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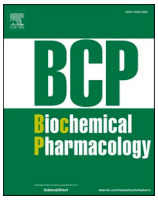

Review

Glycaemia dynamics concepts before and after insulin

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Preface

This commentary is based on the tremendous summary of the knowledge about the elements considered to play a role in the distribution of free D-glucose in the blood in humans reported by Professor John James Rickard Macleod (from the Physiological Department of the University of Toronto, Canada) in 1921 [1]. The available literature already one hundred years ago was deeply into the concept of having convincing measurements of glycaemia and the potential factors that could alter the values of this parameter in individuals with diabetes mellitus. I present a brief discussion highlighting some of the main points raised by Macleod and contemporary colleagues restricted to what was known and how the interpretation under the light of today's knowledge about the blood factors as discussed by Macleod associate with the regulation of the level of this sugar in the blood. Therefore, I did not intend to describe D-glucose homeostasis or anti-hypoglycaemic mechanisms such as glucagon, adrenaline and epinephrine, glucocorticoids, and growth hormone. The mechanisms for these molecules and insulin involved in D-glucose uptake and metabolism in target tissues is nowadays widely described [2–5]. Also, a detailed analysis of D-glucose metabolism and transport mechanisms in red blood cells (RBC), the vascular endothelium, and other tissues are available in excellent recent reviews [4,5].

1. Introduction

John James Rickard Macleod received the 1923 Nobel prize in Physiology or Medicine (The Nobel Foundation, 2021) [6]. Two years before, Macleod addressed the available literature for 1906–1920, discussing the advances and summarising the state-of-the-art knowledge concerning '*The Sugar of the Blood*' [1]. One hundred years ago, there was a need to address the state-of-the-art concept of 'glucose of the blood'. By then, patients affected by diabetes mellitus were the target aiming to understand in a better way the bases of this disease and the mechanisms by which glycaemia changed as a result of altered handling of this hexose by these patients.

One of the main concerns was the content and distribution of D-glucose in the vascular fluid, including the corpuscles. Professor J.J.R. Macleod was asked to address his thoughts on the specific concepts associated with glycaemia, referred to as '*The Sugar of the Blood*' [1]. In this editorial, I intended to summarise several aspects of the elements that were controversial or, definitively, unknown by 1906–1920 (the period covered by Professor Macleod in his review).

Interestingly, several original concepts of Macleod's work were the bases of later confirmed phenomena. These phenomena include today's well-advanced definition of glucose transporters and insulin biological actions and their fundamental roles in the modulation of glucose levels in the blood in healthy individuals (i.e. showing an acute balanced physiological response to glucose overload or deprivation) [2-5] and in individuals affected with diabetes mellitus [5]. Reviewing Macleod's concepts today, after a century, it is vital to notice how the knowledge on this topic has progressed and how the benefits of studying glycaemia dynamics in response to insulin and glucose are applied in the medicine of the present times for the benefit of patients with diabetes mellitus [7].

At the beginning of the twentieth century, the concept of sugar in the blood was hard to understand. Most of the concerns were the difficulty of a precise measurement of sugar concentration, particularly glucose, in blood samples as a molecule contained in a fluid with multiple components more than only a solvent like water. As described by Macleod, in 1921, the great debate was whether glucose in solution, particularly in the blood fluid, was accurately measured by the two more used approaches, *viz*, the colorimetric and titration methods. Also, the need for repeatable results contrasting different research groups was essential to

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the already and strongly but imprecisely defined 'translational medicine'.

The use of colorimetric and titrimetric methods has been exponential since the 1990s. Much more rapid, repeatable, and comparable results for a blood sample are now easier to obtain and are essential for an acute estimation of the metabolic state of patients with metabolic diseases such as type 1 (T1DM) and 2 (T2DM) diabetes mellitus, obesity, metabolic syndrome, gestational diabetes, or gestational diabesity.

One main question that drives research in basic (or fundamental) and clinical science regards how the variations in the glucose concentration in blood, i.e. glycaemia, affect patients' health conditions with abnormal glucose handling. The latter was a worrisome by the early 1900s as it is at present. The quick and accurate determination of glucose concentration in the blood is crucial for healthier outcomes of patients, as stated in the review of the 57th Annual European Association for the Study of Diabetes Congress [7]. Obesity as a condition, intriguingly not yet openly considered a disease, was initially mentioned and documented by 1840s [8,9]. Unfortunately, from 1900 to 1985, the average body mass index (BMI, defined as kg/m^2 of the height) of USA adult subjects increased from BMI ~23 to ~32 kg/m^2 [8,9], leading to a larger population with a BMI over the mean compared with those with a BMI in the established normal weight range (BMI ~18.5 to 24.9 kg/m^2) [10]. Unfortunately, this scenario is also seen and increasing today [10].

Individuals with this abnormal metabolic condition may also show a higher level of glucose in the blood, i.e. hyperglycaemia. Hyperglycaemia results in profound alterations in various cell types, including vascular, adipocyte, hepatocytes, and pancreatic cells. Therefore, it was essential, as it is today, to know the mechanisms of glucose distribution between the extracellular and intracellular environments.

A valid and intriguing question in the scientific and clinical research in the 1920s regarded how sugar is contained in the blood. This question was presented by Macleod [1] as follows:

'…*is all of the sugar in simple solution or is a part of it in some sort of combination with other blood constituents*?…'

The blood is a rich medium containing other sugars than glucose, such as fructose, other aldehydes and ketones and glycuronic acid, and uric acid and creatinine. The distribution of these sugars and other reducing agents in the blood results in a tuned phenomenon of compartmentalisation of D-glucose in the blood. The latter results in a more significant fraction of this hexose in solution (free D-glucose), combined with blood proteins, or forming disaccharides or more elaborated polysaccharides such as dextrin. The compartmentalised distribution of D-glucose in the blood also includes the distribution of a fraction of this sugar within the blood cells of which the RBC play a significant impact mainly because of their large number (4–6 $\times$ 10^6 cells/μL) and cell volume (90–150 fL) [11]. Thus, D-glucose is not only distributed as a free molecule in solution or bound to other structures in the blood (v.g. proteins) but also between the plasma and the blood cells.

Interestingly, the need for a better understanding of the processes involved in the regulation of the content of glucose in the blood led Macleod to highlight the necessity of considering the 'diffusibility' of glucose and other sugars across the plasma membranes of the blood cells, i.e. referred by Macleod as 'corpuscles'. The space to occupy by D-glucose in blood cells is a restriction for this sugar to distribute within the intracellular and extracellular medium. By the 1920s, the mechanism of distribution between these compartments was referred to as diffusibility. The latter concept addressed the efficiency of D-glucose passing through a membrane separating two compartments in a dialysis setup. The dialysis was employed on the knowledge that a given concentration of a molecule was different or the same between one chamber having the sample (v.g. blood) and a second chamber containing water without or with varying contents of the molecule of interest. Diffussibility was to estimate the capacity of the D-glucose in the blood to diffuse across a membrane down a concentration gradient in a time-lapse.

It was 28 years later when LeFevre and colleagues [12] referred to the transmembrane transfer of D-glucose as a phenomenon that required a component allocated at the cellular plasma membrane. Two years later, the concept of diffusibility changed with Widas's proposal of the carrier model [13]. The carrier model regarded a potential dynamic mechanism accounting for the transmembrane distribution of D-glucose, i.e. cells to medium content. The first studies of the existence of a membrane protein mediating D-glucose uptake (or properly transport of D-glucose) in the RBC plasma membranes was described by Kasahara & Hinkle in 1977 [14]. This new concept was also applied to other components of the vasculature, v.g. the endothelium, since they are in direct contact with the blood. The endothelium forms a monolayer of cells contributing to regulating the concentration of this sugar in the blood. D-Glucose is removed from the blood by the endothelial tissue and is used as an immediate source of energy and supply to other tissues for energy generation in the form of adenosine triphosphate (ATP). A concerted co-expression of D-glucose membrane proteins is referred to as transporters or carriers that address a vectorial delivery of D-glucose from the luminal to the abluminal plasma membrane through the metabolic and physical endothelial barrier secures the requested delivery of this sugar to the tissues.

A reduced expression and activity of D-glucose transporters in the endothelium and other blood cells may, at least partially, contribute to a transient state of hyperglycaemia [15,16]. The primary mechanism of D-glucose uptake by blood cells and the endothelium occurs through facilitative glucose transporters (GLUTs) —membrane proteins that mediate D-glucose diffusion from a high to low concentration— [4,5,13-16]. Human endothelial cells express GLUT1, GLUT2, GLUT3, GLUT4, and the fructose highly selective GLUT5 isoforms [16-18]. Expression of GLUTs secures an efficient uptake of D-glucose in a wide range of concentrations since their apparent Michaelis-Menten (Km) values range between 3–25 mmol/L for GLUT1 [17,19-21], 15–20 mmol/L for GLUT2 [19], ~2 mmol/L for GLUT3 [19], and ~5 mmol/L for GLUT4 [19,20,22]. Thus, a change in glycaemia may be counteracted by the activity of these different isoforms of GLUTs covering a range of glucose in the blood (~2–25 mmol/L).

However, it is now accepted that hyperglycaemia, along with the expression and activity of GLUTs, results from the involvement of several other actors [16,21,22]. These actors include the dangerous combination of a higher intake of carbohydrates contained in food with high glycaemic load [22-25], reduced or low effective physical activity [26], thus limiting the energy consumption, and a reduced biological action of insulin, i.e. insulin resistance, in target tissues such as the skeletal muscle, liver, and mature adipocytes [27].

Insulin's most well-described metabolic effect is regulating the expression and localization of GLUT4 in human tissues [4,16,28]. Along with the several metabolic adaptations in response to insulin, increased D-glucose uptake into target tissues via well-defined mechanisms results in rapid (5–10 min) translocation of GLUT4 from intracellular stores to the plasma membrane [16,22]. Insulin biological action results from the activation of isoforms A (associated with preferential activation of protein kinase B (Akt)-mediated signalling) and B (associated with preferential activation of mitogen-activated protein kinases 1 and 2 mediated signalling) of insulin receptors in mammalian cells [29]. GLUT4 redistribution to the plasma membrane results from the activation of insulin receptors-activated signalling cascades mediated by insulin receptor substrates (IRS)/phosphatidylinositol 3-kinase (PI3k, likely PI3k isoform 1A) [30] complex leading to Akt activation in $<$ 1 min [31] and the mechanistic target of rapamycin complex 2 in $<$ 5 min [32]. Also, GLUT4-containing vesicles traffic involving the Rab-GTPase activating protein TBC1D4 by Akt is critical in exposing these membrane transporters to an extracellular medium containing D-glucose [33]. Both the insulin-activated signalling and traffic of vesicles are also affected by the kinetics of generation, activation and trafficking of the GLUT4 storage vesicles, a phenomenon playing a significant role in GLUT4 redistribution [22].

The systemic exposure to long or short-term changes in glycaemia results in abnormal cell metabolism of D-glucose, including the RBC and vascular endothelium [5,16]. The latter is one of the mechanisms which could result from reduced transport of D-glucose into the cells, causing an accumulation of this hexose in the blood and interstitial space [15]. The resultant hyperglycaemia increases the risk of subsequent non-enzymatic reactions between D-glucose and proteins or lipids to form advanced glycated end (AGEs) products. AGEs are found to be elevated in the vasculature in patients with diabetes mellitus contributing to the development of atherosclerosis and other vascular diseases [31].

Insulin resistance is characteristic of patients with T2DM. This condition results in increased insulin release from β-pancreatic cells leading to higher levels of circulating insulin, i.e. hyperinsulinemia, in response to extended periods of hyperglycaemia compared with D-glucose level in the blood at a physiological range (~90 mg/dL fasting in healthy adult subjects) according to the American Diabetes Association (ADA) [32]. However, the over-generated insulin is shown to have a defective action mechanism in target tissues restricting the removal and catabolism of D-glucose and other nutrients (v.g. amino acids). Thus, other mechanisms than being bound to proteins or taken up by the blood cells and the vascular endothelium result in modulating the content of free D-glucose in the blood and the exposure of tissues to an elevated concentration of this sugar.

The described local and specific phenomena involved in regulating sugar in the blood are in concert with the well-known and essential systemic control of glycaemia. The latter is seen in a systemic response triggered after the consumption of a nutrient with a high glycaemic index —a rating system of how quickly and how much the nutrients raise the blood sugar level after eating— [33], such as sugar-enriched beverages and white bread. After food digestion, the derived D-glucose is absorbed by the gastrointestinal epithelium reaching the circulation. A higher level of circulating D-glucose reaches the pancreas to stimulate β-pancreatic cells to release insulin. The increased insulin levels in the circulation increase the uptake of D-glucose in target tissues including the skeletal muscle and fat tissue, lowering its concentration to euglycaemic levels. When the D-glucose in the blood is lower than the expected physiological level, i.e. hypoglycaemia, the α-pancreatic cells react to release glucagon. This hormone mobilises D-glucose from storage, thus increasing the blood glucose levels securing the supply of this substrate to the body. Therefore, the balanced equilibrium of D-glucose in the blood is under the control of systemic adaptations mainly regulated by insulin and glucagon.

The concept that insulin was involved in regulating D-glucose concentration in the blood was not assumed by 1921's. However, Macleod and his colleagues Charles Best and Frederick Banting knew about 'isletin' and its potential actions on sugar regulation in the blood. These researchers initiated the characterisation of isletin, i.e. insulin, by April 1921 and in 1923, Macleod and Banting were awarded the Nobel Prize in Physiology or Medicine [6]. The review published by Macleod regarding sugar in the blood and its control mechanisms and consequences was released in April 1921 [1]. Therefore, much more was to come after they discovered insulin a century ago.

2. Distribution of D-glucose between plasma and blood cells

Another question that attracted the attention of Macleod by 1921 well in advance before Widas's carrier model proposal for mobilisation of substrates across cell membranes was:

'…*how is the sugar distributed between the corpuscles* [blood cells] *and the plasma?* …'

The corpuscles, as mentioned, referred to the blood cells, and more specifically, the RBC. The first assumption for the distribution of D-glucose between these two compartments was that this sugar was in a state of a simple solution; therefore, its concentration should be the same between the plasma and corpuscles. However, other factors, such as the blood proteins, potentially influencing the redistribution of D-glucose between the plasma and the RBC was present.

2.1. Blood proteins

The simple concept of diffusibility addressed in the early 1900s explains the movement of solutes from the vessel lumen to the extra-vessel space, or vice-versa has changed dramatically. Nowadays, the diffusibility term is still used and referred to as the diffusion coefficient (*D*). The *D* of a substrate is determined mainly by the difference in concentration of the solute between two compartments and other intrinsic characteristics of the substrate itself, such as molecular size, lipids solubility, and ionic charge. The substrate also shows an inherent colloid osmotic pressure retaining the water in the vessel lumen, which opposes the hydrostatic forces determining net fluid flux across the vascular endothelium. Thus, a simple phenomenon of diffusion in solution contrast with a more complex mechanism of transmembrane pass of a solute. D-Glucose diffuses in the blood to generate a nearly uniform distribution in solution due to its high *D* value (~2.6×10^6 cm^2 per second) when estimated at 37 °C. Since the blood is a viscous solution due to the presence of other molecules, such as albumin, rather than only D-glucose, the viscosity of the blood is a factor required to consider for the estimation of *D* for D-glucose in solution [34].

Albumin is a small (66.7 kDa), negatively charged globular protein released by the liver into the blood (~12.5 mg per day), where it reaches a concentration of ~40 g/L in the serum of healthy adults [35]. Albumin in the circulation acts as a carrier for other endogenous (v.g. bilirubin, fatty acids, hormones, enzymes, vitamins) or exogenous (v.g. drugs) molecules. It sustains a significant colloid osmotic pressure restricting the blood fluid leak from the vessels to the interstitial space. However, a substantial fraction of the released albumin (60–70%) reaches the interstitial space returning to the blood via the lymphatic system. Only around the 1980s, it was first reported that human serum albumin could get glycated and that this mechanism resulted in being dependent on the plasma D-glucose concentration. Albumin gets glycated via a non-enzymatic process generating the ketoamine glycated albumin, whose grade of glycation negatively correlates with when patients with T1DM or T2DM show glycaemia values within the expected ranges, i.e. time in range [36]. It is estimated that 18–19% of glycated albumin is referred to as patients with T1DM and T2DM having 155–160 mg/dL D-glucose in the plasma.

It is interesting mentioning that patients with a diagnosis of prediabetes (based on fasting plasma glucose 100–125 mg/dL or glycated haemoglobin (HbA1c) 5.7–6.4%) showed regression of this stage to a normal glycaemia and HbA1c in parallel with an increased serum albumin level compared to individuals without this condition [37]. Furthermore, patients with prediabetes that remained with this condition or progressed to T2DM showed lower albumin levels [37]. It was proposed that higher levels of serum albumin protect the patients from developing diabetes mellitus. Serum albumin is the most abundant protein in the blood and, therefore, a major target to bind D-glucose. Thus, an increase in the serum albumin level may result in lower exposure of cells and tissues to free D-glucose in solution. Therefore, it is possible that the fraction of free D-glucose in the blood may be affected by elements, such as albumin contained in this fluid as described for human serum albumin [38].

Interestingly, one hundred years ago, the possibility that D-glucose was in combination with other molecules in the blood reducing its state of free to bound D-glucose was not entirely accepted and matter of discussion (1). To date, McGuigan & Ross in 1917 [39] took samples of blood and subjected these samples to dialysis against an isotonic solution containing increasing concentrations of glucose (expressed as percentages between 0.05 and 0.2, i.e. ~50 and ~200 mg/dL). Their findings showed a perfect sugar equilibrium between the compartments containing the blood and the isotonic solution. These findings allowed Macleod to suggest that:

'…*all sugar in the blood must be in simple solution.*…'

However, other groups criticised these conclusions since the diffusion rate of D-glucose from the blood to the isotonic solution might be different from the D-glucose naturally present in the blood compared with this hexose artificially added to blood samples, as exposed by Kleiner in 1918 [40]. These criticisms pointed out another question regarding whether the combination of D-glucose in the blood would differ in normal individuals versus patients with diabetes mellitus. Thus, Kleiner estimated the diffusion rate of D-glucose using a similar approach with blood samples from these two groups of subjects. The results showed that D-glucose in the blood from patients with diabetes showed a lower rate of diffusion than this sugar in the blood from normal subjects. In the light of these results, Macleod stated the following:

'…*Kleiner thinks that they* [the results] *indicate that some at least of the sugar in the diabetic blood must be in a combined state*…'

and added the following at some stage conclusive sentence:

'…*and he* [Klein] *infers that if such a compound is present in diabetic blood there is a possibility that it also exists in normal blood.* …'

Indeed, the results described above were disrupting the era in thinking about D-glucose distribution in the blood. This sugar may not be only free in solution but associated with other components of the blood. Furthermore, this potential distribution of glucose was proposed to occur not only in blood from patients with diabetes mellitus but also in blood from normal, non-hyperglycaemic subjects. The latter is something that Macleod looked at some sort reluctant and with caution when stating:

'… *These observations are interesting but scarcely conclusive.* …'.

It is worth noting that by 1921 the concept of D-glucose in the urine was firmly established (using Macleod's words) in the sense that the urine from normal subjects does contain D-glucose and that the concentration of this sugar changed likely in parallel with its concentration in the blood [1]. The latter was an argument of Macleod supporting his assumption that D-glucose in the blood was not combined but free, therefore reaching equal concentrations between the blood and urine. Indeed, he commented emphatically the following:

'… *But now that it is firmly established that normal urine does contain sugar and that the concentration of this runs at least approximately parallel with that in the blood, the reason for assuming that some of the blood sugar is combined no longer exists*….'

From this and other statements proposed by Macleod, it is clear how sceptical he was about the possibility of the presence of any degree of combined glucose in the blood. However, Macleod's proposal is nowadays different and measurement of glucose in the urine is considered as an indirect index of the concentration of glucose in the blood [41]. The latter is a matter that is currently better understood. Interestingly, a general consensus is that a reduced tubulo-glomerular feedback and glycosuria in patients with episodes of hyperglycaemia may be indicative of high renal risk for patients with T1DM [42].

2.2. Blood cells ('corpuscles')

2.2.1. Facilitated transport of D-glucose in blood cells

The inclusion of blood cells as an element regulating D-glucose availability in the blood supported the possibility that this sugar was either in a simple solution or redistributed in free or bound D-glucose states. One of the proposals discussed 100 years ago was that an equal concentration of D-glucose in the plasma and blood cells supported the idea that all this sugar should be in a free form in solution generating a state of a simple solution. This assumption may signify that D-glucose will freely move from the plasma to cell compartments based only on its concentration gradient and *D* value. The concept has evolved, and today's knowledge addresses D-glucose as a molecule mobilised from the plasma to blood cells by a facilitated membrane transport mechanism due to the presence of GLUTs [4,5]. The latter mechanism may be involved, at least partially, in keeping glycaemia in the normal ranges in healthy subjects. However, a defective GLUTs expression and transport activity in the skeletal muscle, fat tissue, and other insulin-targeted tissues associated with hyperglycaemia in patients with diabetes mellitus and other abnormal metabolic conditions with impaired D-glucose metabolism such as insulin resistance [43].

GLUTs are a group of at least 14 proteins of the *SLC2* (SoLute Carrier) gene family [4] expressed in all cells in the body. D-Glucose cannot cross the cell plasma membrane by simple diffusion since it is a large and polar, highly hydrophilic molecule, therefore needing an adequate environment sustaining its solubility and keeping its entropy in solution. GLUTs *facilitate* D-glucose diffusion from a higher to lower concentration through the plasma membrane by generating a hydrophilic transmembrane compartment. Therefore, this process is called facilitated transport or diffusion of D-glucose [4]. Thus, the concept of simple diffusion of D-glucose crossing the plasma membrane of blood cells was at least unprecise by the 1920s.

D-Glucose transport across the plasma membrane of cells is possible due to conformational changes experienced in the polar channel formed by GLUTs for this hexose. The latter is a phenomenon that depends on the kinetics of these proteins' changes as initially described in Widas's hypothesis in 1952 [13] which proposes a generalized kinetics for carrier transport systems instead of simple diffusion of D-glucose. The kinetics means different conformational states of the membrane proteins, i.e. the transporters or carriers, in the plasma membrane, a concept revisited by Devés & Boyd in 1998 [44] where the simple carrier model was deeply analysed. Interestingly, the latter model supposes energy required to reach a conformational change of the carriers (*C*) to allow the substrate (D-glucose in this case) movement through the membrane. The influx of a substrate (*S*) into the cells requires the formation of a carrier-substrate complex at the external side of the plasma membrane (i.e. the *CoS*, standing for *S* bound to the outside conformation state of the carrier), an intermediate state in the middle of the membrane (i.e. *CS*), and *S* bound to an inside conformation state of the carrier (i.e. *CiS*). Simplistically, this mechanism starts with *S* in the nearest to *Co* and end with *S* being released in the intracellular (transmembrane passed) space in the nearest of *Ci* described as (see also Fig. 1):

$$S + C_o \leftrightarrow C_oS \cdots CS \cdots C_iS \leftrightarrow C_i + S$$

Due to this sequence of events, D-glucose may enter the cells via GLUTs in favour of a concentration gradient as described in RBC [45,46]. The same happens in other blood cells, including leukocytes [47], lymphocytes [48], platelets [49], monocytes [48,50], neutrophils [51], and the vascular endothelium [52].

The kinetics of the membrane transport mechanism facilitated by carriers is a phenomenon that may reach a state of saturation —a state where the occupation of the binding sites for a substrate is maximal and cannot continue increasing— [16,44]. In the blood, saturation occurs when the concentration of D-glucose in the plasma is higher than the affinity of GLUTs (estimated by their apparent Michaelis-Menten constant, Km) in blood cells. Thus, the simple carrier model for D-glucose transport is finite and eventually not only driven by the concentration difference between the extracellular and intracellular compartments. It should also depend on the availability of sites to bind D-glucose in the outside and inside faces (*Co* and *Ci* conformational states) of GLUTs. Even when simple diffusion may occur for D-glucose being taken up from the plasma by the RBC, this phenomenon is limited by the availability, expression, and dynamics of the kinetics of the *CoS* and *CiS* conformations in the plasma membrane.

Cell catabolism of substrates is also a mechanism that may change the concentration gradient between two compartments lined up by the plasma membrane. D-Glucose is avidly metabolised in the cells, and

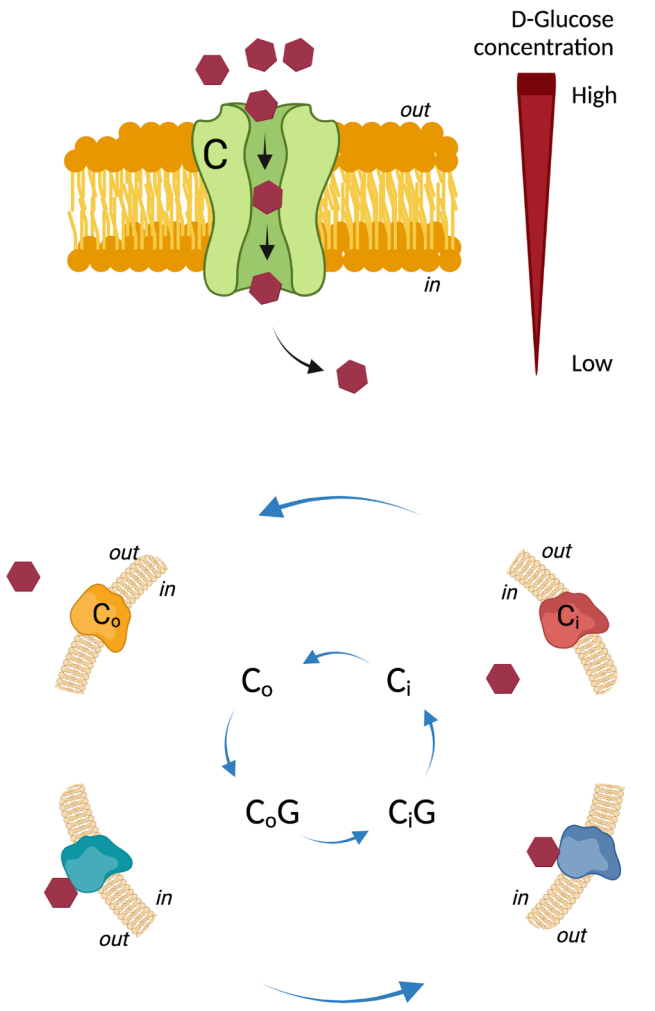

Fig. 1. Uptake of D-glucose in blood cells. The fraction of D-glucose (red hexagon) contained in the blood that is not bound to plasma proteins (i.e. free D-glucose) is taken up by red blood cells via a mechanism described by the general kinetic dynamics of the classical carrier model for the transmembrane transport of a solute (see [13,16,40]). D-Glucose crosses the plasma membrane via membrane transporters (C), also referred to as a carrier, from a high to low concentration favouring an influx transmembrane concentration gradient (*D-Glucose concentration*) from the extracellular (*out*) to the intracellular (*in*) space. Restricting the transmembrane transport of D-glucose (G) to the influx, i.e. *out* to *in* movement of G in the carrier model, this hexose is initially exposed to D-glucose transporters or carriers located in the plasma membrane. The carriers at this side of the plasma membrane show a high affinity for D-glucose generating the external configuration of C (C_o). After C_o binds G, a new conformation predominates, i.e. C_oG. The carrier now complexed with its substrate G allows the displacement of the hexose through a polar channel to generate a new conformation of the complex carrier-glucose that shows high affinity by its inner side, forming the complex carrier in its internal configuration with glucose (C_iG). G detaches from C_i since its affinity is reduced, being released to the intracellular medium. G is then available for metabolism with the latter phenomenon, and C_i stays free of G due to a reduced affinity and rapid translocation to the configuration C_o 'offering' it at the external side of the plasma membrane to initiate the uptake of a new D-glucose molecule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

activation of cell metabolism generates a condition where more energy is requested for cell processes. This phenomenon results in a faster, although limited because of the involvement of enzymes, intracellular D-glucose metabolism into glycolysis —D-glucose breakdown into pyruvate and lactate after the first step of phosphorylation providing substrates for ATP synthesis and storage pathways of glycogenesis and lipogenesis [53]— generating a more pronounced extracellular-*to*-intracellular concentration gradient for D-glucose resulting in higher diffusion through GLUTs. The concept of simple diffusion of D-glucose into blood cells, as proposed by Macleod in 1921, is today recognised as a mechanism subjected to much more direct and indirect regulatory factors than solely the transmembrane concentration gradient of this hexose.

D-Glucose is taken up by mammalian cells, including RBC and other blood cells, and is converted to glucose-6-phosphate (G6P) by hexokinases. The formed G6P is not recognised by the inside conformation (*Ci*) of GLUTs, thus blocking its exit securing the disposal of D-glucose for energy generation. Interestingly, *CoS* → *CiS* dynamics in the plasma membrane is bidirectional or reversible (i.e. *CoS* ↔ *CiS*) for GLUTs-facilitated transport of D-glucose. The latter implies that whether this membrane transport mechanism is operative in blood cells and the endothelium, the effective concentration of this hexose in the plasma may vary as much as the reversibility of transport occurs. There is how the importance of metabolising D-glucose to G6P by the hexokinase in most mammalian cells.

When an extensive period of exposure to hyperglycaemia, the vascular endothelium may respond by regulating the expression of GLUTs. To date, high plasma D-glucose concentrations downregulate, but lower than the physiological levels of plasma D-glucose upregulate the expression of these plasma membrane transporters [54]. Thus, a higher or lower expression of GLUTs may increase or reduce the uptake of D-glucose, respectively, from the plasma into blood cells and the vascular endothelium contributing to the modulation of the levels of free D-glucose in the plasma.

2.2.2. *Active transport of D-glucose in blood cells*

Another group of membrane transporters involved in active D-glucose uptake with a potential effect in the regulation of the concentration of D-glucose in the blood are the sodium-dependent glucose transporters (SGLTs). SGLTs are a family of at least four proteins of the *SLC5* (SoLute Carrier) gene [55] and were first identified in the rabbit intestine in 1987 [56]. These proteins mediate the transport of D-glucose or D-fructose requiring sodium. SGLT2 is a low-affinity, high-capacity cotransporter requiring one sodium for the uptake of one molecule of D-glucose, while SGLT1 is a high-affinity, low-capacity cotransporter requiring two sodium for the uptake of one D-glucose [57,58]. The SGLTs-mediated, sodium-dependent D-glucose uptake is critically regulated by the sodium and D-glucose gradients and the plasma membrane potential, making this process a fully reversible mechanism of D-glucose transport [59].

Interestingly, changes in the activity of SGLTs are expected to cause significant variations in the D-glucose concentration since they are dependent on the existing sodium gradient mobilising D-glucose against a concentration gradient [57,59]. The latter contrast with GLUTs, where the mechanism of D-glucose transport is facilitated as discussed above. Interestingly, human RBC does not express SGLTs; therefore, only GLUTs mediate D-glucose uptake into these cells [4,55]. However, other blood cells express members of the SGLT family, including immortalised lymphocytes Jurkat T cells [60,61], human coronary artery and umbilical vein endothelium [62], and bovine brain microvascular endothelium [63]. Thus, activation or inactivation of SGLTs may result in local, vascular regulation of the level of D-glucose in the blood, confirming the involvement of these types of sodium/D-glucose cotransporters in D-glucose blood homeostasis [64].

3. The role of glycolysis in D-glucose membrane transport

The metabolism of D-glucose in blood cells is a phenomenon that leads to a reduced intracellular concentration of this hexose since this hexose is modified to G6P after hexokinases-mediated phosphorylation. The formation of G6P is a chemical reaction that results in the generation of a localised higher concentration gradient for D-glucose from the

extracellular to the intracellular space (Fig. 2). The more significant concentration gradient is being generated in the nearest of the internal conformation of GLUTs (i.e. *Ci* in the classic carrier model), where permanent sequestration of D-glucose into glycolysis occurs. The immediate consequence of these changes is an increased redistribution of this hexose into the intracellular space compared with the plasma compartments. The facilitated uptake of D-glucose via GLUTs will therefore end in higher uptake of this molecule from the plasma favouring the regulation of its concentration as free D-glucose in the compartment nearby where the uptake mechanism is occurring. Thus, an efficient reaction between D-glucose and hexokinases generating G6P may also be a mechanism to secure the flux of this sugar into the cells, thus keeping its plasma concentration in physiological, not damaging levels.

Based on the classic carrier model [13,44] for D-glucose uptake via GLUTs, the existence of a higher plasma-*to*-intracellular space concentration gradient of D-glucose results in a faster D-glucose uptake rate. This effect may be represented as preferential conformation states of the complexes *CoS* and *CiS*. The *Ci* being preferentially in the inward direction —in a lineal expression as $S + Co \leftrightarrow CoS \rightleftarrows CiS \rightleftarrows Ci + S$)— since the D-glucose is virtually pulled into phosphorylation and glycolysis with no option of binding back to the *Ci* conformation for its efflux (Fig. 2). The increased delivery of D-glucose as G6P to glycolysis results in a higher uptake rate than a hypothetical mechanism where D-glucose uptake was unaltered by an internal driving force exerted by the metabolism. In the latter, the uptake of D-glucose would show a lower rate than when metabolism is involved. Therefore, in a process where the metabolism is not involved in the transport of D-glucose, less efficient regulation of the plasma D-glucose concentration may happen. Interestingly, Macleod proposed that:

'… *glycolysis is an intracorpuscular* [blood cells] *process*. …'

and added that it was not a process present in the plasma. Furthermore, Macleod showed that redistribution of free D-glucose into plasma and blood cell compartments might imply at least these two concepts:

'… *first, the rate of absorption* [uptake] *of glucose into the corpuscle and secondly, the glycolytic power of the corpuscular contents* …'

It is worth mentioning that the enzymatic pathway for glycolysis was not known by 1920s. However, the latter concepts show that an interrelation between membrane transport and metabolism exists and that its dynamics figure out regulation of the plasma concentration of D-glucose. Efficient regulation of the plasma D-glucose concentration is critical when the glycaemia is elevated, as in patients with diabetes mellitus. In these patients, the fasting plasma glucose concentration is at least 1.4–1.6 fold (180–200 mg/dL or 10–11.1 mmol/L) higher than the desired concentration of this hexose in the blood of a healthy subject (fasting plasma glucose < 126 mg/dL or 7.0 mmol/L D-glucose according to ADA) [32]. The abnormally elevated blood concentration of D-glucose in these patients is a clear example of a higher plasma-*to*-

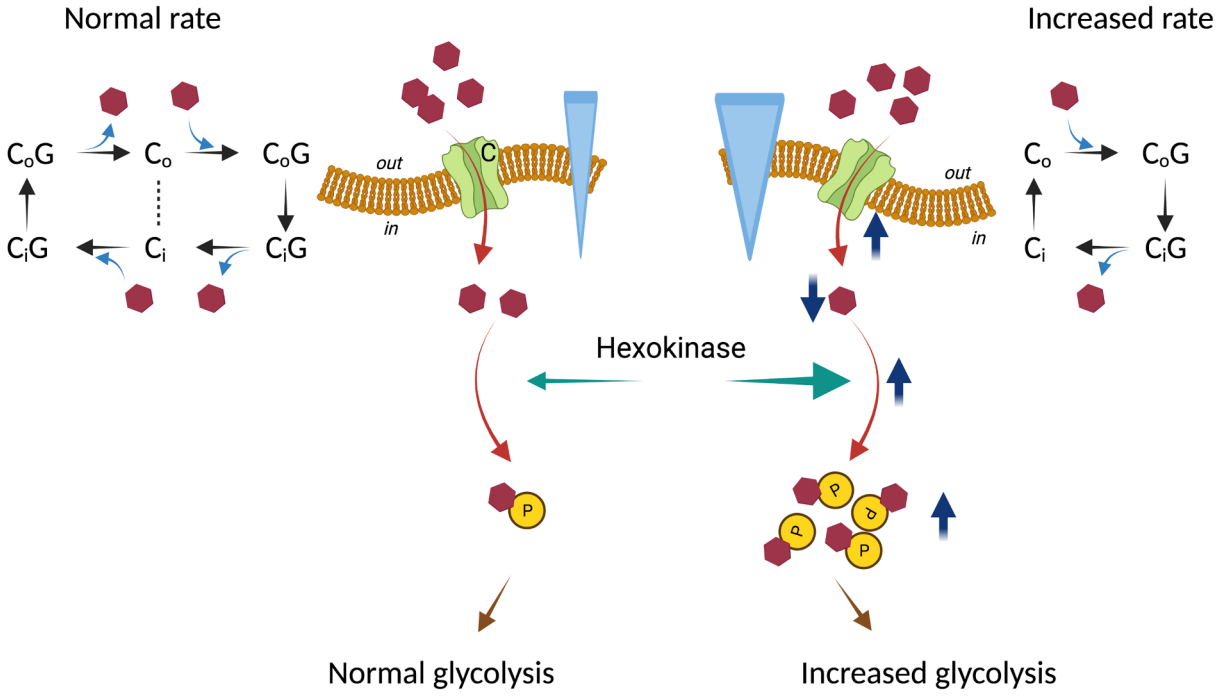

Fig. 2. Impact of glycolysis on D-glucose transport in blood cells. The fraction of D-glucose (red hexagon) contained in the blood that is not bound to plasma proteins (i.e. free D-glucose) crosses the plasma membrane via membrane transporters (C), also referred to as a carrier, from a high to low concentration (light blue triangles) favouring an influx transmembrane concentration gradient from the extracellular (*out*) to the intracellular (*in*) space. Under an average rate of glycolysis (*Normal glycolysis*), the uptake of D-glucose shows a normal rate sustained by the initial phosphorylation (P) of D-glucose by hexokinases. The dynamics of the uptake of D-glucose under the classical carrier model representation (assuming only influx in this example), the transmembrane transport of D-glucose (G) results from the initial exposure of this hexose in the extracellular medium to the transporters located in the plasma membrane. The carriers at this side of the plasma membrane show a high affinity for D-glucose generating the external configuration of C (Co). After Co binds G, a new conformation predominates, i.e. C_oG. The C_oG allows the displacement of G to the intracellular space generating a new conformation of the complex carrier-glucose, i.e. C_iG. D-Glucose is then released to the intracellular medium forming the internal configuration Ci. The existing G in the intracellular medium may rebind to this new Ci species to form C_iG allowing the efflux of this hexose since its affinity as C_i is reduced being released to the extracellular medium. By removing G from C_iG, the Co is reconstituted and becomes available to bind another Co, thus repeating the cycle. An increase (⇧) in the activity of hexokinase driven by increased glycolysis leads to more generation of phosphorylated D-glucose. The latter may cause a reduction (⇩) in the actual concentration of G due to a rapid consumption by hexokinases (notice the larger green arrow compared with *Normal rate*). This phenomenon increases the D-glucose transmembrane gradient, boosting this hexose uptake through the available membrane carriers increasing (*Increased rate*). This mechanism results in the appearance of more Co due to a translocation from Ci not complexed with G since its increased metabolism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

intracellular medium concentration gradient, thus promoting the facilitated diffusion of this sugar via GLUTs into blood cells. However, the metabolic activity, including glycolysis, is reduced in these patients [53], resulting in a diminished influx of D-glucose due to its *trans*-accumulation intracellularly, i.e. reducing the transmembrane concentration gradient. This phenomenon is strengthened by the subsequent saturation of the hexokinases whose Km ~50 μmol/L for D-glucose in human RBC [64], thus, limiting the D-glucose conversion to G6P in these cells [65]. The consequences of these alterations are a state of hyperglycaemia.

The apparent simple phenomenon of a transmembrane lower concentration gradient of D-glucose due to reduced glycolysis is a factor that adds to a reduced capacity of target tissue, i.e. extravascular tissues including skeletal muscle and fat tissue, to respond to insulin [66]. A consequence of this maladaptation of the insulin responding tissues is a reduced driven force for D-glucose to be extracted from the plasma. Furthermore, a potential role of SGLT1, the main isoform mediating absorption of D-glucose by the intestinal epithelium [67], and SGLT2 isoform, highly involved in the reabsorption of the luminal sugar by proximal kidney tubules [67,68], is proposed. Expression of SGLT1 and SGLT2 isoforms is increased as a consequence, at least in part, of the reduced uptake and metabolism of D-glucose by unhealthy tissues showing poor or lack of insulin response. The latter may be an adaptive response to insulin resistance, thus contributing to the accumulation of this sugar in the blood.

It is nowadays possible to point out that several mechanisms may contribute, at least partially, to an increase in the concentration of D-glucose in the blood, *viz*, (*i*) a potential reduction in the uptake by the blood cells and other tissues, (*ii*) reduced glycolysis, (*iii*) less sensitivity to insulin, and (*iv*) increased intestinal absorption and kidney reabsorption of this hexose.

4. Volemia and D-glucose transport

Another point that Macleod discussed in his review referred to the critical role that volemia —the volume of fluid in the vascular system that constitutes an efficiency index of the mechanisms involved in maintaining the body hydration and normal function— plays in the regulation of D-glucose concentration in the blood. Macleod's particular view on volemia as a potential factor modifying the glycaemia was significantly discussed in his publication in 1921. The discussion below is to actually uphold Macleod's thoughts from the 1920s addressing potential mechanistic phenomena associated with volemia that may result in a transient glycaemia regulation. Indeed, these mechanisms were not addressed at Macleods's original review of the available literature by 1920s.

Macleod and other groups referred to changes in the blood volume as:

'… *the increase or decrease in water* [content]…'

and defined this as a mechanism that was critical in changing the percentage of D-glucose in the blood.

Interestingly, one hundred years ago, it was also proposed by Macleod that:

'… *the surfaces of contact between plasma and cells may increase or diminish in proportion as the plasma volume increases or diminishes* …'

The latter proposal referred to the variation of RBC size depending on the state of volemia. An increased or reduced '*surface of contact*' between plasma and cells will result from the degree of deformability of the plasma membrane in RBC in response to a change in the osmotic pressure generated by alterations in the effective concentration of intracellular molecules, including D-glucose. These adaptations are due to the increased or reduced concentration of the fluid colloids and plasma D-glucose in hypovolemia or hypervolemia, respectively.

4.1. Hypovolemia

A drastic reduction in volemia, i.e. hypovolemia, may result from severe dehydration, polyuria, gastrointestinal reduced water absorption, rapid blood loss, or as a consequence of profound vasodilation. Macleod was aware of the concept of volemia and strongly considered the potential effect of severe hypovolemia as a factor that may intervene in the modulation of the concentration of D-glucose in the blood. Under physiological circumstances, normal volemia (or euvolemia) results from the equilibrium between the blood fluid and blood cells water content. The latter is a phenomenon that depends on the content of proteins and other structures with high osmotic activity —in this case, osmotically active substances being solutes that cannot pass across the semi-permeable plasma membrane— in the RBC. Thus, D-glucose distribution between the plasma and intracellular compartments is assumed to be in a basal state of equilibrium (i.e. steady-state). Since the space available for distribution of D-glucose in RBC depend on the volume (referring to size) of these cells under euvolemia, i.e. ~60–100 fL (~0.06–0.1 pL) [11,69], it is expected that a change in the cell size may result in modulating the distribution of D-glucose between these compartments. It is known that RBC can reduce by ~30% or increase by 2–3 fold (~150 fL or ~0.15 pL) their volume [11] when are immersed in the unfavourable osmotic conditions of hypovolemia or hypervolemia, respectively.

Dehydration-associated hypovolemia results in a lower plasma volume becoming an extracellular compartment whose D-glucose effective concentration increases compared with a state of euvolemia. Hypovolemia-associated increase in the plasma D-glucose concentration leads to the generation of a higher plasma-*to*-cell concentration gradient of this hexose. The latter increases the osmotic pressure mobilising the water contained in the RBC to the plasma as a physiological mechanism trying to restore an equilibrated distribution of D-glucose between these two compartments. RBC lose water and shrink under these circumstances, i.e. diminishing the '*surface of contact*'. The latter response of cells helps in reducing the plasma-*to*-RBC D-glucose concentration gradient. Therefore, the facilitated transport of D-glucose via GLUTs is favoured as a cell response expected to reach a new equilibrium steady-state for this hexose between the intracellular and extracellular spaces. In an immediate RBC plasma membrane microdomain, a *momentum* (hereafter defined as a restricted area of the plasma membrane and time) is generated under the concept of the classic carrier model. This *momentum* is assumed to be out of the equilibrium that gets closer to a *zero*-trans influx condition (even when the intracellular concentration of this sugar is not *zero*), thus favouring the uptake of D-glucose [13,16,44]. The latter is a phenomenon that requires changing the microkinetics of transport in terms of the relative formation of the different configurations described in the classical carrier model [44]. The first *momentum* at a state of *zero* (or relatively low) concentration of D-glucose in the intracellular space (*zero S*) may be represented as:

$$S + Co \rightleftarrows CoS \rightleftarrows CiS \cdots (zero\ \ S)$$

followed by rapid, first accumulation of D-glucose in the intracellular medium ending in restoring the equilibrium ($\rightleftharpoons$) for this sugar concentration in the extracellular and intracellular spaces:

$$S + Co \rightleftharpoons CoS \rightleftharpoons CiS \rightleftharpoons Ci + S$$

The resultant equilibrium steady-state for D-glucose distribution ends having a lower sugar content in the hypovolemia-associated shrunk RBC compared with the distribution expected in cells under euvolemia, i.e., RBC having a normal average size.

In severe cases of hypovolemia not associated with haemorrhagic shock, v.g. due to defective gastrointestinal and renal reabsorption of water, the haematocrit might increase due to an unaltered number of RBC in a total lower volume of blood. However, the haematocrit in patients with hypovolemia may result to be in the normal range

(~36–48% in women and ~40–54% in men) when the RBC size and the plasma volume are reduced, thus compensating a potential change in the haematocrit [70].

An interesting example of a reduced RBC size due to plasma volume reduction is described in astronauts that entered microgravity [69]. For the first 22 h in flight, astronauts showed a reduction in the plasma volume (~17%) without alterations in the haematocrit due to increased RBC number. Parallel determinations showed that RBC size was also reduced in this period by ~10% from ~90 to ~81 fL in these subjects. Another approach used as an index of the RBC size is the 'red blood cell distribution width' (RDW) [6771]. Since the RDW values are obtained from the expression $RDW = \frac{1SD}{MCV} \bullet 100\%$, where $1SD$ is one standard deviation, and *MCV* is mean corpuscle volume (or size), any change in the *MCV* will result in modifying the RDW (normal range is 11–15%) [71,72]. A reduction in MCV by ~10% results in an increase in the RDW value by ~9.7%, considering the RBC size in the astronauts described above [69]. Whether the RDW values in the astronauts were in the lower *cut-off* for the range considered normal, the reduction in RBC size will increase this parameter from ~11 to ~12.1%, i.e. a value still within the normal range. However, whether the astronauts showed RDW $\geq$ 13.7% a reduction in the RBC size of ~10% will put them out of the normal upper *cut-off* range based on this parameter.

Hypervolemia describes an excess of fluid in the intravascular compartment. This condition may result from increased sodium content in the body due to impaired function of the kidneys and liver or as a response to blood transfusion. Hypervolemia can also result in healthy individuals having a high sodium intake or being subjected to extreme conditions such as high-intensity exercise [73]. Hypervolemia has been a concern in patients subjected to dialysis since this condition is associated with an increased risk of cardiovascular disease and dysfunction of the vascular endothelium [74]. Fortunately, the consensus is that patients subjected to haemodynamic or peritoneal dialysis do not show hypervolemia [75].

4.2. Hypervolemia

Hypervolemia results in higher osmotic pressure generated by an effective increased concentration of intracellular molecules, including D-glucose. This adaptation is due to the dilution of the fluid colloids and plasma D-glucose by excess plasma volume. The plasma dilution generates a higher RBC-*to*-plasma D-glucose gradient concentration making the RBC gain water and swells, i.e. increasing the '*surface of contact*'. The latter responses help diminish the relative increased intracellular D-glucose concentration, thus, reducing the elevation in D-glucose gradient concentration. Contrary to the hypovolemia-increased influx of D-glucose, GLUT's facilitated efflux of D-glucose from a high (cells) to a lower (plasma) concentration is favoured in hypervolemia. A new *momentu*m is generated in the immediate plasma membrane microdomain holding GLUTs under the classic carrier model [13,16,44].

Adaptations of GLUTs activity to hypervolemia-increased RBC size configures a hypothetical condition out of the equilibrium that gets closer to a *zero*-trans efflux condition (even when the extracellular concentration of D-glucose is not *zero*), thus favouring the efflux of D-glucose. This phenomenon may be described as a first *momentum* at a state of *zero* (or relatively low) concentration of D-glucose in the extracellular space (*zero S*) and may be represented as:

$$(zeroS)\cdots CoS \leftrightarrows CiS \leftrightarrows CiS$$

followed by a rapid, first redistribution of D-glucose in the plasma, ending in restoring the equilibrium for the concentration of this sugar in the extracellular and intracellular spaces. As for hypovolemia, the resultant equilibrium steady-state for D-glucose distribution ends having a lower concentration of this sugar in the hypervolemia-associated swelled RBC compared with the distribution expected in cells under euvolemia.

Summarising the concepts mentioned above, the concentration of free D-glucose in the plasma is also subjected to changes in volemia, with hypovolemia favouring an increase in the effective concentration (not the total amount of D-glucose) and hypervolemia favouring a decrease in the concentration of this sugar in the plasma. As suspected and superficially touched by Macleod in his proposal in 1921, volemia is an added factor to consider in estimating free D-glucose in the plasma. The volemia is of extreme importance in patients needing dialysis. These patients include those affected by renal disease or patients with diabetes mellitus that are unable to control their blood glucose levels by a controlled and restricted diet, exercise, healthy life habits, or those treated with insulin or hypoglycaemic pharmaceutical drugs [76,77].

It was also visionary that Macleod continues his analysis of the available results indicating that a change in the '*surface of contact*' between the plasma and RBC should be a case in which:

> '… *the total number of glucose molecules impinging on the total cell surface will remain the same.*'

The latter sentence clearly stated that changes in RBC size due to hypovolemia or hypervolemia would not affect the quantity but the concentration of D-glucose, which was interacting with the cell surface. In 1921 the existence of GLUTs was still unknown. Thus, a potential interpretation for the statement mentioned above from Macleod of a change in the concentration of D-glucose affecting the transport mechanisms for this hexose in the plasma membrane of RBC was not possible. At present, and since 1986 the knowledge of the kinetics of GLUTs in human RBC is known [78]. Under equilibrium exchange of D-glucose transport, the calculated Km value (K_m^{ee}) for GLUT1 activity, the main form of facilitated D-glucose transporters expressed in human RBC [78-80], was ~17 mmol/L with a maximal velocity (Vmax) of ~5.9 mmol/L cell water/second (at 20 °C) [78]. Therefore, under the equilibrium exchange steady-state, a change in plasma concentration of D-glucose within the range of K_m^{ee} value will not significantly alter the maximal D-glucose transport capacity (influx or efflux) defined as the Vmax/Km ratio [76].

Interestingly, reducing the plasma volume by 10% will increase the plasma D-glucose concentration from 5.5 to ~6.11 mmol/L (i.e. ~0.61 mmol/L, in the absence of compensatory mechanisms). The magnitude of the change in the plasma D-glucose concentration will most likely not significantly alter the K_m^{ee} value (17 mmol/L at 5.5 mmol/L compared with a plasma D-glucose concentration ~6.11 mmol/L). However, changes in microkinetics for D-glucose transport via GLUT1 in the microenvironment of the plasma membrane where these proteins are located might occur due to this hexose's more significant plasma-*to*-RBC concentration gradient. The alterations in the microkinetics of D-glucose transport are reflected as a preferential *CoS* to *CiS* conformation, resulting in removing the excess of D-glucose from the extracellular space, thus buffering the relatively small increase in the plasma concentration of this hexose due to a reduced plasma volume.

GLUT1 is a transporter that shows asymmetry in influx and efflux kinetics. In human RBC, the affinity of GLUT1 for the *zero*-trans influx is higher than for *zero*-trans efflux [78]. The D-glucose transport under *zero*-trans influx shows a Km value ($K_m^{zt/oi}$, *oi* indicating extracellular-*to*-intracellular transport) for GLUT1 activity of ~1.6 mmol/L [78]. Thus, an increase of ~0.61 mmol/L D-glucose in plasma when a 10% reduction of the plasma volume may not significantly modify the uptake of D-glucose. The latter result from the possibility that GLUT1 in their external configuration (*Co*) will be saturated (i.e. all the binding sites in GLUT1 for D-glucose should be occupied) by an excess of ~2.9 mmol/L D-glucose (assuming that the phenomenon occurs without alterations in the Vmax and therefore the Vmax/Km). The same happens considering that the zero-trans efflux shows $K_m^{zt/io}$ (*io* indicating intracellular-*to*-extracellular transport) of 4.6 mmol/L in RBC. However, as for transport of D-glucose under an equilibrium exchange steady-state, the potential effect of a lower plasma volume on the *zero*-trans influx and *zero*-trans

efflux is likely occurring as part of the modifications of GLUT1 transport microkinetics. The latter may reflect changes in the local concentration of D-glucose in the microenvironment at the plasma membrane where these phenomena are occurring.

5. The concept of glycosuria as blood sugar index in diabetes

As mentioned by Macleod, the applicability of the discussed concepts concerning the distribution of sugar in the blood may be put in context in patients with altered D-glucose levels in the plasma and the urine. The pattern of changes in glycaemia and glycosuria —the appearance of glucose in the urine— were not understood by the 1920s. However, the data available in the 1910–1920s was the base allowing researchers and clinicians to understand the glycaemia link with glycosuria in patients with diabetes mellitus. A series of experiments performed by 1915–1921 was extensively described by Macleod, compiling previous data from Williams & Humphreys in 1919 to address the variations of D-glucose in the urine in healthy subjects and patients with very mild or mild diabetes [81]. The results in those experiments show that postprandial hyperglycaemia after an oral load of 100 g glucose in subjects with mild diabetes and severe cases of diabetes was seen for more extended periods (>3 h afterload) compared with the change in glycaemia detected in normal subjects (Fig. 3).

Interestingly, as emphasised by Macleod, hyperglycaemia in patients with very mild and mild diabetes was paralleled by increased glycosuria. A higher expression and activity of SGLTs transporters at the brush-border (apical or luminal) membrane in the cortical proximal tubules segment in the kidney (SGLT1 and SGLT2) and the absorptive enterocytes in the small intestine (SGLT1) result in higher reabsorption and absorption of D-glucose, respectively [67]. One of the first consequences of higher intestinal absorption and kidney reabsorption of D-glucose is an increase in this hexose concentration in the blood. An excess in carbohydrate and sodium load contained in the digested food increases the expression of SGLT1 in the intestine [82,83]. The latter raises the possibility that the absorptive enterocytes take up more D-glucose molecules from the digested food in the intestinal lumen due to a higher number of binding sites (or recognition sites) for this hexose at the apical plasma membrane in these cells.

SGLT1 is a membrane transporter with low capacity and high affinity showing a Km ~0.5–2 mmol/L for D-glucose requiring two sodium per molecule of D-glucose to energise its transport activity [84]. Therefore, SGLT1 role in the absorptive intestinal epithelium is key in recognising small changes in D-glucose concentration to get activated. Internalised D-glucose is subjected to a cycle of phosphorylation to form G6P by hexokinase and dephosphorylation, restoring D-glucose from G6P by the glucose-6-phosphatase. The hexokinase/glucose-6-phosphatase concerted activity is maintained in equilibrium since these enzymes show similar activity [85]. The available glucose in the enterocyte leaves these cells through the basolateral (abluminal) plasma membrane via GLUT2. The facilitated glucose transporters GLUT2 work favouring an enterocyte-*to*-interstitial space concentration gradient of this sugar, increasing its delivery to the blood. The exit of this ion efficiently keeps the driving force given by sodium to take up D-glucose through SGLT1 via the sodium/potassium ATPase at the basolateral membrane of these epithelial cells. An increase in the expression of SGLT1 and the higher and well equilibrated and interdependent activity of SGLT1 and GLUT2 also contribute to reducing the high D-glucose–derived osmotic activity in the intestinal lumen. Consequently, the loss of water due to the initial hyperosmotic content caused by D-glucose in the intestinal lumen is reduced.

Interestingly, patients with diabetes mellitus may also present with cellular and tissue dehydration, a condition that may result from lower water absorption in the colon [86]. The latter is a phenomenon seen in patients with the autosome recessive glucose/galactose malabsorption showing a mutation in SGLT1 and impaired function [87]. Intestine SGLT1 altered expression and activity without evident defects in the reabsorption of water in the kidney, resulting in water loss and dehydration.

Reduced reabsorption of D-glucose by the kidney leads to the appearance of detectable levels of this hexose in the urine [88]. The latter is a concept elaborated and reported by Macleod based on published findings by Bailey in 1919 [89] referred to as the threshold of the appearance of D-glucose in the urine. The proposal by Macleod was that:

> '… *the renal threshold represents a critical point in blood sugar below which the sugars of blood* [glycaemia] *and urine* [glycosuria] *run parallel but above which a large excess of sugar escapes into the urine.* …'

The dynamics of this concept regards a proposed renal threshold for glycaemia of ~170 mg/dL until the glycosuria increased in parallel in normal subjects [89]. In the present times, i.e. 100 years after, we refer

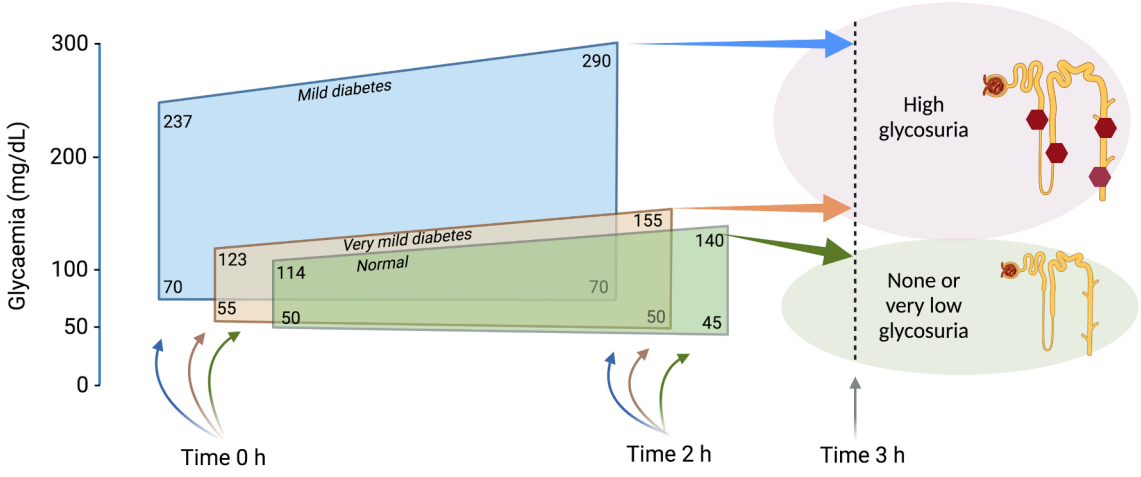

Fig. 3. Glycaemia and glycosuria after glucose load in subjects with very mild and mild diabetes. After an oral glucose tolerance test, the glycaemia in healthy subjects (*Normal*) or individuals with very mild (*very mild diabetes*) or mild (*mild diabetes*) diabetes was measured at the beginning (0 h) and after 2 h. The ranges of values expected for fasting plasma glucose are those indicated at time 0 h in *Normal* (green panel), *very mild diabetes* (brown panel), and in patients with decided but *mild diabetes* (blue panel). Two hours after the glucose load, the glycaemia ended with the indicated range of values, a phenomenon that is extended up to 3 h in individuals with *very mild diabetes* or *mild diabetes*, compared with the return to the normal range of glycaemia in *Normal* individuals. (information extracted from Figs. 3 and 4 in Macleod [1] and from the original data reported by Williams and Humphreys in 1919 [66]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to it as the renal threshold for glucose, and the most accepted glycaemia for this parameter is ~180 mg/dL [90,91]. Between glycaemia values of 180–270 mg/dL, glycosuria increases, which gets linear when glycaemia > 270 mg/dL [84].

In a healthy subject, the increased glycosuria is reverted by the efficient reabsorption of D-glucose (99–100% of D-glucose in the urine) via at least two active sodium-dependent transporters, *viz*, SGLT1 and SGLT2 located at the S1/S2 segments (i.e. the proximal convoluted tubule) and S3 segment (i.e. the proximal straight tubule) of the proximal tubules [67,84,92]. At the S1/S2 segment, the SGLT2 at the apical membrane runs in concert with GLUT2 activity and the sodium/potassium ATPase (keeping sodium gradient) at the basolateral membrane of the renal epithelium. However, at the S3 segment, the SGLT1 is preferentially expressed and acts in concert with GLUT1 at the basolateral membrane [67,84].

The human kidney SGLT2 shows low affinity (Km ~2–5 mmol/L), high-capacity transport activity requiring one sodium per molecule of D-glucose [84,93]. The role of SGLT2 in the D-glucose reabsorption by kidney epithelium at the S2 segment recognises more considerable changes in D-glucose concentration than those recognised by SGLT1 in the intestine enterocytes. GLUT2 located at the basolateral membrane in the kidney epithelium in the S2 segment of the proximal tubule secures the export of D-glucose from this tissue to the portal circulation to enter the blood. The rationale of translocation of D-glucose from the luminal to the basolateral (abluminal) membrane of the S2 segment renal epithelium is that it lacks aerobic and anaerobic glycolysis [84], thus offering this hexose from SGLT2 to GLUT2 for its efflux. GLUT2 are highly expressed at the S1 and S2 segments and to a lesser extent at the S3 segment. GLUT2 activity is of low affinity for D-glucose (Km ~15–20 mmol/L); therefore, the flux from SGLT2, which limits the influx of D-glucose over ~4–10 mmol/L (twice its Km and near or slightly higher than their Vmax), to these facilitative glucose transporters is preferent. Most D-glucose concentration in the nearest GLUT2 should not be far from that concentration that saturates the efflux activity of these transporters.

SGLT1 is abundantly expressed in the S3 segment of the kidney proximal straight tubule [92]. The activity of SGLT1 is of high affinity (Km ~0.5–2 mmol/L), low capacity, and requires one sodium to energise the reabsorption of D-glucose in the kidney [67]. The D-glucose uptake by the kidney epithelium through the apical membrane is also maintained by a permanent efflux of this hexose at the basolateral membrane via GLUT1, thus supporting the transcellular movement of this hexose. SGLT1 and GLUT1 show affinity values that are within the same range (Km from ~0.5 mmol/L in SGLT1 and ~1 mmol/L in GLUT1, both reaching maximal values of ~2 mmol/L). Therefore, the maximal SGLT1-mediated supply of D-glucose to GLUT1 may never overpass the Vmax of GLUT1, thus allowing the efficient facilitated removal of this hexose from the kidney epithelium to the portal circulation reaching the blood. It is worth mentioning that SGLT1 plays a minor role in the reabsorption of D-glucose (~10% versus ~90% via SGLT2) when SGLT2 expression and function is normal but increases (~3–4 fold) when SGLT2 activity is absent or drastically reduced by inhibitors [94].

The concerted expression and activity of SGLT2 and GLUT2 in the proximal convoluted tubule and SGLT1 and GLUT1 in the proximal straight tubule allows reabsorption of D-glucose from the urine. In this process, the combined activity of SGLT2/GLUTs result in the reabsorption of almost all the D-glucose released to the urine, with SGLT1/GLUT1 playing a less prominent but influential role in this process in normal subjects.

Patients with T2DM show a higher renal threshold for D-glucose (i.e. > 180 mg/dL), which is shown to increase the expression of SGLT2 in the proximal convoluted tubule [95,96]. The latter is a mechanism leading to higher reabsorption of D-glucose, aiming to minimise D-glucose excretion via the urine, resulting in a higher delivery of this hexose to the blood [96,97]. Nowadays, it is well known that the use of SGLT2 inhibitors may reduce the renal threshold for D-glucose to values between ~40–120 mg/dL leading to higher excretion of D-glucose, i.e. glycosuria resulting in reduced glycaemia. Interestingly, the use of SGLT2 inhibitors also results in lower systolic blood pressure, likely due to the resulting hypovolemia in critical cases by the hyperglycaemic osmotic diuresis-associated increase in kidney sodium and water clearance. The higher expression and activity of SGLT2 is paralleled by increased expression of GLUT2 as reported in human exfoliated proximal tubular epithelial cells [95]. The increase in GLUT2 expression is also proposed as a factor acting as sensor of basolateral hyperglycaemia to induce upregulation of the SGLT2 expression [98].

The pattern of SGLT1 expression in kidneys from patients with diabetes mellitus is less clear [99,100]. It was shown that SGLT1 mRNA level increased in patients with T2DM compared with subjects with normoglycaemia [101] and in patients with diabetes cardiomyopathy [102]. However, other studies show unaltered or reduced mRNA expression for this membrane transporter in T2DM [101,103]. A similar disparity of results is available for SGLT1 expression in the intestine in patients with diabetes mellitus [99,102]. GLUT1 expression in the kidney in patients with diabetes mellitus is still uncertain.

Final thoughts

It has been amazing, and a real adventure to address the concepts of sugar (referring to D-glucose) found in humans under different compartments (i.e. under the idea of compartmentalisation) in the blood. The separation between plasma and other blood elements, v.g. blood cells and proteins, is a concept extensively discussed by Professor Macleod contrasting the different groups working on this topic and the methodologies applied to determine the D-glucose level in the blood. Among other factors involved in regulating glycaemia, the review by Macleod referred to specific elements to detect the potential distribution of D-glucose in the blood that may influence glycaemia (Fig. 4). Macleod and contemporaneous groups discussed the existence of free D-glucose, i.e. not trapped in other molecules or structures, bound D-glucose, redistribution of D-glucose between the plasma and RBC, changes in the free D-glucose according to volemia, and other factors. Several aspects of these mechanisms are better known today, allowing different interpretations in normal subjects and patients with diabetes mellitus.

The concept of translational medicine was present by then but not openly defined as we know it today. However, the experimental approaches done by the 1920s and their interpretation in healthy subjects and patients with diabetes mellitus was translational medicine. The fact that analytical chemistry was limited may make us think that today's technology is tremendously developed; however, the principles are the same.

Several external factors may change the capacity of the individuals to manage proper glycaemia. Some of these factors include altered metabolic conditions such as obesity which was documented half a century earlier than when Macleod wrote his review. Most clinicians and basic researchers accept today that obesity might (should) be considered a pathology. External (v.g. air pollution, fatty food, contaminated water) and internal (v.g. hyperglycaemia) factors included in the exposome —internal and external environmental factors that delineate human health differently but complement the effects mediated by genetic background [104-106]— play a role in modulating the capacity of the body to maintain the glycaemia in the physiological ranges at fasting and postprandial behaviour. The concept of exogenous and endogenous exposome is recent in the literature, and its association with diabetes mellitus [103,104,105–108], including gestational diabetes mellitus [106] and gestational diabesity [75] assign to hyperglycaemia a key role in the regulation of D-glucose in the blood and glucosuria. Notably, the characterization of the permanently changing glycaemia dynamics in an individual with diabetes mellitus is more useful and somehow crucial in the decision making for their treatment compared with decisions based on a single capillary glycaemic measurement [109].

Diffusion of D-glucose was a mechanism not well understood in thermodynamics regarding the presence of membrane transporters in

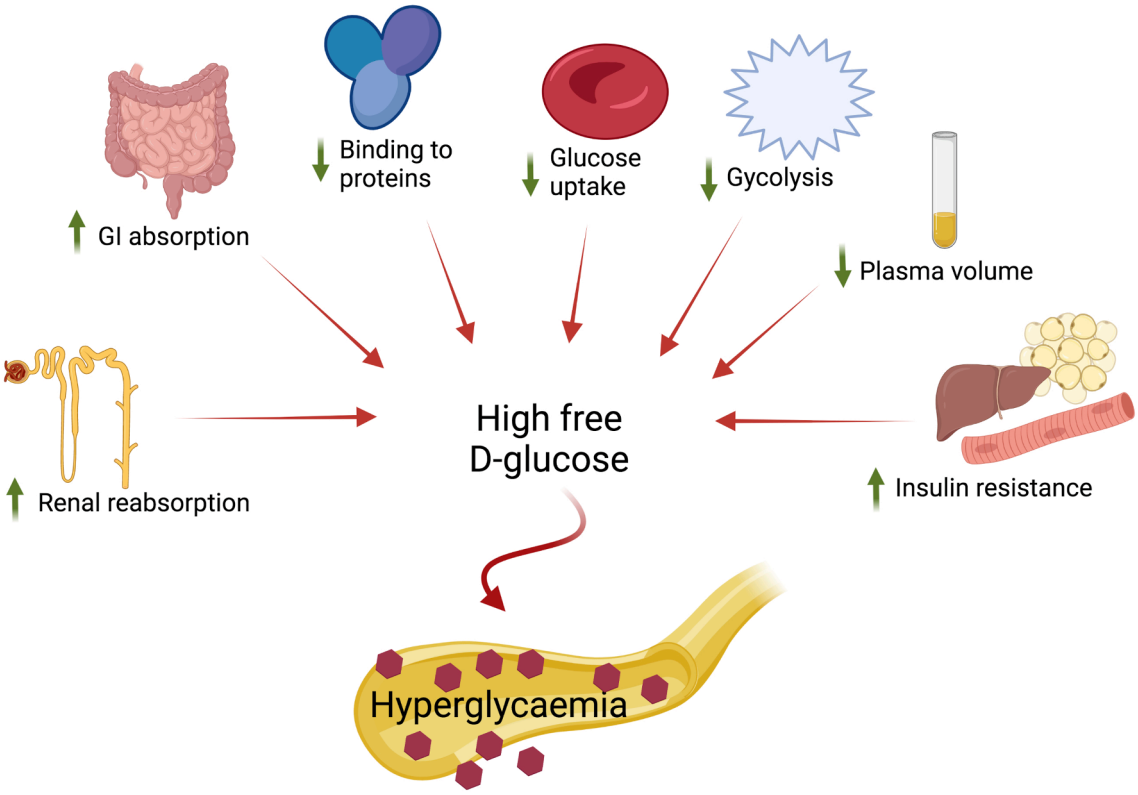

Fig. 4. Potential mechanisms that could increase the D-glucose in the blood. The glycaemia might increase (*Hyperglycaemia*) due to free D-glucose in the blood. The latter may result from higher (⇧) gastrointestinal absorption of D-glucose (*GI absorption*) and renal reabsorption, and insulin resistance by major organs (v.g. liver, skeletal muscle, and adipose tissue). Hyperglycaemia may also result from a lower (⇩) uptake of free D-glucose by red blood cells, reduced binding of this hexose to plasma proteins (v.g. albumin), diminished glycolysis, or increased plasma volume. See body text for details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

blood cells. We now know about the classical carrier model for transport substrates (including D-glucose) through the plasma membrane. The findings reported by Widas [13] and other contemporary groups allowed us to clarify several aspects of the dynamics of redistribution of D-glucose between the plasma blood cells. Also, it allowed the better knowledge of a dynamic movement of D-glucose in the plasma membrane by the proposed conceptual existence of different conformational states of the membrane transporters for this hexose and other molecules. The concept of diffusion of D-glucose from the plasma to blood cells favours a plasma-*to*-blood cells concentration gradient via GLUTs, and D-glucose absorption in the intestine and reabsorption in the kidneys by SGLTs are concerted in terms of kinetics. The concerted functional kinetics between these membrane transporters allows the interpretation of several highly specific inhibitors for the variety of proteins mediating D-glucose uptake (influx) and release (efflux) and the dynamic role in the SGLTs/GLUTS axis allowing transcellular transport.

Imagination, creativity, perseverance, and acute criticism of our and others work, as Macleod did, is and will continue being the key to understanding phenomena for which the tools (understood as knowledge) were not present and perhaps not even imagined. As the Nobel laureate Gabriel García Márquez mentioned in his masterpiece *One Hundred Years of Solitude*: '*…The world was so recent that many things lacked names, and to name them you had to point your finger at them…*' [110], the science behind '*The Sugar of the Blood*' by Macleod contained a bit (perhaps more than a bit) of this absolutely necessary component of life.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] J.J.R. Macleod, The sugar of the blood, Physiol. Rev. 1 (2) (1921) 208–238.
[2] P. Falcetta, M. Aragona, A. Bertolotto, C. Bianchi, F. Campi, M. Garofolo, S. Del Prato, Insulin discovery: A pivotal point in medical history 2022, Metabolism 127 (2022), 154941, https://doi.org/10.1016/j.metabol.2021.154941.
[3] S.K. Venugopal, P. Sankar, I. Jialal, Physiology, Glucagon. [Updated 2021 Mar 7], in: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2022 Jan-. https://www.ncbi.nlm.nih.gov/books/NBK537082/.
[4] G.D. Holman, Structure, function and regulation of mammalian glucose transporters of the SLC2 family, Pflugers Arch. 472 (2020) 1155–1175, https://doi.org/10.1007/s00424-020-02411-3.
[5] P.J. Stanirowski, D. Szukiewicz, A. Majewska, M. Wątroba, M. Pyzlak, D. Bomba-Opoń, M. Wielgoś, Placental expression of glucose transporters GLUT-1, GLUT-3, GLUT-8 and GLUT-12 in pregnancies complicated by gestational and type 1 diabetes mellitus, J. Diabetes Investig. 13 (3) (2022) 560–570.
[6] The Nobel Foundation, 2021. https://www.nobelprize.org/prizes/medicine/1923/summary/.
[7] EMJ Diabet. Review of the 57th Annual European Association for the Study of Diabetes Congress 9 (2021) 11-22. https://www.nobelprize.org/prizes/medicine/1923/summary/.
[8] K. Segrave, Obesity in America, 1850-1939. A history of Social Attitudes and Treatment. Ed. McFarlan & Company, Inc., Publishers, USA, 2008.
[9] J. Komlos, M. Brabec, The trend of mean BMI values of US adults, birth cohorts 1882–1986 indicates that the obesity epidemic began earlier than hitherto thought, Am. J. Hum. Biol. 22 (2010) 631–638, https://doi.org/10.1002/ajhb.21055.

[10] World Health Orgnization (WHO). Obesity and overweight, 2021. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
[11] C.E. McLaren, G.M. Brittenham, V. Hasselblad, Statistical and graphical evaluation of erythrocyte volume distributions, Am. J. Physiol. 252 (1987) H857–H866, https://doi.org/10.1152/ajpheart.1987.252.4.H857.
[12] P.G. LeFevre, Evidence of active transfer of certain non-electrolytes across the human red cell membrane, J. Gen. Physiol. 31 (1948) 505–527, https://doi.org/10.1085/jgp.31.6.505.
[13] W.F. Widdas, Inability of diffusion to account for placental glucose transfer in the sheep and consideration of the kinetics of a possible carrier transfer, J. Physiol. 118 (1952) 23–39, https://doi.org/10.1113/jphysiol.1952.sp004770.
[14] M. Kasahara, P.C. Hinkle, Reconstitution and purification of the D-glucose transporter from human erythrocytes, J. Biol. Chem. 252 (20) (1977) 7384–7390.
[15] Y. Zhang, T. Liu, Y. Chen, Z. Dong, J. Zhang, Y. Sun, B. Jin, F. Gao, S. Guo, R. Zhuang, CD226 reduces endothelial cell glucose uptake under hyperglycemic conditions with inflammation in type 2 diabetes mellitus, Oncotarget 7 (2016) 12010–12023, https://doi.org/10.18632/oncotarget.7505.
[16] G.E. Mann, D.L. Yudilevich, L. Sobrevia, Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells, Physiol. Rev. 83 (2003) 183–252, https://doi.org/10.1152/physrev.00022.2002.
[17] A.A. Mamun, H. Hayashi, A. Yamamura, M.J. Nayeem, M. Sato, Hypoxia induces the translocation of glucose transporter 1 to the plasma membrane in vascular endothelial cells, J. Physiol. Sci. 70 (2020) 44, https://doi.org/10.1186/s12576-020-00773-y.
[18] N. Gaudreault, D.R. Scriven, I. Laher, E.D. Moore, Subcellular characterization of glucose uptake in coronary endothelial cells, Microvasc. Res. 75 (2008) 73–82, https://doi.org/10.1016/j.mvr.2007.04.006.
[19] C.F. Burant, G.I. Bell, Mammalian facilitative glucose transporters: evidence for similar substrate recognition sites in functionally monomeric proteins, Biochemistry 31 (1992) 10414–10420, https://doi.org/10.1021/bi00157a032.
[20] H. Nishimura, F.V. Pallardo, G.A. Seidner, S. Vannucci, I.A. Simpson, M. J. Birnbaum, Kinetics of GLUT1 and GLUT4 glucose transporters expressed in Xenopus oocytes, J. Biol. Chem. 268 (12) (1993) 8514–8520.
[21] P.E. Day, J.K. Cleal, E.M. Lofthouse, M.A. Hanson, R.M. Lewis, What factors determine placental glucose transfer kinetics? Placenta 34 (2013) 953–1058, https://doi.org/10.1016/j.placenta.2013.07.001.
[22] D.J. Fazakerley, F. Koumanov, G.D. Holman, GLUT4 On the move, Biochem. J. 479 (2022) 445–462, https://doi.org/10.1042/BCJ20210073.
[23] W. Willett, J. Manson, S. Liu, Glycemic index, glycemic load, and risk of type 2 diabetes, Am. J. Clin. Nutr. 76 (2002) 274S–280S, https://doi.org/10.1093/ajcn/76/1.274S.
[24] H. Lee Lee, M. Um, K. Nam, S.J. Chung, Y. Park, Development of a prediction model to estimate the glycemic load of ready-to-eat meals, Foods 10 (2021) 2626, https://doi.org/10.3390/foods10112626.
[25] M.K. Leow, C.J. Henry, Glycemic index, glycemic load, and cardiovascular disease and mortality, N. Engl. J. Med. 385 (2021) 378, https://doi.org/10.1056/NEJMc2107926.
[26] J. Frampton, B. Cobbold, M. Nozdrin, H.T.H. Oo, H. Wilson, K.G. Murphy, G. Frost, E.S. Chambers, The effect of a single bout of continuous aerobic exercise on glucose, insulin and glucagon concentrations compared to resting conditions in healthy adults: a systematic review, meta-analysis and meta-regression, Sports Med. 51 (2021) 1949–1966, https://doi.org/10.1007/s40279-021-01473-2.
[27] R. Villalobos-Labra, M. Subiabre, F. Toledo, F. Pardo, L. Sobrevia, Endoplasmic reticulum stress and development of insulin resistance in adipose, skeletal, liver, and foetoplacental tissue in diabesity, Mol. Aspects Med. 66 (2019) 49–61, https://doi.org/10.1016/j.mam.2018.11.001.
[28] A. Klip, T.E. McGraw, D.E. James, Thirty sweet years of GLUT4, J. Biol. Chem. 294 (2019) 11369–11381, https://doi.org/10.1074/jbc.REV119.008351.
[29] F. Westermeier, T. Sáez, P. Arroyo, F. Toledo, J. Gutiérrez, C. Sanhueza, F. Pardo, A. Leiva, L. Sobrevia, Insulin receptor isoforms: an integrated view focused on gestational diabetes mellitus, Diabetes Metab Res Rev. 32 (2016) 350–365, https://doi.org/10.1002/dmrr.2729.
[30] Z.A. Knight, B. Gonzalez, M.E. Feldman, E.R. Zunder, D.D. Goldenberg, O. Williams, R. Loewith, D. Stokoe, A. Balla, B. Toth, T. Balla, W.A. Weiss, R. L. Williams, K.M. Shokat, A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling, Cell 125 (2006) 733–747, https://doi.org/10.1016/j.cell.2006.03.035.
[31] A. Goldin, J.A. Beckman, A.M. Schmidt, M.A. Creager, Advanced glycation end products: sparking the development of diabetic vascular injury, Circulation 114 (2006) 597–605, https://doi.org/10.1161/CIRCULATIONAHA.106.621854.
[32] Diabetes Care 44 (2021) S15–S33, https://doi.org/10.2337/dc21-S002.
[33] D.J. Jenkins, T.M. Wolever, R.H. Taylor, H. Barker, H. Fielden, J.M. Baldwin, A. C. Bowling, H.C. Newman, A.L. Jenkins, D.V. Goff, Glycemic index of foods: a physiological basis for carbohydrate exchange, Am. J. Clin. Nutr. 34 (1981) 362–366, https://doi.org/10.1093/ajcn/34.3.362.
[34] A.N. Bashkatov, E.A. Genina, Y.P. Sinichkin, V.I. Kochubey, N.A. Lakodina, V. V. Tuchin, Glucose and mannitol diffusion in human dura mater, Biophys. J. 85 (2003) 3310–3318, https://doi.org/10.1016/S0006-3495(03)74750-X.
[35] H. Chen, S. Huang, H. Wang, X. Chen, H. Zhang, Y. Xu, W. Fan, Y. Pan, Q. Wen, Z. Lin, X. Wang, Y. Gu, B. Ding, J. Chen, X. Wu, Preparation and characterization of paclitaxel palmitate albumin nanoparticles with high loading efficacy: an in vitro and in vivo anti-tumor study in mouse models, Drug Deliv. 28 (2021) 1067–1079, https://doi.org/10.1080/10717544.2021.1921078.
[36] M. Ohigashi, K. Osugi, Y. Kusunoki, K. Washio, S. Matsutani, T. Tsunoda, T. Matsuo, K. Konishi, T. Katsuno, M. Namba, H. Koyama, Association of time in range with hemoglobin A1c, glycated albumin and 1,5-anhydro-D-glucitol, J. Diabetes Investig. 12 (2021) 940–949, https://doi.org/10.1111/jdi.13437.
[37] J.E. Jun, S.-E. Lee, Y.-B. Lee, J.H. Jee, J.C. Bae, S.-M. Jin, K.Y. Hur, M.-K. Lee, J. H. Kim, V. Grolmusz, Increase in serum albumin concentration is associated with prediabetes development and progression to overt diabetes independently of metabolic syndrome, PLoS ONE 12 (4) (2017) e0176209.
[38] A. Mohamadi-Nejad, A.A. Moosavi-Movahedi, G.H. Hakimelahi, N. Sheibani, Thermodynamic analysis of human serum albumin interactions with glucose: insights into the diabetic range of glucose concentration, Int. J. Biochem. Cell Biol. 34 (2002) 1115–1124, https://doi.org/10.1016/s1357-2725(02)00031-6.
[39] H. McGuigan, E.L. Ross, Methods for the determination of blood sugar in reference to its condition in the blood, J. Biol. Chem. 31 (3) (1917) 533–547.
[40] I.S. Kleiner, The rate of dialysis of the blood sugar in experimental diabetes, J. Biol. Chem. 34 (3) (1918) 471–487.
[41] S.L. Cowart, M.E. Stachura, Glucosuria, in: H.K. Walker, W.D. Hall, J.W. Hurst (Eds.), Clinical Methods: The History, Physical, and Laboratory Examinations, 3rd ed., Butterworths, Boston, 1990. Chapter 139.
[42] C. Carpentier, S. Dubois, K. Mohammedi, N. Belhatem, B. Bouhanick, V. Rohmer, C. Briet, A. Bumbu, S. Hadjadj, R. Roussel, L. Potier, G. Velho, M. Marre, Glycosuria amount in response to hyperglycaemia and risk for diabetic kidney disease and related events in Type 1 diabetic patients, Nephrol. Dial. Transplant. 34 (2019) 1731–1738, https://doi.org/10.1093/ndt/gfy197.
[43] F.H. Epstein, P.R. Shepherd, B.B. Kahn, Glucose transporters and insulin action–implications for insulin resistance and diabetes mellitus, N. Engl. J. Med. 341 (4) (1999) 248–257.
[44] R. Devés, C.A. Boyd, Transporters for cationic amino acids in animal cells: discovery, structure, and function, Physiol. Rev. 78 (1998) 487–545, https://doi.org/10.1152/physrev.1998.78.2.487.
[45] W.R. Lieb, W.D. Stein, Carrier and non-carrier models for sugar transport in the human red blood cell, Biochim. Biophys. Acta, Lipids Lipid Metab. 265 (1972) 187–207, https://doi.org/10.1016/0304-4157(72)90002-0.
[46] A.G. Lowe, A.R. Walmsley, The kinetics of glucose transport in human red blood cells, Biochim. Biophys. Acta, Lipids Lipid Metab. 857 (1986) 146–1454, https://doi.org/10.1016/0005-2736(86)90342-1.
[47] M. Güven, H. Hatemi, E. Taşan, Y. Altuntaş, T. Ulutin, V. Tezcan, G. Kanigür-Sultuybek, The modulation of glucocorticoid receptor content by 3-O-methyl-D-glucose transport in human mononuclear leukocyte in obesity, J. Endocrinol. Invest. 21 (1998) 656–661, https://doi.org/10.1007/BF03350794.
[48] Y. Fu, L. Maianu, B.R. Melbert, W.T. Garvey, Facilitative glucose transporter gene expression in human lymphocytes, monocytes, and macrophages: a role for GLUT isoforms 1, 3, and 5 in the immune response and foam cell formation, Blood Cells Mol. Dis. 32 (2004) 182–190, https://doi.org/10.1016/j.bcmd.2003.09.002.
[49] H.F. Heijnen, V. Oorschot, J.J. Sixma, J.W. Slot, D.E. James, Thrombin stimulates glucose transport in human platelets via the translocation of the glucose transporter GLUT-3 from alpha-granules to the cell surface, 323-230, J. Cell Biol. 138 (1997), https://doi.org/10.1083/jcb.138.2.323.
[50] W. Bieger, H. Weicker, J. Michl, Transport and utilization of amino acids and glucose in human monocytes: activation of glucose metabolism by insulin, J. Clin. Endocrinol. Metab. 50 (1980) 1121–1126, https://doi.org/10.1210/jcem-50-6-1121.
[51] R. Bigley, M. Wirth, D. Layman, M. Riddle, L. Stankova, Interaction between glucose and dehydroascorbate transport in human neutrophils and fibroblasts, Diabetes 32 (1983) 545–548, https://doi.org/10.2337/diab.32.6.545.
[52] R.F. Corkey, B.E. Corkey, M.A. Gimbrone Jr., Hexose transport in normal and SV40-transformed human endothelial cells in culture, J. Cell. Physiol. 106 (1981) 425–434, https://doi.org/10.1002/jcp.1041060312.
[53] T. Guo, Y. Mao, H. Li, X. Wang, W. Xu, R. Song, J. Jia, Z. Lei, D.M. Irwin, G. Niu, H. Tan, Characterization of the gene expression profile of heterozygous liver-specific glucokinase knockout mice at a young age, Biomed. Pharmacother. 66 (2012) 587–596, https://doi.org/10.1016/j.biopha.2012.07.002.
[54] N. Kaiser, S. Sasson, E.P. Feener, N. Boukobza-Vardi, S. Higashi, D.E. Moller, S. Davidheiser, R.J. Przybylski, G.L. King, Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells, Diabetes 42 (1993) 80–89, https://doi.org/10.2337/diab.42.1.80.
[55] K.B. Gagnon, E. Delpire, Sodium transporters in human health and disease, Front. Physiol. 11 (2021), 588664, https://doi.org/10.3389/fphys.2020.588664.
[56] M.A. Hediger, M.J. Coady, T.S. Ikeda, E.M. Wright, Expression cloning and cDNA sequencing of the Na+/glucose co-transporter, Nature 330 (1987) 379–381, https://doi.org/10.1038/330379a0.
[57] E.M. Wright, Renal Na(+)-glucose cotransporters, Am. J. Physiol. Renal. Physiol. 280 (2001) F10–F18, https://doi.org/10.1152/ajprenal.2001.280.1.F10.
[58] E.M. Wright, D.D. Loo, B.A. Hirayama, Biology of human sodium glucose transporters, Physiol. Rev. 91 (2011) 733–794, https://doi.org/10.1152/physrev.00055.2009.
[59] E.M. Wright, C. Ghezzi, D.D.F. Loo, Novel and unexpected functions of SGLTs, Physiology (Bethesda) 32 (2017) 435–443, https://doi.org/10.1152/physiol.00021.2017.
[60] S.K. Bhavsar, Y. Singh, P. Sharma, V. Khairnar, Z. Hosseinzadeh, S. Zhang, M. Palmada, I. Sabolic, H. Koepsell, K.S. Lang, P.A. Lang, F. Lang, Expression of JAK3 sensitive Na+ coupled glucose carrier SGLT1 in activated cytotoxic T lymphocytes, Cell. Physiol. Biochem. 39 (2016) 1209–1228, https://doi.org/10.1159/000447827.
[61] F. Lang, Y. Singh, M.S. Salker, K. Ma, A.A. Pandyra, P.A. Lang, K.S. Lang, Glucose transport in lymphocytes, Pflugers Arch. 472 (2020) 1401–1406, https://doi.org/10.1007/s00424-020-02416-y.

[62] L. Uthman, A. Homayr, R.P. Juni, E.L. Spin, R. Kerindongo, M. Boomsma, M. W. Hollmann, B. Preckel, P. Koolwijk, V.W.M. van Hinsbergh, C.J. Zuurbier, M. Albrecht, N.C. Weber, Empagliflozin and dapagliflozin reduce ROS generation and restore NO bioavailability in tumor necrosis factor α-stimulated human coronary arterial endothelial cells, Cell. Physiol. Biochem. 53 (2019) 865–886, https://doi.org/10.33594/000000178.
[63] S. Vemula, K.E. Roder, T. Yang, G.J. Bhat, T.J. Thekkumkara, T.J. Abbruscato, A functional role for sodium-dependent glucose transport across the blood-brain barrier during oxygen glucose deprivation, J. Pharmacol. Exp. Ther. 328 (2009) 487–495, https://doi.org/10.1124/jpet.108.146589.
[64] C. Berger, D. Zdzieblo, Glucose transporters in pancreatic islets, Pflugers Arch. 472 (2020) 1249–1272, https://doi.org/10.1007/s00424-020-02383-4.
[65] S. Bhise, J. Rao, M. Hegde, S. Katyare, Type 2 diabetes differentially affects the substrate saturation kinetic attributes of erythrocyte hexokinase and phosphofructokinase, FEBS Lett. 594 (2020) 240–250, https://doi.org/10.1002/1873-3468.13604.
[66] D.E. James, J. Stöckli, M.J. Birnbaum, The aetiology and molecular landscape of insulin resistance, Nat. Rev. Mol. Cell Biol. 22 (2021) 751–771, https://doi.org/10.1038/s41580-021-00390-6.
[67] R. Sano, Y. Shinozaki, T. Ohta, Sodium-glucose cotransporters: Functional properties and pharmaceutical potential, J. Diabetes Investig. 11 (2020) 770–782, https://doi.org/10.1111/jdi.13255.
[68] L. Ferté, A. Marino, S. Battault, L. Bultot, A. Van Steenbergen, A. Bol, J. Cumps, A. Ginion, H. Koepsell, L. Dumoutier, L. Hue, S. Horman, L. Bertrand, C. Beauloye, New insight in understanding the contribution of SGLT1 in cardiac glucose uptake: evidence for a truncated form in mice and humans, Am. J. Physiol. Heart Circ. Physiol. 320 (2021) H838–H853, https://doi.org/10.1152/ajpheart.00736.2019.
[69] C.P. Alfrey, M.M. Udden, C.L. Huntoon, T. Driscoll, Destruction of newly released red blood cells in space flight, Med. Sci. Sports Exerc. 28 (1996) S42–S44, https://doi.org/10.1097/00005768-199610000-00032.
[70] C.R. Valeri, R.C. Dennis, G. Ragno, H. Macgregor, J.O. Menzoian, S.F. Khuri, Limitations of the hematocrit level to assess the need for red blood cell transfusion in hypovolemic anemic patients, Transfusion 46 (2006) 365–371, https://doi.org/10.1111/j.1537-2995.2006.00730.x.
[71] N. Li, H. Zhou, Q. Tang, Red blood cell distribution width: a novel predictive indicator for cardiovascular and cerebrovascular diseases, Dis. Markers 2017 (2017) 7089493, https://doi.org/10.1155/2017/7089493.
[72] E. Danese, G. Lippi, M. Montagnana, Red blood cell distribution width and cardiovascular diseases, J. Thorac. Dis. 7 (2015) E402–E411, https://doi.org/10.3978/j.issn.2072-1439.2015.10.04.
[73] H.J. Green, J.A. Thomson, M.E. Ball, R.L. Hughson, M.E. Houston, M.T. Sharratt, Alterations in blood volume following short-term supramaximal exercise, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 56 (1984) 145–149, https://doi.org/10.1152/jappl.1984.56.1.145.
[74] W. Tang, T. Xue, X.H. Lu, Y.J. Luo, T. Wang, Factors contributing to formation of edema in volume overloaded continuous ambulatory peritoneal dialysis patients, Perit. Dial. Int. 31 (2011) 160–167, https://doi.org/10.3747/pdi.2010.00055.
[75] P.V. Aguiar, O. Santos, L. Teixeira, F. Silva, P. Azevedo, J. Vidinha, F. Ferrer, M. J. Carvalho, A. Cabrita, A. Rodrigues, Overhydration prevalence in peritoneal dialysis - A 2 year longitudinal analysis, Nefrologia. 35 (2015) 189–196, https://doi.org/10.1016/j.nefro.2015.05.020.
[76] L. Sobrevia, R. Salsoso, T. Sáez, C. Sanhueza, F. Pardo, A. Leiva, Insulin therapy and fetoplacental vascular function in gestational diabetes mellitus, Exp. Physiol. 100 (2015) 231–238, https://doi.org/10.1113/expphysiol.2014.082743.
[77] M. Cornejo, G. Fuentes, P. Valero, S. Vega, A. Grismaldo, F. Toledo, F. Pardo, R. Moore-Carrasco, M. Subiabre, P. Casanello, M.M. Faas, H. van Goor, L. Sobrevia, Gestational diabesity and foetoplacental vascular dysfunction, Acta Physiol. (Oxf.) 232 (2021), e13671, https://doi.org/10.1111/apha.13671.
[78] I.I. Concha, F.V. Velásquez, J.M. Martínez, C. Angulo, A. Droppelmann, A. M. Reyes, J.C. Slebe, J.C. Vera, D.W. Golde, Human erythrocytes express GLUT5 and transport fructose, Blood 89 (1997) 4190–4195.
[79] E. Szabó, A. Kulin, L. Korányi, B. Literáti-Nagy, J. Cserepes, A. Somogyi, B. Sarkadi, G. Várady, Alterations in erythrocyte membrane transporter expression levels in type 2 diabetic patients, Sci. Rep. 11 (2021) 2765, https://doi.org/10.1038/s41598-021-82417-8.
[80] L.V. Maggiotto, M. Sondhi, B.C. Shin, M. Garg, S.U. Devaskar, Circulating blood cellular glucose transporters - Surrogate biomarkers for neonatal hypoxic-ischemic encephalopathy assessed by novel scoring systems, Mol. Genet. Metab. 127 (2019) 166–173, https://doi.org/10.1016/j.ymgme.2019.05.013.
[81] J.R. Williams, E.M. Humphreys, The clinical significance of blood sugar in diabetes mellitus, Arch. Int. Med. 23 (1919) 546–558.
[82] J. Dyer, I.S. Wood, A. Palejwala, A. Ellis, S.P. Shirazi-Beechey, Expression of monosaccharide transporters in intestine of diabetic humans, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2002) G241–G248, https://doi.org/10.1152/ajpgi.00310.2001.
[83] T.V. Fiorentino, E. Suraci, G.P. Arcidiacono, A. Cimellaro, C. Mignogna, I. Presta, F. Andreozzi, M.L. Hribal, F. Perticone, G. Donato, F. Luzza, G. Sesti, Duodenal sodium/glucose cotransporter 1 expression under fasting conditions is associated with postload hyperglycemia, J. Clin. Endocrinol. Metab. 102 (2017) 3979–3989, https://doi.org/10.1210/jc.2017-00348.
[84] V. Vallon, Glucose transporters in the kidney in health and disease, Pflugers Arch. 472 (2020) 1345–1370, https://doi.org/10.1007/s00424-020-02361-w.
[85] E.A. Newsholme, A.L. Carrié, Quantitative aspects of glucose and glutamine metabolism by intestinal cells, Gut 35 (1994) S13–S17, https://doi.org/10.1136/gut.35.1_suppl.s13.
[86] R. Gundamaraju, R. Vemuri, Pathophysiology of greedy colon and diabetes: role of atropine in worsening of diabetes, Euroasian J. Hepatogastroenterol. 4 (2014) 51–54, https://doi.org/10.5005/jp-journals-10018-1096.
[87] E. Turk, B. Zabel, S. Mundlos, J. Dyer, E.M. Wright, Glucose/galactose malabsorption caused by a defect in the Na+/glucose cotransporter, Nature 350 (1991) 354–356, https://doi.org/10.1038/350354a0.
[88] J.E. Gerich, Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications, Diabet. Med. 27 (2010) 136–142, https://doi.org/10.1111/j.1464-5491.2009.02894.x.
[89] C.V. Bailey, Studies on alimentary hyperglycemia and glycosuria, Arch. Intern. Med. (Chic.) 23 (1919) 455–483, https://doi.org/10.1001/archinte.1919.00090210049005.
[90] S.L. Cowart, M.E. Stachura, Glucosuria, in: H.K. Walker, W.D. Hall, J.W. Hurst (Eds.), Clinical Methods: The History, Physical, and Laboratory Examinations, 3rd ed., Butterworths, Boston, 1990. Chapter 139.
[91] S.S. Cui, L.J. Duan, J.F. Li, Y.Z. Qin, S.Q. Bao, X. Jiang, The factors influencing the renal glucose threshold in patients with newly diagnosed type 2 diabetes mellitus, Diabetes Metab. Syndr. Obes. 14 (2021) 4497–4503, https://doi.org/10.2147/DMSO.S336791.
[92] I. Vrhovac, D. Balen Eror, D. Klessen, C. Burger, D. Breljak, O. Kraus, N. Radović, S. Jadrijević, I. Aleksic, T. Walles, C. Sauvant, I. Sabolić, H. Koepsell, Localizations of Na(+)-D-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart, Pflugers Arch. 467 (2015) 1881–1898, https://doi.org/10.1007/s00424-014-1619-7.
[93] Y. Kanai, W.S. Lee, G. You, D. Brown, M.A. Hediger, The human kidney low affinity Na+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose, J. Clin. Invest. 93 (1994) 397–404, https://doi.org/10.1172/JCI116972.
[94] G. Gyimesi, J. Pujol-Giménez, Y. Kanai, M.A. Hediger, Sodium-coupled glucose transport, the SLC5 family, and therapeutically relevant inhibitors: from molecular discovery to clinical application, Pflugers Arch. 472 (2020) 1177–1206, https://doi.org/10.1007/s00424-020-02433-x.
[95] H. Rahmoune, P.W. Thompson, J.M. Ward, C.D. Smith, G. Hong, J. Brown, Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes, Diabetes 54 (2005) 3427–3434, https://doi.org/10.2337/diabetes.54.12.3427.
[96] R.G. Moses, S. Colagiuri, C. Pollock, SGLT2 inhibitors: New medicines for addressing unmet needs in type 2 diabetes, Australas Med. J. 7 (2014) 405–415, https://doi.org/10.4066/AMJ.2014.2181.
[97] D.S. Hsia, O. Grove, W.T. Cefalu, An update on sodium-glucose co-transporter-2 inhibitors for the treatment of diabetes mellitus, Curr. Opin. Endocrinol. Diabetes Obes. 24 (2017) 73–79, https://doi.org/10.1097/MED.0000000000000311.
[98] J. Nespoux, V. Vallon, SGLT2 inhibition and kidney protection, Clin. Sci. (Lond.) 132 (2018) 1329–1339, https://doi.org/10.1042/CS20171298.
[99] J.A. Dominguez Rieg, T. Rieg, What does sodium-glucose co-transporter 1 inhibition add: Prospects for dual inhibition, Diabetes Obes. Metab. 21 (2019) 43–52, https://doi.org/10.1111/dom.13630.
[100] V. Srinivasan Sridhar, J.P.N. Ambinathan, M. Kretzler, L.L. Pyle, P. Bjornstad, S. Eddy, D.Z. Cherney, H.N. Reich, European Renal cDNA Bank (ERCB); Nephrotic Syndrome Study Network (NEPTUNE). Renal SGLT mRNA expression in human health and disease: a study in two cohorts, Am. J. Physiol. Renal. Physiol. 317 (5) (2019) F1224–F1230.
[101] L. Norton, C.E. Shannon, M. Fourcaudot, C. Hu, N. Wang, W. Ren, J. Song, M. Abdul-Ghani, R.A. DeFronzo, J. Ren, W. Jia, Sodium-glucose co-transporter (SGLT) and glucose transporter (GLUT) expression in the kidney of type 2 diabetic subjects, Diabetes Obes. Metab. 19 (2017) 1322–1326, https://doi.org/10.1111/dom.13003.
[102] B. Pitt, D.L. Bhatt, Does SGLT1 inhibition add benefit to SGLT2 inhibition in type 2 diabetes? Circulation 144 (2021) 4–6, https://doi.org/10.1161/CIRCULATIONAHA.121.054442.
[103] A. Solini, C. Rossi, C.M. Mazzanti, A. Proietti, H. Koepsell, E. Ferrannini, Sodium-glucose co-transporter (SGLT)2 and SGLT1 renal expression in patients with type 2 diabetes, Diabetes Obes. Metab. 19 (2017) 1289–1294, https://doi.org/10.1111/dom.12970.
[104] C.P. Wild, Complementing the genome with an “exposome”: The outstanding challenge of environmental exposure measurement in molecular epidemiology, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 1847–1850, https://doi.org/10.1158/1055-9965.EPI-05-0456.
[105] C.P. Wild, The exposome: From concept to utility, Int. J. Epidemiol. 41 (2012) 24–32, https://doi.org/10.1093/ije/dyr236.
[106] P. Valero, G. Fuentes, M. Cornejo, S. Vega, A. Grismaldo, F. Pardo, G. García-Rivas, J.L. Hillebrands, M.M. Faas, P. Casanello, E.M. van der Beek, H. van Goor, L. Sobrevia, Exposome and foetoplacental vascular dysfunction in gestational diabetes mellitus, Mol. Aspects Med. 101019 (2021), https://doi.org/10.1016/j.mam.2021.101019.
[107] D.J. Hill, Impact of the exposome on the development and function of pancreatic β-cells, Mol. Aspects Med. 100965 (2021), https://doi.org/10.1016/j.mam.2021.100965.
[108] N. Kupper, B. Huppertz, The endogenous exposome of the pregnant mother: Placental extracellular vesicles and their effect on the maternal system, Mol. Aspects Med. 100955 (2021), https://doi.org/10.1016/j.mam.2021.100955.
[109] P. Valero, R. Salas, F. Pardo, M. Cornejo, G. Fuentes, S. Vega, A. Grismaldo, J.-L. Hillebrands, E.M. van der Beek, H. van Goor, L. Sobrevia, Glycaemia dynamics in gestational diabetes mellitus, Biochim. Biophys. Acta, Gen. Subj. 1866 (7) (2022) 130134, https://doi.org/10.1016/j.bbagen.2022.130134.
[110] G.G. Márquez, One Hundred Years of Solitude, Harper, USA, 2003.