MEMBRANE FLEXIBILITY AND CELLULAR ENERGY MANAGEMENT IN TYPE 2 DIABETES, GESTATIONAL DIABETES, AND OBESITY

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ABSTRACT

In the search to understand the onset of life’s biochemistry, scientists attached a great deal of importance to unravelling the replication mechanism of a cell, and the cell membrane was accepted as an indispensable entity. In this review, we give an account of the recent progress of the understanding that the cell membrane has also continuously evolved over the long-term, leading to the important insight that the membrane unsaturation index (USI) - a measure of unsaturation - plays a pivotal role in the basal metabolic rate of a cell and in the aetiology of Type 2 diabetes mellitus (T2DM). It is now clear that increasing the USI with long-term interventions - in the form of aerobic exercise and caloric restriction - can contribute to the prevention or postponement of the onset of T2DM, gestational diabetes, and prediabetic obesity.

Keywords: Membrane flexibility, exercise, gestational diabetes, glucose transporter, obesity, phospholipids, Type 2 diabetes, unsaturated fatty acid.

INTRODUCTION

About 20 years ago, Shulman et al. reported that in vivo carbon-13 nuclear magnetic resonance spectroscopy had measured human muscle glycogen synthesis rates in patients with Type 2 diabetes mellitus (T2DM) and matched controls. They showed that the muscle glycogen synthesis rates in the patients were approximately 50% of the rate observed in controls. The same group investigated, under hyperglycaemic-hyperinsulinaemic conditions, the pathway: transmembrane glucose transport into the muscle cell, conversion of intracellular glucose into glucose-6-phosphate, and then, after two more intermediates, the addition of the latter through glycogen synthase to the glycogen polymer. They concluded that the transmembrane glucose transport into the muscle cell is the rate-controlling step; this is newsworthy biochemistry because it indicated that there must be an essential difference in plasma membrane function between patients with T2DM and the matched healthy controls. Two important questions arise: first, is it an isolated event that transmembrane glucose transport is the rate-controlling step or does this reflect the inherent nature of an evolutionary process? Second, what is the relationship between the chemical structure and physical properties of the various phospholipid molecules, and can this relationship elucidate how membrane function might be altered? To shed more light on the molecular processes underlying these phenomena, we summarise current knowledge about cell membranes.

CELL MEMBRANES

Phospholipid bilayers form rapidly and spontaneously when phospholipids are added to water. The two acyl chains yield a roughly cylindrical molecule that can easily pack in parallel arrays to form extended sheets of membranes composed of a mosaic of proteins and phospholipids in a fluid phospholipid matrix. The driving force of this aggregation phenomenon is the weak, noncovalent bond (van der Waals force) between a
pair of carbon atoms, which can be calculated with the Lennard-Jones (L-J) potential: \( U = (11.5 \times 10^{-6})/r^{12} - (5.96 \times 10^{-3})/r^6 \). The interaction energy \( U \) is related to the distance \( r \) between two carbon atoms, as shown graphically in Figure 1. Recently, Sun et al.\(^6\) provided experimental data indicating the correctness of the L-J potential. What we can conclude from this graph is, firstly, that the minimum energy principle favours a carbon-carbon distance of \(-4\) Å, which is the most stable distance between the centres of two carbon atoms, with a minimum interaction energy of \(-0.77\) kJ/mol. Secondly, when the carbon atoms in two acyl chains of a phospholipid diverge, their interaction energy decreases, and when they approach each other, their interaction energy increases. Thus, the sum of weak noncovalent forces of many carbon-carbon interactions creates flexibility in a lipid bilayer.

**Membrane Flexibility**

An exciting result recently achieved by the discipline of physical chemistry was the notion that (poly)unsaturated acyl chains of phospholipid membranes have an intrinsic propensity toward cell membrane flexibility. Saturated fatty acids possess essentially linear alkyl chains, with no double bonds. Conversely, double bonds in unsaturated fatty acids are nearly in the \( ciss \) configuration, which produces a bend in the fatty acid chain.\(^7\)\(^8\) This bend makes it more difficult for phospholipids with unsaturated acyl chains to pack close together, thus promoting bilayer flexibility. The most basic structural result obtained from X-ray scattering analyses of oriented bilayers in model phospholipid membrane systems is the area \( A \) per lipid molecule, which denotes the cross-section of the cylindrical space occupied by a phospholipid. Various studies of fully hydrated, fluid phase, model phosphatidylcholine bilayers (Table 1) have demonstrated that introducing one or more carbon-carbon \( ciss \) double bonds into the saturated acyl chains will increase the cross-sectional area \( A \) by approximately 18%.\(^9\)\(^12\) Thus, an 8.5% increased interchain distance results in a 33% decreased attraction energy per pair of fatty acyl carbon atoms. Consequently, due to reduced van der Waals interactions, an increased interchain distance of a phospholipid results in greater membrane flexibility.

![Figure 1: The van der Waals interaction energy profile as a function of the distance \( r \) between the centres of two carbon atoms.](image)

The energy was calculated using the empirical equation \( U = B/r^{12} - A/r^6 \). Values for the parameters \( B = 11.5 \times 10^{-6} \) kJ nm\(^{12}\)/mol and \( A = 5.96 \times 10^{-3} \) kJ nm\(^6\)/mol for the interaction between two carbon atoms.

*Adapted from Levitt.\(^5\)*
A number of papers have reported examples of reduced membrane flexibility; a review by Cho et al.\textsuperscript{13} indicated that patients with T2DM exhibited reduced erythrocyte deformability compared to healthy controls. Also, Gupta et al.\textsuperscript{14} showed that reduced microvascular endothelial flexibility was characteristic of asymptomatic obesity combined with prediabetes. The erythrocyte membrane is compositionally very similar to the vascular endothelium. This has crucial implications because, in capillaries, the size of red blood cells is of the same order of magnitude as the capillary lumen (∼8 μm); thus, deformability is an important determinant of blood flow. Increased stiffness of both the microvascular endothelium and the erythrocyte membrane decreases the microcirculatory flow, which leads to reduced oxygen supply, and consequently, to chronic tissue hypoxia, reduced adenosine triphosphate (ATP) production, and ultimately, increased endothelial dysfunction. Also, reduced oxygen uptake may be the cause of the failure of the endoplasmatic reticulum to generate sufficient oxidative potential for disulphide bonds to be formed, as mentioned by Watson\textsuperscript{15} in a recent paper. It is noteworthy that a decrease in microcirculatory flow leading to reduced oxygen uptake was demonstrated in the 6-year Malmö feasibility study; i.e. at baseline, subjects with newly-detected T2DM and those with impaired glucose tolerance showed a significantly reduced maximal oxygen uptake compared to strictly healthy individuals.\textsuperscript{16}

**Table 1: Experimental data of fully hydrated fluid phase phosphatidylcholine lipid bilayers.**

<table>
<thead>
<tr>
<th></th>
<th>DLPC</th>
<th>DMPC</th>
<th>DPPC</th>
<th>DOPC</th>
<th>PDPC</th>
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<td>10,11</td>
<td>10</td>
<td>12</td>
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<td>[C14:0]\textsuperscript{2}</td>
<td>[C16:0]\textsuperscript{2}</td>
<td>[C18:1]\textsuperscript{2}</td>
<td>C16:0,C22:6</td>
</tr>
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<td>30</td>
<td>50</td>
<td>30</td>
<td>30</td>
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<td>Area A per lipid molecule (Å)\textsuperscript{2}</td>
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<td>64.0</td>
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<td>74.8</td>
</tr>
<tr>
<td>Mean Area A per lipid molecule (Å)\textsuperscript{2}</td>
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<td>73.6</td>
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<td>Carbon interchain distance (Å)</td>
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<tr>
<td>Attraction energy U (kJ/mol)</td>
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<td>-0.39</td>
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<tr>
<td>Attraction energy decrease (%)</td>
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<td>32.9</td>
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DLPC: dilauroylphosphatidylcholine; DMPC: dimyristoylphosphatidylcholine; DPPC: dipalmitoylphosphatidylcholine; DOPC: dioleoylphosphatidylcholine; PDPC: palmitoyl-docosahexaenoic-phosphatidylcholine.

**Allometry**

With the technique of biological scaling (allometry), the discipline of biology achieved an innovative result. Allometry, in its broadest sense, describes how the characteristics of living creatures change with size. For example, the observation that mammalian basal metabolic rate (BMR) changes with increases in species size, has been the subject of routine investigations for over a century. White et al.\textsuperscript{17} compiled relevant data from the literature for 619 species with masses that ranged from 3-300,000 g. From that data, they derived the relationship between mammalian body mass (M, g) and BMR (ml of O\textsubscript{2} per h) in an allometric equation of the form: BMR = 4.12 M\textsuperscript{0.69}. The result is endlessly fascinating because this relationship, with an allometric coefficient of 0.69, means that the BMR grows at a slower rate than the body mass (the slow-down principle). To understand the rationale behind the slow-down principle, let us suppose the existence of an extinct cubic species that consists of one unit cell with a length of 1 cm. The outer surfaces of this species are used for, among other things, exchange of metabolic heat with the environment. Then, the cubic species evolved over time to a larger cubic species with a total volume of 8 cm\textsuperscript{3}. Each of the 8 unit cells of this larger cubic species exposes only 3 outer surfaces for heat exchange with the environment, which means a 50% decrease in outer surface per unit cell, compared to the original cubic cell. To prevent
overheating of the larger cubic cell, evolution developed the slow-down principle.

A consequence of the allometric equation is that a doubling of body mass involves a 19.3% decrease in the mass-specific basal metabolic rate. For the reader’s mind-set, our common ancestor - the species Homo habilis - had a body mass of approximately 32 kg. Thus, after a period of about 2 million years, the species Homo sapiens arrived with a body mass of about 70 kg and a 19.3% decreased mass-specific BMR compared to that of Homo habilis; i.e. a 1 per mille decrease in the BMR per 13,350 years. This is a fine example of regulated cellular basic metabolic rate sensing, but even more importantly, it is an indication of the existence of a genetically-regulated species-dependent set point of basic metabolic rate. The answer to the question: ‘What is the principal cause of the slow-down mechanism for BMR?’ starts with the observation that, heart, skeletal muscle, liver, and kidney tissue phospholipids, which have been shown to have significant influence on many aspects of membrane function, have also exhibited allometric trends. Phospholipid acyl chains have shown a significant decrease in the unsaturation index (USI) (i.e. the mean number of cis double bonds per fatty acid residue, multiplied by 100) with increases in species body size from 7-370,000 g. Also, although membrane bilayers showed essentially no change in the percentage of saturated acyl chains with changes in species size, the membrane bilayers of small mammals were generally high in docosahexaenoic acid (DHA) (C22:6) and low in oleic acid (C18:1), and the opposite was observed in large mammals.

This observation gave rise to the membrane pacemaker theory of metabolism, which suggests that the relative balance between monounsaturated and polyunsaturated acyl chains, particularly DHA, in cell membranes is a fundamental determinant of the metabolic rate of a species. In other words, the BMR of a cell, an important characteristic of a cell’s energy management, depends on its cell membrane flexibility. Because the thermoregulation of a species may be the driving force behind the pacemaker theory, it is attractive to hypothesise that the primary cause of T2DM is a hypothalamic dysfunction, critically involved in thermoregulation, which in turn, results in a decrease in the USI, in keeping with the slow-down principle.

Transmembrane Glucose Transport

Focusing on membrane flexibility, we will discuss some aspects of membrane insertion of both the non-insulin-mediated glucose transporter Type 1, GLUT1, and the insulin-mediated glucose transporter, GLUT4. GLUT1 is a monomeric protein with 12 transmembrane helical segments. A fundamental aspect of the transmembrane insertion machinery, located in the endoplasmic reticulum, is that the transporter protein must traverse the plasma cell membrane 12 times in a zigzag fashion, before initiating the folding necessary to form the three-dimensional structure. Moreover, hydrodynamic size analysis and electron microscopy of GLUT1 proteoliposomes support the hypothesis that GLUT1 is a multimeric (probably tetrameric) complex of GLUT1 proteins. Thus, the process of inserting this glucose transporter into a bilayer membrane requires high membrane flexibility.

GLUT4 insertion into the plasma membrane follows a somewhat more complicated route that consists of two important phases (Figure 2). In the first phase, GLUT4 is inserted into the membrane of intracellular vesicles. As discussed in the aforementioned paragraph, this process demands flexibility of the vesicular membrane. In the second phase, the vesicles that contain GLUT4 take part in a fusion process with the cell membrane. This process of membrane fusion is described by the ‘stalk-pore’ hypothesis, and involves three decisive steps. In the first step, opposing membranes of the vesicle and the cell membrane are separated by at least a 10-20 nm gap. Their contact involves specialised tethering molecules. Next, fusion proteins induce bending of the plasma membrane bilayer, which establishes a very close contact between the two membranes; and finally, activated fusion proteins drive fusion pore formation by assembling into an interconnected protein coat surrounding the fusion gate. Clearly, high flexibility of the cell membrane plays a central role in this fusion process. Thus, high flexibility of the cell membrane represents a key determinant in glucose transport due to its influence on all Class 1 GLUT proteins.

Replies to the Questions

Now we have summarised the current knowledge about cell membranes, we can answer the two questions raised in the Introduction. First, the presented data show that the BMR of a cell
depends on its plasma membrane flexibility, and this flexibility, in turn, determines the number of GLUTs present at the cell surface. These findings are consistent with the observation that transmembrane glucose flux (TGF) is the rate-controlling step in muscle glycogen production, as demonstrated in the experimental work of Shulman’s group.² Those results have provided an impressive indication of the correctness of the pacemaker hypothesis. Second, this mechanism did not arise from an isolated event. Far from it, they shed light on the inherent nature of an evolutionary process, which is based on maintaining the relative balance between monounsaturated and long-chain polyunsaturated acyl chains in membrane bilayers. With elegant simplicity, this is a fine example of energy management over the long-term.

Now, we can quite simply answer the open question of how an elevation in plasma-free fatty acids (FFAs) causes inhibition of transmembrane glucose transport, as reported by Shulman’s group.²³ In that study, an increase in plasma-FFA levels in healthy subjects was created with intravenous infusions of a triglyceride emulsion, Liposyn II. Liposyn II comprises a 50/50 safflower/soybean oil mixture, where the major component fatty acids are approximately 65.8% linoleic (C18:2), 17.7% oleic (C18:1), 8.8% palmitic (C16:0), 3.4% stearic (C18:0), and 4.2% α-linolenic (C18:3) acid. Due to the absence of long-chain polyunsaturated fatty acids in Liposyn II, its intravenous infusion caused a decreased USI, decreased membrane flexibility, and finally, a decrease in all functional Class 1 GLUTs.

**A NEW ROUTE TO THE DEVELOPMENT OF T2DM**

In the aetiology of T2DM, a new working hypothesis that attempts to accommodate findings from numerous laboratories is presented in Figure 3. Shulman’s group demonstrated that a reduced ATP production in the skeletal muscle of young, lean insulin-resistant offspring of parents with T2DM probably occurs due to decreased mitochondrial activity,²⁴,²⁵ which is a characteristic for T2DM individuals.²⁶ To acquire extra ATP, this leads to gradual elevation of plasma-FFAs, which in turn, causes a shift from unsaturated to saturated fatty acyl chains in membrane phospholipids, and a decreased USI. Consequently, there is harmful increased membrane stiffness with a concomitant reduction in all functional Class I GLUTs. The net effect would be a decrease in glucose flux into cells, which would further stimulate hepatic lipolysis.

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![Figure 2: Glucose transporter type 4 (GLUT4) protein biogenesis.](image-url)

The membrane protein is modified by engineering of the structural gene, where the critical steps in the biosynthetic pathway, leading to the folded GLUT4 in the membrane, are its insertion into the vesicle and the vesicle fusion with the acceptor membrane where events are under control of membrane flexibility.
The progress of these events sets up a vicious cycle. The reduced glucose uptake causes increases in plasma glucose and insulin concentrations, which are positively related up to a plasma glucose concentration of about 10 mmol/L. Thereafter, β-cell failure occurs, and due to insufficient insulin, the condition of glucose intolerance gives way to frank T2DM. From the Liposyn II experiments, we may conclude that the increasing level of plasma-FFAs is the initiating cause of the vicious cycle. Moreover, this logical sequence of events explains the time-dependent increase in both glucose and insulin concentrations during the prediabetic phase. Additional experimental data in favour of the new working hypothesis has been summarised in a recent study.

Obesity is characterised by an elevation in plasma-FFAs because enlarged, stressed adipose tissue releases more FFAs, and FFA clearance may be reduced compared to the non-obese condition. According to our proposed scheme (Figure 3), this elevation of FFAs would reduce the USI, which would finally result in a reduction in all functional Class I GLUTs and the development of T2DM. In the case of obese, but otherwise healthy individuals, an over-secretion in insulin compensates for an increased plasma glucose concentration. However, in prediabetic individuals, this compensation fails and the consequence is overt T2DM. Gestational diabetes mellitus (GDM) arises from two underlying phenomena: first, a temporary increased plasma concentration of FFAs, which induces a reduced glucose flux into maternal cells, along with a concomitant increased insulin level during pregnancy, and ensures an adequate glucose supply for foetal growth and development; second, a chronic increase in plasma-FFAs due to the presence of a prediabetic state. Together, these independent increases in FFAs result in serious decreased membrane flexibility that causes metabolic abnormalities to culminate in the characteristics of GDM. After delivery, the temporary increased FFAs ceases, which results in apparently normal glucose homeostasis.

Another argument for our proposed scheme (Figure 3) is the observation that individuals with T2DM have higher basal metabolic rates than nondiabetic control subjects. After all, in individuals with T2DM, the reduction in all Class 1 GLUTs, followed by an increase in plasma-FFAs induces a shift from glucose oxidation towards fatty acid oxidation. In eukaryotes, complete glucose oxidation involves the breakdown of the glucose carbon-carbon bonds, which require (per carbon-carbon bond) $6/6 = 1.00$ molecule of $O_2$; in contrast, the complete oxidation of palmitoyl-coenzyme A involves the breakdown of the palmitoyl carbon-carbon bonds, which require (per carbon-carbon bond) $23/16 = 1.43$ molecules of $O_2$. Consequently, oxygen consumption, which is a measure of basal

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**Figure 3: Hypothetical pathway of the development of Type 2 diabetes.**

ATP: adenosine triphosphate; FFA: free fatty acid; GLUT: glucose transporter.
metabolic rate, should be higher in individuals with T2DM than in non-diabetic control subjects. This hypothesis had to be demonstrated.

**Aerobic Exercise**

We speculate that aerobic exercise is essential for restoring flexibility to stiff membranes, a common characteristic of T2DM, GDM, and prediabetic obesity. Exercise training showed direct effects on the ‘browning’ of white fat through irisin. The function of brown adipose tissue is to transfer energy from fatty acids into heat. Because brown adipose tissue is more saturated than white adipose tissue, and because exercise burns mostly brown fat, exercise acts to reduce the body's saturated fatty acid content, and consequently, promotes an increase in membrane flexibility and TGF. A number of studies that focused on lifestyle changes, such as diet and physical exercise, have supported the hypothesis that T2DM can be prevented or delayed with exercise in individuals at high risk of the disease, and that exercise can be beneficial as an adjuvant therapy in GDM.

Although the study designs differed from each other to some extent, the results may be summarised as follows: a 5-year protocol which aimed to achieve and maintain at least 5% weight reduction through aerobic exercise: the 6-year Malmö feasibility study, the 5-year Diabetes Prevention Programme, and the 5-year Finnish Diabetes Prevention Study. The study periods differed from each other, but the results showed that these programmes were beneficial in the prevention of diabetes mellitus in individuals at high risk of developing T2DM.

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**CONCLUSION**

Notwithstanding the fact that diabetes is a disease with a genetic component of unknown origin, the good news is that an individual with high risk of T2DM, or with T2DM, is the conductor of his/her own USI, with all its benefits. This control provides an individual with a unique position because normalisation of both glycaemia and the USI are cornerstones of effective T2DM management. Knowledge and understanding of the presented concept are essential to informing public health programmes and policy, based on the expectation that these concepts will affect governmental decision-making regarding public health issues.

**REFERENCES**