliva samples may hinder assay performance and result in low accuracy. If the technique is responsible, saliva-based testing with this method would be limited to monitoring glycemic control in already diagnosed patients ***(2).***

This evidences the need to validate specific techniques using more accurate and sensitive techniques for determining glucose in unconventional fluids, like saliva. More in-depth studies of the relationship between blood glucose levels and sweat in healthy and individuals with diabetes are also needed ***(7).***

Clinically Validated Biomarkers:

Traditional glycemic markers, such as glucose and HbA1c, present several limitations that can lead to under-diagnosis and poor disease prognosis in people with T2DM. As previously stated, HbA1c concentration cannot measure transitory hyperglycemic changes and is altered by patient characteristics (medical conditions and ethnicity). Furthermore, fasting glucose alone does not give enough information to fully understand the glycemic state of the patient ***(1).***

Evidence from studies comparing the performance of new glycemic markers such as glycated albumin (GA), fructosamine (FA), and 1,5-anhydroglucitol (1,5-AHG) have shown that they provide independent clinical information and can improve the prognostic value of conventional markers ***(8).***

Previous studies in the Atherosclerosis Risk in Communities (ARIC) Study framework have confirmed that FA, GA, and 1,5-AHG markers are strongly related to the risk of developing diabetes. These intermediate markers can be used to determine the risk of T2DM and its complications independently of fasting blood glucose and HbA1c values ***(9).***

The moderate correlation and clinical variations between non-traditional markers such as GA, FA, and 1,5-AHG and conventional markers might be due to the fact that they are more strongly influenced by postprandial excursions than HbA1c, which is more affected by long-term glycemia as well as by the differential effect of oxidative stress ***(9).***

The ability to evaluate blood glucose in the short-, intermediate-, and long-term is critical to face the health challenges posed by T2DM. The selective and combined use of these tools will allow access to more timely diabetes prevention, early diagnosis, and timely management of T2DM ***(3).***

Fructosamine:

Fructosamine (FA) refers to all stable ketoamines produced through the non-enzymatic glycation of circulating serum proteins (albumins, globulins, and other minority proteins). After a complex cascade of reactions, the early glycation products generate irreversible conjugates, called advanced glycation end products (AGEs) ***(9).***

The concentration of FA in serum increases in T2DM due to the higher sugar concentration in the blood. Therefore, it could be useful as a glycemic marker that allows discrimination between normoglycemic and individuals with diabetes. Also, its application as a biomarker for screening or diagnosis of gestational diabetes mellitus compared against OGTT has been reported ***(10).***

Unlike the determination of HbA1c, which measures long-term changes because of the longer circulating lifetime of hemoglobin, FA reflects glucose levels over 2 to 3 weeks. Furthermore, FA assays are more affordable and less complicated than HbA1c. The most widely employed methods for assessing FA are colorimetric-based, which are fast, technically easy, inexpensive, and available for automation. Several studies have shown strong correlations between FA and HbA1c in T2DM with high sensitivity and specificity to distinguish between normoglycemic and individuals with diabetes ***(10).***

Also, FA does not require fasting. In addition to its clinical application as a marker for diagnosing and monitoring hyperglycemia, high FA levels are associated with an increased incidence of vascular complications associated with T2DM, and persistently high FA levels indicate a more aggressive disease progression. The FA assay can be applied to detect and monitor T2DM, although it is currently only used in combination with the traditional marker. The use of FA as a risk predictor for the development of T2DM was evaluated ***(10).***

FA seems to have its application as a risk biomarker rather than a diagnostic or monitoring marker. FA as an intermediate marker is particularly beneficial for monitoring the glycemic status in patients with poor glycemic control or those starting a new therapeutic regimen. The assessment of salivary FA levels has been proposed as a possible biomarker that can be measured non-invasively, but more evidence is needed before its clinical application is established ***(9).***

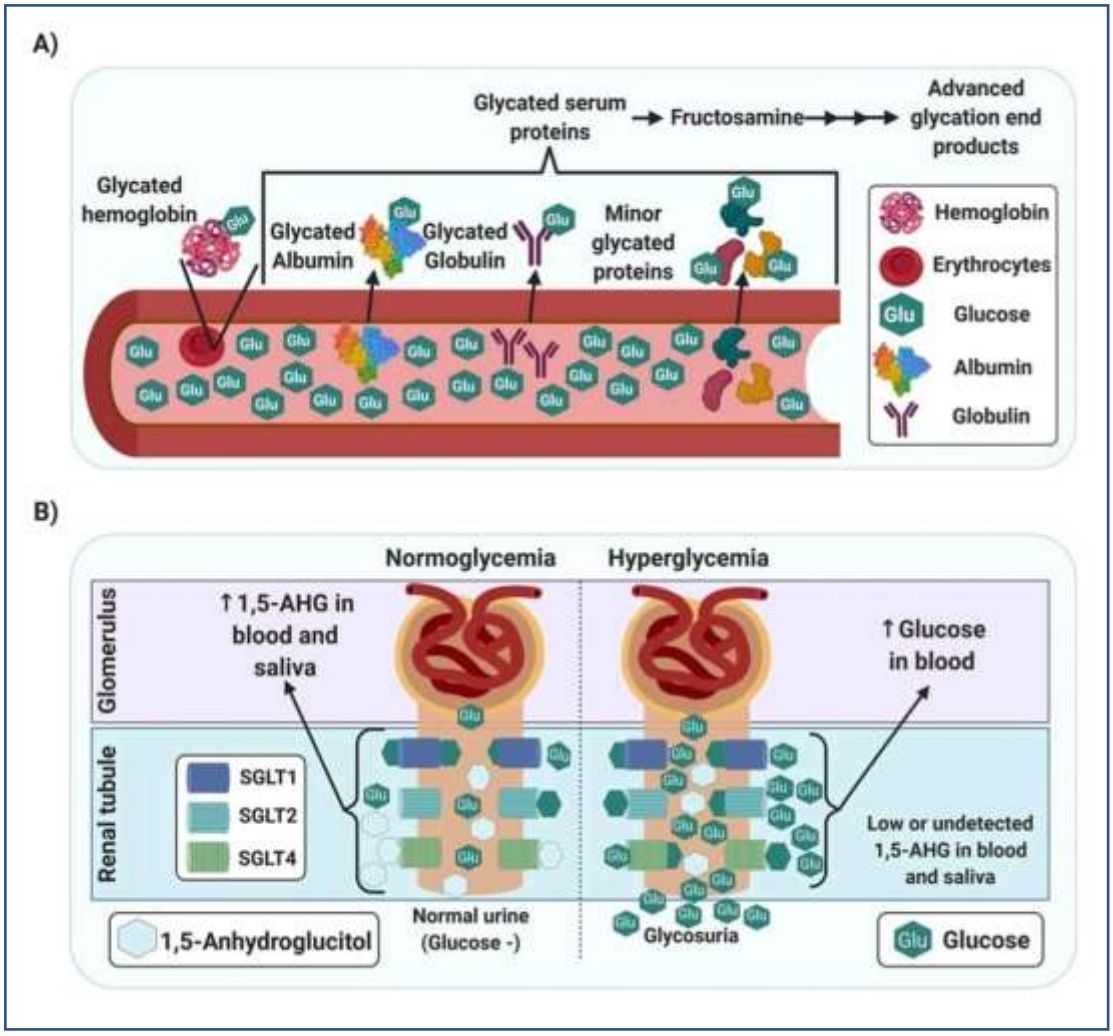

Figure (2): *'A' Graphical representation of the mechanism by which glycated proteins and fructosamine correlate to hyperglycemia. 'B' Kidney reuptake of 1,5-anhydroglucitol and glucose under normoglycemia and hyperglycemia. Abbreviations: 1,5-AHG, 1,5-anhydroglucitol; SGLT, sodium-glucose linked transporter* ***(2).***

Glycated Albumin:

Human serum albumin, the major circulating protein in blood, can undergo increased glycation due to hyperglycemia. Glycation is the non-enzymatic addition of reducing sugars, in this case, glucose, to amine groups in proteins. This addition creates an intermediate product that subsequently undergoes a rearrangement to create a more stable derivative, either an Amadori product or a ketoamine ***(11).***

There is a direct relationship between hyperglycemic states and the generation of glycated albumin; this, combined with the half-life of albumin, is the reason why the GA ratio can be used as an intermediate-term biomarker of glycemic control. The generation of AGEs from albumin and other serum proteins is directly related to the development and progression of diabetic complications. Thus, measurement of FA and GA can assess not only glycemic status for screening and diagnostics of T2DM but for its progression. The main methods for the isolation and quantification of GA are boronate affinity chromatography, ion-exchange chromatography, high-performance liquid chromatography, immunoassays, and a two-steps enzymatic assay; the first step is AGEs and peroxide elimination by ketoamine oxidase (KO) then hydrolyzation (proteinase) and oxidation by KO to produce hydrogen peroxide measured by a colorimetric method, the latter being the most reported ***(12).***

Additionally, GA as a biomarker of glycemic control has shown higher sensitivity and specificity than the gold standard for long-term monitoring, HbA1c. Furthermore, GA has been explored as a measure of risk for developing nephropathy and cardiovascular diseases. The possibility of supplementing self-

monitoring through capillary glucose measurements with a GA test on a POCT platform has been evaluated with great interest since it would reduce health expenses and improve patients' quality of life ***(11).***

Measurement of GA is especially relevant for controlling postprandial hyperglycemia and glycemic fluctuations. Because of its atherogenic potential, GA is a marker of cardiovascular risk, as well as a glycemic marker. GA has demonstrated similar diagnostic performance to HbA1c ***(11).***

It is important to mention that despite their promising results, the conventional markers HbA1c and OGTT and the new clinically validated marker GA do not detect T2DM in the same individuals strengthening the hypothesis that the best approach is the combined use of the available markers. However, GA has an advantage over HbA1c in that GA can be measured in both plasma and serum, so unlike HbA1c, it could be measured with the rest of the biochemical biomarker panel from a single blood sample ***(12).***

Despite evidence showing GA's clinical utility and superior efficiency over FA and HbA1c in a broad range of clinical settings, there are no commercial GA assays currently available. However, due to the high incidence of T2DM, healthcare professionals recognize the need for auxiliary indicators for their screening and early diagnosis that can add to traditional tools or, in an ideal case, a new biomarker that can improve prevention schemes and patient care. This need can be an impetus for a unified GA quantification method and comprehensive studies regarding its clinical application ***(12).***

Microalbuminuria

In an attempt to control the consequent increase of DN prevalence, screening for diabetic kidney disease are essential in diabetic patients. The early screening of diabetic nephropathy has focused currently on microalbuminuria which is considered as the most widely investigated biomarker for the screening of DN. Microalbuminuria allows the detection of patients with an increased risk for the development of overt diabetic nephropathy with persistent macroalbuminuria. However, Albuminuria has numerous confusing concerns linked with it such as exercise, acute illness, cardiac failure, and urinary tract infection. Moreover, it has been reported to occur in the urine of non-diabetic subjects, signifying the non-specificity of albuminuria for the exact estimate of the diabetic kidney disorder. Moreover, impaired renal function may be present even in patients with normal urinary albumin excretion rate .Therefore, the diagnostic value of microalbuminuria in early-stage DN is limited as renal injury commonly precedes proteinuria . ***(13)***

1,5-Anhydroglucitol:

The glycemic biomarker 1,5-anhydroglucitol (1,5-AHG), or 1-deoxyglucose, is a six-carbon monosaccharide which also is known as 1-deoxyglucose. As one of the major polyols in the human body, 1,5-AHG was first isolated from the Polygala amara plant in 1888, and its structure was defined in 1943. This biomarker is metabolically stable, originates mainly from the diet (where it is found in low concentrations) and is well absorbed intestinally ***(2).***

Also, its tissue concentrations reach steady-state levels due to the absence of a metabolic pathway for 1,5-AHG degradation and its renal reabsorption. Therefore, its levels in different biological fluids are stable and correlated with blood glucose. 5-AHG was proposed as a novel biomarker for diabetes. The concentration of systemic 1,5-AHG is kept in balance by urine excretion ***(14).***

In normoglycemic individuals, about 99.9% of 1,5-AHG is renally absorbed, competing with glucose at the sodium-glucose linked transporters (SGLT) for kidney reuptake; thus, it is retained in detectable concentrations in blood and saliva. Under hyperglycemia, the glucose transporters are monopolized by the excess glucose ***(14).***

The 1,5-AHG is not reabsorbed at the tubular level, reducing its concentration in serum and saliva. 1,5-AHG in serum decreases while glucose levels rise above the renal glucose threshold; thus, it has been reported that 1,5-AHG represents postprandial hyperglycemia in individuals with diabetes more robustly than HbA1c or FA. Low concentrations of 1,5-AHG reflect poor glycemic control in the preceding 1–2 weeks ***(14).***

1,5-AHG acts not only as a glycemic marker but can also be integrated into a model that considers other risk factors or combined with conventional markers to improve its T2DM diagnostic potential. It has been proven that 1,5-AHG levels increase as glycemic control is achieved independent of body weight, sex, age, treatment, and diabetes evolution among non-insulin-dependent diabetes patients ***(9).***

Controlled glycemia in individuals with diabetes due to combined treatment schemes (pharmacological and non-pharmacological) generates a sustained increase in 1,5-AHG values up to the expected range for normoglycemic individuals. This ability allows 1,5-AHG to exert a differential function in the different stages of T2DM management, screening, diagnosis, and monitoring ***(15).***

Blood 1,5-AHG quantification of at-risk individuals could provide a targeted screening strategy to prevent the development of T2DM or identify those with asymptomatic diabetes. The glycemic biomarker 1,5-AHG not only has a diagnostic application for diabetes, but it has the potential to evaluate the risk of long-term complications, including the most documented association with cardiovascular diseases and mortality in people with T2DM ***(9).***

There is also evidence of its prognostic value for microvascular complications such as retinopathy and CKD. Furthermore, there is evidence that 1,5-AHG is a valuable marker of diabetes progression for individuals affected by diabetic nephropathy in which the determination of HbA1c is not recommended. Furthermore, 1,5-AHG can be used as a marker of remission in patients with T2DM treated with insulin ***(14).***

Salivary 1,5-AHG has been previously shown to be strongly correlated with serum 1,5-AHG and inversely correlated with fasting glucose, OGTT, and HbA1c. A clear advantage of using saliva as a sample is that its collection is non-invasive compared to traditional blood collection. 1,5-AHG isn't a good marker in unconventional biological fluids, such as saliva, tears, and sweat. ***(15).***

Table (1): Comparison of the three clinically validated biomarkers ***(2).***

	Fructosamine (FA)	Glycated albumin (GA)	1,5-Anhydroglucitol (1,5-AHG)
Time required for significant change	1–2 weeks	1–2 weeks	24–72 h
Length of glycemic observation	2–3 weeks	2–3 weeks	1–2 weeks
Reflection of fasting glucose levels	+	+	+
Reflection of postprandial glucose and glucose excursions	+	+	+
Correlation to diabetes complications	+	+	+
Determination by enzymatic methods	Available	Available	Available
Optimal detection range	Medium to high hyperglycemia	Medium to high hyperglycemia	Modest hyperglycemia to near normoglycemia
Point of care testing status	Biosensors and paper-based platforms have been evaluated		
	Paper-based platforms	Paper-based platforms Electrochemical biosensors	Paper-based platforms Electrochemical biosensors
Most common sources of error	Falsely low levels: hypothyroidism and liver cirrhosis		Falsely high levels: chronic kidney disease stages 4–5
	Falsely high levels: hypoalbuminemia, hyperthyroidism, hyperuricemia, hypertriglyceridemia, nonalcoholic fatty liver disease		Falsely low levels: pregnancy, chronic liver disease, glucokinase-maturity-onset diabetes of the young

Novel biomarkers:

Few novel biomarkers have shown significant advantages over those already established and validated, such as FPG, OGTT, and HbA1c. However, it is expected that more extensive studies will lead to new resources in the management of the T2DM epidemic. No biomarker studied so far is the perfect marker for all T2DM patients in all conditions ***(16).***

The most effective approach to search for new biomarkers and exploit their differences with conventional ones is to stop looking for a perfect marker that achieves the status of a universal gold standard for glycemic control and work on multivariable panels that consider the combination of biomarkers, anthropometric characteristics, and lifestyle habits that allow from risk assessment to continuous monitoring of individuals with diabetes ***(17).***

More in-depth studies involving subpopulations of interest are also required, considering pathological conditions and underlying diseases of high incidence to define which biomarkers are the best option for each case. A pivotal approach in the search for new markers of T2DM is the use of metabolomics to generate profiles by monitoring the various metabolic pathways for evidence of deregulated metabolites whose study may lead to potential biomarkers for screening, diagnosis, and monitoring ***(17).***

These metabolomic profiles may also provide insight into potential therapeutic targets and contribute to generating risk profiles for complications. Currently, the search for novel biomarkers for T2DM is primarily based on metabolomic studies. Identifying novel biomarkers that predict the risk, incidence, or complications associated with T2DM usually starts with non-targeted metabolomic analyses. Metabolites that show strong correlations with the diagnosis of diabetes, its validated risk factors, or its complications are later analyzed by targeted metabolomic analyses. Usually, these metabolomic strategies must be carried out in large populations to increase the results' significance and validity—sometimes over long periods ***(18).***

Several metabolomic studies have found characteristic patterns and specific biomarkers associated with the deregulation of energy metabolism in T2DM. The most reported metabolic alterations in the profiling of patients with metabolic disorders and specifically individuals with diabetes are high levels of branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs), as well as the ketosis marker β-hydroxybutyrate (β-HB) ***(2).***

Altered amino acid metabolism appears to be a link between T2DM and the development of cardiovascular disease. It is known that the physiological mechanism of this alteration is the relationship of amino acid metabolism with insulin secretion and tolerance, so monitoring the amino acid profile can provide information about cardiometabolic health ***(18).***

In contrast, the metabolites 1,5-AHG, lysophosphatidylcholine (LysoPC), Linoleoylglyglycerophosphocholine (L-GPC), glutamine (Gln), and glutamine/glutamate ratio present a decrease. In these studies, the roles of clinically validated biomarkers such as 1,5-AHG have been confirmed, in addition to new markers such as fetuin-A, BCAAs, adipokines, L-GPC, LysoPC, among others ***(16).***

Despite the substantial data generated by metabolomic studies, before a potential biomarker is successfully introduced into the clinic, the marker must undergo rigorous clinical validation for its predictive power independently and in conjunction with traditional risk assessment tools ***(17).***

Clinical evaluation of biochemical markers should focus primarily on individuals identified as high risk, as this will allow greater coverage with less expense, which is crucial in resource-limited settings. Identification of these high-risk individuals can be made on a mass scale with the help of standardized tools such as risk assessment questionnaires that usually consider the most recognized risk factors such as age, sex, family history, level of physical activity, as well as family history of hypertension ***(16).***

In some more extensive studies, such as FINDRISC, fruit and vegetable consumption and anthropometric measures such as BMI and waist circumference are also included. These parameters can be used in combination with conventional biomarkers such as FPG and HbA1c in predictive models, and cardiovascular risk markers such as triglycerides and HDL cholesterol can be added to improve their performance and assess the risk of developing complications ***(2).***

It has been shown that using a multi-metabolite score consisting of phenylalanine, non-esterified cholesterol in large HDL, and the ratio of cholesteryl ester to total lipid in large VLDL allows the determination of long-term risk for T2DM in young adults with better performance than any individual metabolite ***(18).***

Amino acids:

A different amino acid profile was found for patients with impaired fasting glucose and T2DM compared to a control population. In subjects with impaired FPG and T2DM, the fasting levels of BCAAs, glutamic acid, lysine, phenylalanine, arginine, alanine, tyrosine, and aspartic acid increased as glycemic control was lost ***(19).***

The concentration of these amino acids correlates significantly with FPG and HbA1c classical markers of T2DM and pro-inflammatory cytokines TNF-α and IL-6. These amino acids demonstrated the ability to discriminate normoglycemic subjects from those with impaired FPG or T2DM ***(19).***

The ability of amino acid levels, including BCAAs (isoleucine, leucine, valine) and aromatic amino acids (tyrosine and phenylalanine), to predict prediabetes risk was evaluated. Levels of aspartic acid, asparagine, and histidine significantly predicted the incidence of prediabetes, with the increased risk differing between African Americans and European Americans. The evidence observed in prediabetes suggests that changes in the amino acid profile occur in the transition from normoglycemia to the development of T2DM ***(2).***

β-hydroxybutyrate (β-HB):

In many metabolomic studies, the α-HB was the biomarker with the best performance to identify individuals with insulin resistance. This behaviour was consistent in both screening and targeted assays. α-HB predictive potential can be explained both by its metabolic relevance and that its synthesis is stimulated by the elevation of the NADH/NAD+ ratio due to increased lipid oxidation ***(20).***

Linoleoylglyglycerophosphocholine(L-GPC)

The potential of L-GPC values as a biomarker of insulin resistance during fasting and a five-point OGTT was evaluated. Despite not showing a linear correlation with classic risk markers such as BMI, fat tissue distribution, lipids, fasting glucose, and HbA1c, subjects with high L-GPC showed higher glycemic excursions during a five-point OGTT ***(21).***

L-GPC has a strong negative correlation with glucose disposal and is negatively associated with insulin sensitivity, showing that it may be used as a biomarker for insulin resistance, especially in patients who do not present the classic risk factors ***(21).***

Leptin:

The relationship between leptin and microvascular complications caused by diabetes progression in a population of T2DM patients was explored. Leptin serum values showed a positive correlation with duration of diabetes, BMI, waist circumference, blood pressure, fasting glucose, HbA1c, serum insulin levels, cholesterol, triglycerides, and LDL cholesterol, consistent with previous reports identifying leptin as a marker of insulin resistance and a possible diagnostic marker for T2DM ***(22).***

Regarding its potential as a predictor of microvascular complications, leptin concentration is positively correlated with urinary albumin-creatinine ratio, peripheral neuropathy, and retinopathy. eGFR showed a negative correlation with serum leptin ***(22).***

Irisin marker

Background:

Irisin, a novel myokine produced in response to physical activity, promotes white-to-brown fat transdifferentiation. The name irisin referred to the ancient Greek goddess Iris, the messenger who delivered (bad) news from the gods. In mice, it has been demonstrated that irisin plays a key role in metabolic regulation, energy expenditure and glucose homeostasis. ***(23).***

New findings from various studies carried out in both animals and humans suggest that irisin might also have other favourable effects, such as increasing bone cortical mass, preventing hepatic lipid accumulation, and improving cognitive functions, thus mediating many exercise-induced health benefits. ***(24)*** However, data on the role and function of irisin in humans have prompted controversy, due mostly to the only recent confirmation of the presence of irisin in humans. Another strong limitation to the understanding of irisin mechanisms of action is the lack of knowledge about its receptor, which until now rem
ains unidentified in humans and in animals. ***(25)***

Adipose tissue has a key role in energy balance due to its ability to store and release lipids, also contributing to regulating thermogenesis and heat production. It is possible to distinguish at least two types of adipose tissue with very different histological and functional characteristics. The white adipose tissue (WAT) is an energy depot where free fatty acids and triglycerides are stored, while brown adipose tissue (BAT) has thermogenic properties containing specialized mitochondria with uncoupling protein-1 (UCP-1), thus producing heat and, consequently, dissipating energy. ***(23).***

Different factors may induce the formation of new brown adipose tissue (BAT). It is now well established that under appropriate stimuli, white adipose tissue (WAT) can transdifferentiate into BAT and vice versa, a phenomenon also known as fat browning, and that is of great interest, especially in the field of obesity and diabetes. Much research has been produced in order to understand the mechanisms regulating fat browning. ***(24)***

Skeletal muscle seems to play an important role in this context due to its ability to produce cytokines, also known as myokines, which act as hormones and influence energy homeostasis. Irisin is a recently discovered myokine, and animal studies have demonstrated that it is produced and released in response to physical activity, being able to promote the browning of WAT and improving the overall metabolic status. ***(24)***

The name irisin referred to the ancient Greek goddess Iris, the messenger who delivered (bad) news from the gods. Animal studies also suggest that irisin may have additional favourable health effects, improving cognitive function and bone metabolism. Irisin may therefore also be a fascinating link between physical exercise and mental faculties, thus confirming the ancient Romans saying mens sana in corpore sano. ***(25)***

Discovery and doubts about irisin:

In 2012, Bostrom and colleagues first reported that transgenic mice, whose characteristic is an enhanced expression of the muscular peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), which is among other things involved in both thermogenesis and biogenesis of BAT, exhibited a high production of UCP-1, the biomarker of BAT. ***(23).***

In particular, they observed an enhanced mRNA expression of BAT-related genes, and a 25-fold increase in UCP-1 levels in subcutaneous inguinal fat of mice following 3 weeks of wheel running when compared to resting mice. Five target genes whose expression was controlled by PGC-1α were identified in muscle cells, but only fibronectin type III domain containing 5 (FNDC5), a type I membrane protein, was able to induce a remarkable brown fat gene program. ***(25)***

Using Western blot through antibodies against FNDC5 protein, they detected a 32 kDa band that decreased to 20 kDa after deglycosylation. Further analysis by mass spectrometry revealed that FNDC5 protein was cleaved and secreted into the bloodstream as a polypeptide, which they named irisin. In the study, irisin was identified also in human plasma, and healthy adults undergoing 10 weeks of endurance exercise exhibited twofold higher circulating irisin levels than resting individuals, suggesting that this protein was produced by the human skeletal muscle in response to physical exercise. ***(23).***

Irisin is cleaved from the extracellular ectodomain of FNDC5 by an unknown protease at the C-terminal, and then released into the bloodstream as a 112 AA polypeptide. This myokine exists as a dimer and consists of an N-terminal fibronectin type-III-like region, containing a four-stranded β-sheet packed to a three-stranded β-sheet, attached to a small C-terminal tail. ***(24)***

In detail, irisin forms a continuous eight-stranded β-sheet dimer containing each subunit four-stranded β-sheet, probably binding to its receptor just as a preformed dimer. Moreover, the inner 10 H-bonds between the β-sheets, the two salt bridges between Arg-75 and Glu-79′ (the prime stands for the second subunit of the dimer), and the tight interaction among the remaining three-stranded β-sheets Trp-90 and Trp-90′ gives great stability to the irisin dimer. The fibronectin type III domain accounts for the thermodynamic stability showed by proteins with this domain, as irisin itself. ***(24)***

These findings might contribute to explaining the interesting data reported by several authors who observed no significant differences in detectable circulating levels of irisin in sera that had undergone multiple freezing and thawing cycles. At present, an important limitation is that the irisin receptor has not been identified. However, some studies have shown that irisin promotes fat-browning by activating both the

p38 mitogen-activated protein kinase (p38 MAPK) and the extracellular signal-related kinase (ERK) signalling pathways. ***(25)***

Irisin might also act by inducing PPARα expression and, if confirmed, this effect may suggest that irisin is to some extent able to influence lipid metabolism. Despite the fact that irisin secretion in skeletal muscle following exercise has been described, data from several studies focusing on which kind of physical activity is able to promote the secretion of this myokine are conflicting. ***(23).***

While irisin has been isolated and identified in urine, lower levels of circulating irisin have been found in patients with renal impairment compared with individuals with normal renal function, thus hypothesizing an exclusive role of the liver in irisin metabolism and elimination. ***(24)***

Irisin exerts its effects mainly on white adipocytes, inducing the brown fat-like gene expression that leads to a phenotypical switch with adipocytes, by which they lose their single large fat storage, and fill with multiple small lipid droplets, and turn into fat cells with characteristics between white and brown adipocytes that have been called brite or beige adipocytes. ***(23).***

Irisin in diabetes and obesity:

Since its discovery, it has been hypothesized that irisin may have beneficial effects on chronic diseases such as obesity and type 2 diabetes (T2D), as well as protective effects against cardiovascular risk. However, attempts to reproduce in humans the encouraging findings obtained in animal studies have had conflicting results. ***(25)***

First, the relationship between irisin and body mass index is still unclear. Although, as expected, an inverse association between irisin concentrations and body mass index has been reported by many authors, there are studies that have showed either opposite or conflicting results. ***(25)***

There are also inconsistencies concerning the relationship between irisin, T2D, and insulin resistance. High serum concentrations of irisin have been observed in patients with T2D, although the bulk of the studies have reported opposite results. ***(23).***

Irisin and cardiovascular risk:

Few studies have investigated the relationship between irisin and cardiovascular risk, and much of data do not refer to the global risk, but rather to its association with some of the established cardiovascular risk factors, of which T2D and insulin resistance have already been described above. ***(26)***

Other possible effects of irisin:

Both animal and in vitro studies have suggested that irisin exerts anti-inflammatory effects modulating the production of cytokines as interleukin-6, interleukin-1β and tumor necrosis factor-α, influencing transcription factors as MAPK and nuclear factor-kappa B, or reducing the production of reactive oxygen species. ***(25)***

Interestingly, studies in vitro demonstrated that irisin protects pancreatic β-cells from high glucose-induced apoptosis. Irisin has also been shown to protect against palmitic acid-induced apoptosis in hepatocytes or against lipopolysaccharide-induced apoptosis of alveolar epithelial cells in lung. ***(24)***

Some favourable effects of irisin on extra-adipose tissue have been reported, mainly concerning bone metabolism and cognitive capacities. Irisin also improves cortical bone mass by stimulating bone formation, reducing the number of osteoclasts and preventing bone loss. It has been demonstrated in vitro that irisin stimulates the differentiation of osteoblasts. If these actions are confirmed, one could hypothesize that irisin promotes changes aimed at making the bone able to support greater loads due to an increased physical activity. ***(23).***

Guo et al. cleared that irisin is a muscle factor that skeletal muscles release after exercise. It can boost the body's energy expenditure, encourage metabolism, and cause the conversion of white fat, which serves as storage, into brown fat, which produces heat. Irisin can thereby increase insulin sensitivity ***(27).***

Irisin has the ability to stimulate thermogenesis and the production of white adipose tissue that resembles brown fat in myokines. The pleiotropic features of irisin have been extensively studied, indicating its crucial function in the control of energy metabolism by acting on several tissues and interfering in numerous metabolic processes. Irisin has been suggested to have actions by way of integrin V receptors.

mostly produced by skeletal muscle, making up around seventy two percent of the total amount in circulation. However, studies indicate that irisin can also be produced by the pancreatic islets, making it a new potential intra-islet hormone ***(28).***

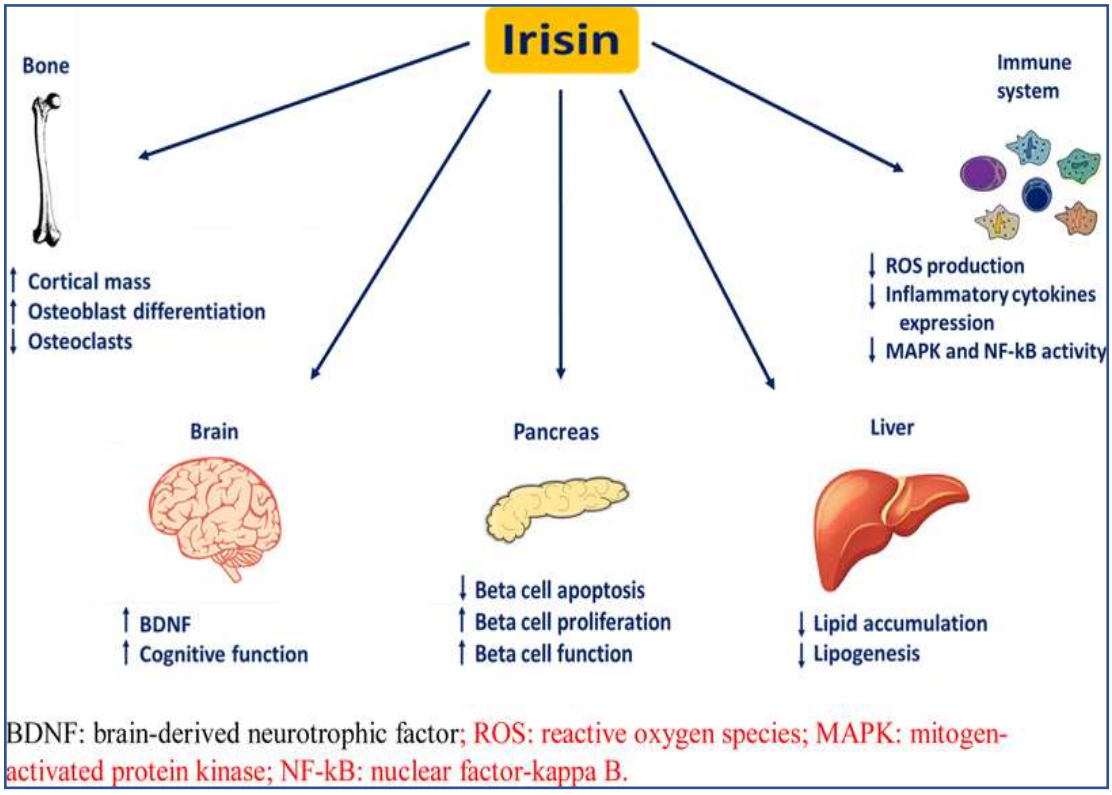

Figure (3): Effects of irisin. ***(23).***

Prehistoric man, much more than today, had the need to activate thermogenesis (at least in part guaranteed by BAT) for vital needs, an even greater need in the winter when forced to search for food. Interestingly, even Bostrom asked why exercise should induce a program leading to heat generation and energy consumption when people engage in physical activity in search of food for accomplishing energy intake requests. ***(23).***

In that case, it should be considered that muscle contractions produce heat, and muscles themselves uses lipids as fuel. Interestingly, it was demonstrated that exposure to the cold induced irisin secretion proportionally to the intensity of shivering, likely an evolutionary defensive mechanism against hypothermia. ***(23).***

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