

Glucose transport in human red blood cells

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Abstract: The penetration of D-[¹⁴C] glucose into human red blood cells (RBCs) features kinetic parameters which are readily distinguishable from passive permeation. It would be expected to require activation energy above 80 kJ/mol for permeation of glucose with five hydroxyls capable of forming hydrogen bonds, but the measured activation energy is approximately 16 kJ/mol. As a consequence, glucose permeates RBC membrane about five orders of magnitude faster than would be expected for passive permeation. Glucose transporter protein 1, or GLUT1 and SGLT1, present in all human tissues, but especially in RBCs. It is also anchored in the protective sheet of flat cells that line up the blood vessels of the brain. GLUT1 has a strong affinity for glucose and it ensures that both RBCs and the brain receive appropriate levels of glucose that they need to be able to function. The brain consumes ~120g of glucose per day; the blood glucose level in a typical person 80mg/100ml. The binding site of glucose faces intracellular and extracellular of the membrane alternately when it is loaded by a glucose. The transport is accomplished by conformational changes within GLUT1, and not by rotation of the whole single long polypeptide chain (55kD, ~500 residues) with the presence of 12 trans membrane α -helices segments. The super family of related GLUT sugar transporters comprises 14 identified isoforms in the human genome, all adopting a 12-membrane-spanning domain structure that delineate 6 extracellular loops. The erythrocyte glucose transporter GLUT1 has an ~10-fold-lower affinity for D-glucose, $K_m \approx 10$ –15 mM, at the inside face for net export than on the outside ($K_m = 1$ –2 mM) for net import of glucose (zero-*trans* net flux) at 24°C, pertaining a liganded consequential asymmetric transporter.

Keywords: D-[¹⁴C]Glucose Transporter (GLUT1), Secondary Active Glucose Transporter (SGLT), Membrane Transport, Glucose Transporter Gene (GLUT1 cDNA), Erythrocytes, Glycogenolysis, *Diabetes Mellitus*, D-Glucose, Facilitated Transport, Cytochalasin B (CB), Na⁺/K⁺-ATPase Pump, Carbamazepine, Simvastatin.

1. Introduction

The mature RBC (erythrocyte) of the normal allele Hb^A is flexible and oval biconcave disc 8 x 8 x 1 to 2 μ m with a volume of 80 μ m³ [1,2]. A 0.9% NaCl solution is isotonic with plasma [3]. RBC lacks a cell nucleus and most organelles. New erythrocytes are produced ~2.4 million per second, and an erythrocyte circulates for about 100–120 days in the body before its components are recycled by macrophages [4]. Since RBC has no mitochondria and enzymes of citric acid cycle and oxidative phosphorylation, adenine triphosphate synthesis takes place through glycolysis.

Passive and mediated permeations are more generally distinguishable on the basis of differences in the kinetics and specificities of the two processes [5]. When the change in the intracellular concentration is measured as a function of time in a facilitated transport system in a single

experiment, the results appear very much like a simple diffusion process (Fig.1), since the final intracellular concentration equals the extracellular concentration, $[C]_i = [C]_o$. It is only when the concentration of extracellular is varied and competing chemical molecules are added to the system that specificity, saturation and competition are demonstrated. A system that moves substrate by facilitated diffusion carriers across the membrane of RBCs is invariably of limited capacity and it should eventually become saturated [6]. As the substrate gradient is increased, the rate of substrate permeation would be expected to approach and maintain a maximum provided that the substrate concentration can be raised to an appropriate level. This unidirectional flux of substrate across the RBC membrane is described approximately by adopting a Michaelis-Menten [7] type expression:

$$J_{1 \rightarrow 2} = J_{max} [C]_1 / K_m + [C]_1$$

where $J_{1 \rightarrow 2}$ is flux from compartment 1 to compartment 2, J_{\max} is maximum rate of flux,

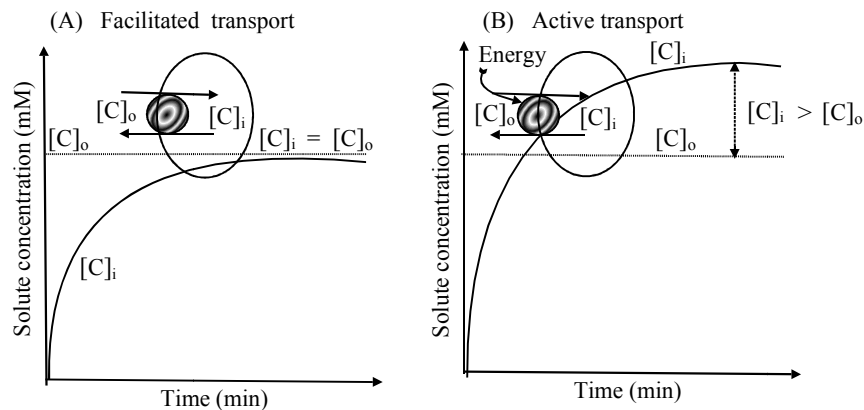


Fig 1. Time course of changes in human RBC intracellular concentration $[C]_i$ during (A) facilitated transport, resulting from the permeation of extracellular solute molecules; (B) active transport, resulting in an intracellular concentration of solute higher than the extracellular concentration $[C]_o$, i.e. the value at saturation of the mediated system and $[C]_i$ is the concentration of glucose in compartment 1. The net flux in these terms would be:

$$J_{1 \rightarrow 2} = J_{\max} \left\{ \left(\frac{[C]_1}{K_m + [C]_1} \right) - \left(\frac{[C]_2}{K_m + [C]_2} \right) \right\}$$

The electrochemical gradient in many mediated permeation systems is maintained by removing the substrate from the second compartment, and the rate of mediated permeation is then closely approximated by:

$$J_{1 \rightarrow 2} = J_{\max} \frac{[C]_1}{K_m + [C]_1}$$

This formal similarity to enzyme kinetics means that certain types of mathematical calculations can be used to determine J_{\max} and K_m , and also to characteristics such as specificity, competitive inhibition, pH dependence,

temperature dependence, specific inhibition of the mediated permeability [8].

Glucose is transported across RBC membranes by a uniporter; that is, transporters carry only a single type of substrate across the membrane [9,p.395]. Glucose concentration in the blood is carefully regulated (80mg/100ml, 4.4-8mM), so that it is normally higher than intracellular concentrations. As soon as glucose enters the cell, it is converted into other chemicals (Fig.2) needed for energy production or biosynthesis and to maintain intracellular concentration much lower than 5mM normal level in the blood.

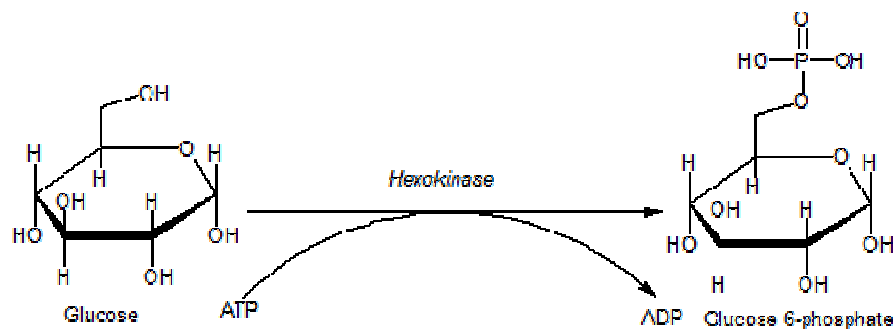


Fig 2. The formation of a phosphate ester of glucose. Adenosine triphosphate is the phosphate group donor. The enzyme [2.7.1.1 or 2.7.1.2.] specifies the interaction with $-CH_2OH$ on carbon 6 of α -D- Glucose (α -D- Glucopyranose). Adapted from: Stryer, L. (1995) *Biochemistry*, 4th edn., p.571. New York, NY: H.W. Freeman and Company.

In hereditary spherocytosis (congenital hemolytic icterus), the cells are spherocytic in normal plasma and hemolyze more readily than normal cells in hypotonic NaCl solutions. RBCs can also be lysed by drugs and infections. The susceptibility of RBCs to hemolysis by these agents is increased by deficiency of the enzyme glucose-6-phosphate dehydrogenase [10, 11], which catalyzes the initial step in the oxidation of glucose via hexose monophosphate shunt aerobic minor pathway [9]. This pathway generates nicotinamide adenine dinucleotide phosphate (NADPH) that is required to sustain normal fragility of the cell [12, 13]. Congenital deficiency of this enzyme is the

commonest known genetically determined human enzyme abnormality [14].

However, glucose-6-phosphate dehydrogenase [1.1.1.49], triose phosphate isomerase [5.3.1.1.], pyruvate kinase [2.7.1.40] and glutathione reductase [E.C.1.6.4.2] are essential human RBC enzymes in the pentose phosphate pathway in preventing haemoglobin oxidation [15]. The deficiency of these enzymes cause the failure of this mechanism that would in turn result in oxidizing, denaturing and precipitating haemoglobin as Heinz bodies [16,17].

2. RBC Membrane Defect

The cell membrane of the erythrocyte is a trilaminar bipolar phospholipid structure in which are anchored many types of integral structural proteins; it is flexible and it is often referred to as “fluid mosaic structure” [18].

Rhesus (Rh) blood group antigens are transmembrane proteins with loops exposed at the surface of RBCs [19,20]. Very rare patients with the lack of Rh antigens have severe membrane abnormalities that result in deformed RBCs known as stomatocytes with an elongated (mouth-like) area of central pallor that shortens survival times [21]. The ABO glycoprotein blood group antigens are located on the outer surface of the membrane and they are not integral structural proteins of the membrane [22].

Spherocytosis congenital autosomal dominant disease, i.e. a familial hemolytic disorder condition that causes an abnormality in the RBC membrane [23,24]. Elliptocytosis (ovalocytosis) is a rare autosomal dominant disorder that resembles spherocytosis except that the RBCs are varying in shape from elongated to oval and the disease is less severe, associated with mild splenomegaly [25]. They can be seen in hereditary disorders or in acquired disorders, such as iron deficiency anemia, infectious anemia, thalassemia, and in newborn babies [26]. Paroxysmal nocturnal haemoglobinuria is an acquired disorder characterized by a defect in the glycosylphosphatidylinositol anchor that leads to the premature death and impaired production of RBCs [27, 28].

3. Materials and Methods

D- ^{12}C Glucose and $[\text{U-}^3\text{H}]$ inulin were purchased from Radiochemical Centre, Amersham, Buck, UK; Silicon oil was purchased from Hopkin and Williams, Romford, Essex, UK. All other materials were supplied by Sigma (London) Chemical Co., Poole, Dorset, UK, or by BDH Ltd., Poole, Dorset, UK, and were of AnalaR grade whenever possible. All solutions were prepared with glass-distilled water.

4. Erythrocyte Preparation

Freshly outdated blood containing citric acid, sodium citrate, dextrose and adenosine was collected from Essex Country Hospital, Colchester, UK. Unfortunately, the gender, age and lifestyle of blood donors were anonymous for making comparative analysis. Human erythrocytes were separated by sedimentation at 2500 g (at r_{av} 13 cm) and 4°C for 5 min using MSE Mistral 4L. The plasma and buffy coat were removed by aspiration. The erythrocytes were then washed three times by re-suspension and sedimentation in ice-cold NaCl (152 mM). Finally, erythrocytes were suspended in Krebs-Ringer bicarbonate [29] at pH 7.4, 1:1 (v/v) for isotope flux studies, and kept on ice.

Erythrocytes were always used for experiments immediately after preparation. The volume of erythrocyte

intracellular water (68.8%) was determined with the use of $[\text{H}^3]$ inulin as described by Winter and Christensen [30] for rabbit erythrocytes. The standard dry weight of erythrocyte suspension (typically ~30%) was used to calculate the intracellular fluid for each experiment.

5. D- ^{12}C Glucose Flux Studies

D- ^{12}C Glucose influx was measured using a slight modification of the procedure reported by Eavenson and Christensen [31] for pigeon erythrocytes. An iso osmotic glucose/sucrose solution (1ml, pH 7.4) containing 0.05-0.25 $\mu\text{Ci/ml}$ was pipetted into a test tube and pre-incubated at 37 °C for 15 min. An erythrocyte suspension (packed cells: Krebs Ringer bicarbonate buffer, 1:1 by vol, 1 ml) was then added, oxygenated and incubated. The media was shaken mechanically throughout the incubation period. At predetermined intervals 0.5 ml of incubation medium was added to polypropylene conical tubes (1.5 ml) containing 0.5 ml of silicon oil (ρ 1.07) and centrifuged for 20 s in a Beckmann Microfuge B [32]. Aliquots of the supernatant fluid (0.1 ml) were taken for scintillation counting, the remainder being removed by aspiration. Extra care was taken not to aspirate portions of the pellet while removing the oil. H_2O (0.25 ml) was then added to the packed cells with vigorous mixing. The resulting haemolyzed erythrocytes were bleached with 10% trichloroacetic acid (0.25 ml). The denatured material was sedimented by centrifugation and a 0.1 ml sample of supernatant fluid was taken for scintillation counting.

Under these incubation conditions, with extracellular chloride ~ 40mM, chloride ions efflux from the cell at the onset of the incubation due to the chloride concentration gradient across the erythrocyte membrane. This net flux creates a diffusion potential which is positive inside. This diffusion potential increases both the initial rate and the distribution ratio of glucose (unpublished observations). These incubation conditions were used throughout the study. Similar results were obtained by substituting Krebs Ringer bicarbonate buffer by phosphate/citrate, Tris-HCl, Sorenson's phosphate or glycine/NaOH buffers (pH 7.4). During the initial phase of uptake experiments, the influx of glucose into human erythrocytes is effectively irreversible and is pseudo-zero order [7]. The rate of influx of glucose (1 to 20 mM) was linear for time period up to 5 min. Fixed time assays were run either for 1 min or 2 min intervals. Values are presented as mean \pm SD, based on 3 measurements per sample.

It is assumed that the properties of the D- ^{12}C glucose tracer do not differ from those of the nonradioactive D- ^{12}C glucose.

6. Results

Miller [33] investigated several methods of measuring glucose flux across human red blood cell membranes, using the Ørskov [34] light scattering technique.

Table 1. Glucose transport across the human red blood cell membrane

Procedure	Direction	V_{\max} (mM/min)	K_m (mM)
zero-trans	inside \rightarrow outside outside \rightarrow inside	$V_{1 \rightarrow 2}^{zt}$ 129.0	$K_{1 \rightarrow 2}^{zt}$ 25.0
		$V_{2 \rightarrow 1}^{zt}$ 32.0	$K_{2 \rightarrow 1}^{zt}$ 1.8
Infinite-cis	inside \rightarrow outside	$V_{1 \rightarrow 2}^{ic}$ 132.0	$K_{1 \rightarrow 2}^{ic}$ 1.9
	outside \rightarrow inside	$V_{2 \rightarrow 1}^{ic}$ 85.0	$K_{2 \rightarrow 1}^{ic}$ 2.8
equilibrium exchange	D- $[^{12}C]$ glucose in or out	$V_{1 \rightarrow 2} = V_{2 \rightarrow 1}$	
$[glucose]_{in} = [glucose]_o$	both directions	$=V^{ec}$ 310.0	K_m^{ec} 31.0

NB: Fluxes are always considered to be measured from the cis to the trans solution. So, either solution could be the cis for influx or efflux ; the other solution would be then the trans for efflux or influx.

Adapted from: Naftalin, R.J. and Holman, G.D. (1977) Transport of Sugars in Human Red Blood Cells, in Membrane Transport in Red Cells, Ellory, J.C. and Lew, V.L. (eds), pp.257-300. London: Academic Press; Lieb, W.R. (1982) A Kinetic Approach to

Transport Studies, in Red Cell Membranes A Methodological Approach, Ellory, J.C. and Young, J.D. (eds), pp.135-164. London: Academic Press ; Naftalin, R.J. (2008)

“Alternating carrier model of asymmetric glucose transport violate the energy conservation laws”, Biophysical J., vol.95, pp.4300-4314.

The transport of D-glucose mediated by GLUT1 [35] is inhibited by cytochalasin B, sugar transport inhibitor, at a stoichiometric ratio of 1:1 [36, 37]. Also, since haemoglobin is by the far the major protein constituent of

the RBC cytoplasm, the removal of the internal contents of RBC has a marked effect on glucose flux asymmetry system because haemoglobin affects the glucose transport kinetics [38].

The presence of very significant trans acceleration immediately supports the model of facilitated transport for consideration. Also, the various K_m values are not uniformed, and the human RBC glucose system is overwhelmingly complicated than even a simple carrier though heterosaccharides fluxes [39] supports the notion of fixed number of carrier molecules within the membrane [40].

7. The Carrier Hypothesis

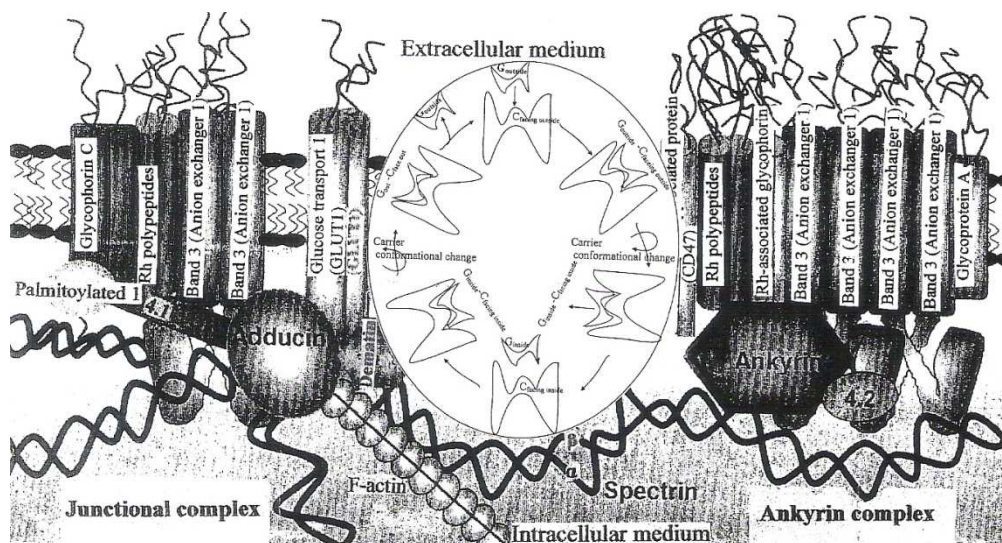



Fig 3. The alternating conformation model of α -D- glucose () carrier in human erythrocyte by GLUT1. The glucose cooperative tetramer of proteins carrier is proposed to exist in one of two conformation, with a binding site for glucose facing either outside or inside of the cell. A complex of glucose-carrier results from the binding of glucose to its site that cause re-orientation of the protein carrier, so that the glucose gains access to the opposite side of the RBC membrane in a three-mode sequential cycle.

The properties of specificity, saturation and competition suggests that a specific chemical site within the membrane called GLUT1 is able to combine with the transported glucose [39, 41, 42]. GLUT1 associates with stomatin, a cholesterol-binding, structural/scaffolding protein that forms large oligomeric complexes associated with cholesterol-rich membrane domains; its absence in RBC

disorder may condition the differential recruitment of channel and transporter glycoproteins to structured membrane microdomains [43, 44]. The carrier hypothesis is a model that attempts to explain the properties of facilitated transport in terms of the movements of the carrier and the carrier-glucose complex (Fig.3). The interaction of lipid raft-associated membrane protein with GLUT1 regulates

the distinctive transport properties of GLUT1 in human RBC. The simultaneous existence of both an outward-facing and inward-facing carrier binding sites cannot occur within a single glucose transporter [45, 46] of three mediated events [47].

The phenomenon of trans-acceleration of glucose flux through RBC membranes is very interesting; unlabeled D-glucose on one side of the membrane stimulates the transport of [^{12}C] glucose from the other (trans) side [23, 48, 49]. This is ramified on the basis that rate constant of an empty carrier re-orientation is less than that of a loaded

carrier. Also, this phenomenon rules out inside / outside two sites load carrier re-orientation [40].

The mature human RBCs express functional insulin receptors, as demonstrated by increased calcium flux and tyrosine phosphorylation in response to insulin [50].

Adapted from: Hajjawi, O.S. (2013) "Ionic and osmotic equilibria of human red blood cells", American J. Sci. Res., vol.86, p.181; Hajjawi, O.S. (2013) "Human red blood cells-2", American Journal of life Sciences, vol.1(5), p.221.

8. Sources of Blood Glucose

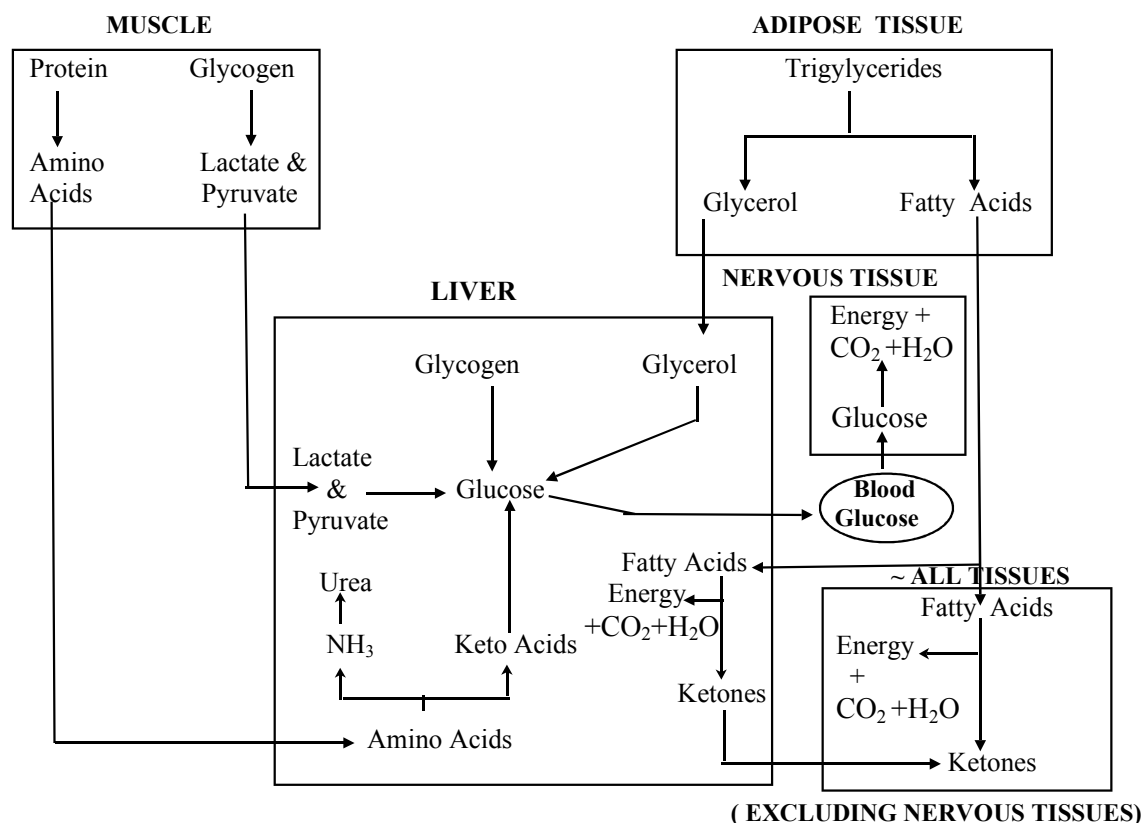


Fig 4. Major metabolic pathways of glucose during fasting to regulate blood glucose concentration (~5.5 mM).

The central focus is the regulation of blood glucose concentration through major metabolic pathways (Fig. 4) of the post absorptive (fasting) phase is as follows:

1. Glycogen (animal starch) is glucose residues linked by α -1, 4 glycosidic bonds. It branches out at ~every tenth to fourteenth residue by a α -1, 6 glycosidic bonds. It is stored in the liver and it breaks down (glycogenolysis) by glycogen phosphorylase [EC 2.4.1.1] to release glucose 1-phosphate from glycogen for 83 min [51]. The normal liver contains less than 100 g of glycogen (at 4 kcal/g, giving 400kcal or 1674 kJ \rightarrow enough energy to meet body needs for ~240min), 2. Glycogen in muscle provides a similar amount of glucose as that of the liver. Since muscle lacks glycogen phosphorylase necessary to form free glucose from glycogen, glycolysis breaks down glycogen into pyruvate and lactate which are then transported to liver and they are synthesized into glucose [52], (3) Adipose a type

of connective tissue which fat is stored and which has the cells distended by droplets of fat (Lin et al., 2010). The catabolism of triglycerides produces both glycerol and fatty acids. The glycerol is transported to the liver and can be converted to glucose, whereas the fatty acids are the energy source in almost all tissues excluding nervous tissue though they may permeate the liver producing energy and forming ketones which are organic compounds that result when body fat is broken down for energy [9]. So, the liver that converts fatty acid into ketone through ketogenesis, it converts also ketone bodies to acetoacetate and β -hydroxybutyrate, are amphipathic and can be transported into the brain (and muscles) and broken down into acetyl-CoA for use in the TCA cycle, and (4) Protein muscle is the largest source of protein in the body, and it is a major source of blood glucose through amino acid precursors i.e. gluconeogenesis [53]. A sizable fraction catabolized into

amino acids that permeate liver to be converted into keto acids and in turn to glucose [54].

Adapted from: Campbell, M.K. and Farrell, S.O. (2006) Biochemistry, 5th edn. Belmont, CA: Thomson Learning, Inc.; Nelson, D.L. and Cox, M.M. (2008) Lehninger Principles of Biochemistry, 5th edn. New York, NY: W.H. Freeman and Company.

A simple calculation reveals that the 180g of glucose per day [4] produced by the liver during fasting would not be enough to meet all the body's energy needs:

$180 \text{ g/day} \times 4 \text{ kcal/g} = 720 \text{ kcal/day}$ (3013kJ/day). The normal total energy expenditure is $\sim 1800 \text{ kcal/day}$ (7531 kJ/day) and the brain itself consumes about 18% of the basal metabolic rate that equates to 324 kcal (1356 kJ), or $\sim 80 \text{ g}$ of glucose ([55]). The brain consumes $\sim 25\%$ of total body glucose consumption.

An approximately 1.334 calories (5.581 Joule) is required every hour for every 1000 g of body weight [56]. Hence, a man weighing 60Kg would require $1.334 \times 24\text{hrs} \times 60\text{Kg} = 1921 \text{ cal/day}$.

The Dietary Guidelines for Americans [57] recommends the following blend:

- 45-65% Carbohydrates (sugar, sweets, bread, cakes)
- 20-35% Fat (dairy products, oil)
- 10-35% Protein (eggs, milk, meat, poultry, fish)

Sadava and Orians [55] and Begum [58] reported Let us consider for the purpose of this example and calculate the values for a man of 60 kg in body weight as follows:

Carbohydrates : 45%	Fat : 20%	Proteins : 10%
Energy yield : 4.2 cal/g	Energy yield: 9.5 cal/g	Energy yield: 4.1 cal/g
Diet input : 45% x 2941 = 1323 cal = 5535 Joule = 315 g	Diet input : 20% x 2941 = 588 cal = 2460 Joule = 62 g	Diet input : 10% x 2941 = 294 cal = 1230 Joule = 72 g

Glucose is the principal source of energy for mammalian cells and its transport is mediated by a group of membrane transport proteins and it is a universally conserved property (Fig. 4). Human RBC expresses the highest level of GLUT1 with more than 2×10^5 molecules per cell where GLUT1 accounts for 10% of total protein mass [59]. The GLUT4 is an insulin-regulator glucose membrane transporter found in adipose tissues whereas GLUT4 is insulin dependent [60].

Several other hormones (e.g. glucagon, growth hormone, catecholamines, and corticosteroids) oppose insulin action and they increase blood glucose [61].

9. Insulin

The blood concentration is influenced by hormones which facilitate its entry or removal from the circulation (Fig. 4). Insulin that its functions are anabolic, is produced by beta (β) cells and peptide hormone glucagon is made and secreted in alpha (α) cells of the endocrine pancreas within specialized areas called the islets of Langerhans

[4,62]. The most important hormone involved in glucose metabolism is insulin for the following reasons : (1) it decreases blood glucose by enhancing glucose uptake through GLUT4 and its in metabolism by liver, muscle, adipose tissue and other tissues, as well as its ability to inhibit gluconeogenesis and glycolysis, (2) it increases fatty acids and triglycerides synthesis, thus increasing fat storage (adipogenesis) and promoting glycogen synthesis in the liver, (3) it stimulates the transport of free amino acids into the liver and muscle cells for protein synthesis; it inhibits the catabolism of proteins, and (4) it induces the cellular uptake of K^+ , HPO_4^{2-} and Mg^{2+} .

Insulin release into the blood stream is usually triggered as a consequence of blood glucose level increase after a carbohydrate-rich meal [63]; insulin does not facilitate glucose uptake in human RBC [64,65]. Unfortunately, insulin deficit will have the opposite results, especially as high rates of glucose uptake and net anabolism of glycogen, protein and triglycerides (Fig. 4) which depend on the presence of high concentration of insulin in the blood stream [66]. The glucose can be utilized by the brain because its glucose uptake is not insulin dependent [67].

10. Diabetes Mellitus

It is a variable disorder (disease) of carbohydrate metabolism caused by a combination of hereditary and environmental factors and usually characterized by inadequate secretion or utilization of endogenous insulin; so that excess blood glucose ($> 126 \text{ mg/dl}$ or 7.0 mmol) cannot be mediated nor absorbed into the target cells that require it in the body, hyperglycemia [68]. The classical symptoms of diabetes are: polyuria, polydipsia, polyphagia and weight loss [58,69]. F. Banting and J.J.R. MacLeod shared the 1923 Nobel Prize for Physiology and Medicine for their work on "discovering" insulin. Since insulin's discovery, medical breakthroughs continued to prolong and ease the lives of people with diabetes. Roger Hinsworth in 1935 discovered there were two types of diabetes: "insulin sensitive" (TI DM) resulted from autoimmune destruction of insulin producing beta cells of the pancreas due to low level of protein interleukin-2, and "insulin insensitive" (TII DM) that is related to high fat levels in the pancreas and the liver and it is characterized by peripheral insulin resistance and impaired insulin secretion from pancreatic β cells [23, 68]. By differentiating between the two types of diabetes, Hinsworth helped open up new avenues of diabetes treatment [69], as the primary concern is that there is cell degeneration.

The reasons for inadequate insulin may include lack of hormone production by β -cells of the pancreas, reduction in insulin receptor signaling, or insufficient active GLUT4 [68, 70, 71]. Simvastatin is a medicine that is used to reduce the amount of cholesterol produced in the body in order to minimize the chances of a heart attack or stroke in people who have heart disease, dyslipidaemia and to minimize the chances of a heart attack or stroke in people who have

diabetes [9].

Various cytokines, including tumor necrosis factor (TNF), growth hormone (GH) and interleukin (IL)-6, induce insulin resistance. It was demonstrated that induction of suppressor of cytokine signaling (SOCS)-3 by TNF and GH is an important mechanism by which these cytokines, obesity and pregnancy impair insulin sensitivity [72, 73, 74]. In pregnancy, progesterone can cause insulin resistance and results in gestational diabetes [75]; pregnancy-associated hormones at 55-200ng/ml during third trimester may contribute to insulin resistance and secondary hyperlipidemic [76]. Prediabetes is when people have blood glucose levels higher than normal but not high enough for a diagnosis of diabetes; this condition raises the risk of developing TII DM diabetes, heart disease, and stroke [68,69].

Diabetes treatment varies depending on the type and severity of disease, but there is no cure short of a pancreas transplant that can lead to a variety of serious complications and risks. Could be a future without diabetes? The hyperglycaemia part of diabetes is conceptually simple, i.e. restoring insulin producing cells through Islet cell transplantation, artificial pancreas, immunotherapy, vaccination, stem cells treatment and others trends in future research in pursue of a cure.

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