AIMS Medical Science

AIMS Medical Science, 9(3): 424–432.
DOI: 10.3934/medsci.2022021
Received: 02 May 2022
Revised: 26 July 2022
Accepted: 27 July 2022
Published: 15 August 2022

http://www.aimspress.com/journal/medicalScience

Opinion paper

Unraveling the Gordian knot of insulin resistance in type 2 diabetes mellitus

Rob NM Weijers*

Teaching Hospital, OLVG, Amsterdam 95500, Netherlands

* **Correspondence:** Email: robw01@xs4all.nl.

Abstract: β-cells play an important role in unraveling the Gordian knot of insulin resistance in type 2 diabetes. Firstly, a key feature of the etiology of type 2 diabetes, which appears in the prediabetic phase, is a reduction in the unsaturation index (number of cis carbon-carbon double bonds per 100 acyl chains of membrane phospholipids) compared to healthy controls, which leads to a lower rate of transmembrane glucose transport, and consequently causes reduced glucose effectiveness. Thus, the amount of glucose entering the β-cell via glucose transporter-2 reduces insulin production, leading to reduced insulin sensitivity. Secondly, after synthesis of monomer insulin, six monomer insulin molecules can join together in the presence of zinc ions. The mature hexamers are packed inside mature intracellular vesicles that are transported to the β-cell plasma membrane. Fusion of the intracellular vesicle membrane with the β-cell plasma membrane creates a fusion pore that allows expulsion of monomer insulin molecules into the blood circulation. The large dimensions of the monomer insulin molecule (30 Å wide and 35 Å high) require substantial flexibility of the vesicle membrane and the β-cell plasma membrane. Reduction in the unsaturation indexes leads to a lower rate of insulin transport into the blood circulation, which results in a further decrease in insulin sensitivity.

That brings us to a crucial point. The conceit behind the term "insulin resistance" is wrong. It suggests that cells do not respond well to insulin, but the fact is that this term ignores the essential reduction, compared to the plasma glucose concentration, in the amount of glucose entering the β-cell via glucose transporter-2, resulting in reduced insulin production. We now know that an increase in glucose effectiveness, powered by an increased unsaturation index, reframes fundamentally the mechanisms that participate in the glucose homeostasis during type 2 diabetes mellitus.

Keywords: β-cell; glucose effectiveness; glycerophospholipids; insulin resistance; insulin sensitivity; type 2 diabetes mellitus; unsaturation index

Although the term "insulin resistance" was used as early as 1931, there was no general agreement on the definition, and thus gaps in research and clinical care persisted [1]. Over the years, accumulating data have been published, enabling reconsideration of the meaning of "insulin resistance".

Computer modelling of glucose and insulin kinetics after intravenous glucose challenge has demonstrated that compared to normoglycemic individuals, patients with type 2 diabetes show essential reductions in both insulin sensitivity and the insulin-independent glucose removal rate (Table 1) [2]. Insulin sensitivity reflects the ability of insulin to enhance the effect of glucose to normalize its own concentration and glucose effectiveness is the ability of glucose, independent of a dynamic insulin response, to enhance net glucose disappearance. Thus, these patients exhibit a state of reduced responsiveness to circulating insulin, as well as a state of reduced glucose effectiveness. In the latter condition, glucose—independent of changes in the insulin concentration—is less able to stimulate its own uptake through a mass action effect, and less able to suppress its own release. A prospective study investigated the development of type 2 diabetes in normoglycemic offspring of parents who both had type 2 diabetes, and revealed that the offspring exhibited essential defects in both insulin sensitivity and glucose effectiveness more than 10 years before disease development (Table 1) [2].

Table 1. Measures of glucose effectiveness and insulin sensitivity for a one- and two-compartment minimal models [2].

Units	Control subjects	Type 2 diabetes	P value	Δ (%)	Com.	Tracer
S_G						
min^{-1}	0.023 ± 0.012	0.016 ± 0.009	< 0.001	30.4	One	No
h^{-1}	0.41 ± 0.04	0.33 ± 0.02	< 0.001	19.5	Two	^{13}C
h^{-1}	0.52 ± 0.05	0.37 ± 0.02	< 0.001	28.8	Two	^{2}H
average				26.2		
S_I						
$10^{-4} \times min^{-1} \times (mU/L)^{-1}$	13.45 ± 11.12	5.31 ± 3.98	< 0.01	60.5	One	No
$pmol \times L^{-1} \times (h^{-1})$	0.0082 ± 0.0012	0.0036 ± 0.0006	< 0.001	56.1	Two	^{13}C
$pmol \times L^{-1} \times (h^{-1})$	0.0098 ± 0.0013	0.0042 ± 0.0008	< 0.001	57.1	Two	^{2}H
average				57.9		

Note: S_G: glucose effectiveness; S_I: insulin sensitivity; Com.: number of compartments.

What do these findings mean? Shulman et al. partly answered this question by using in vivo carbon-13 nuclear magnetic resonance spectroscopy to study muscle glycogen synthesis in subjects with type 2 diabetes and matched controls [3]. Their data revealed that the muscle glycogen synthesis rate in subjects with type 2 diabetes was about 43% of the rate observed in healthy controls. Six years later, this research group hypothesized that insulin resistance in humans is induced by free fatty acids, through the initial inhibition of glucose transport/phosphorylation, followed by an approximately 50% reduction in the rates of both muscle glycogen synthesis and glucose oxidation [4].

There are two plausible explanations for these changes: a reduction in the amount of glucose transporter-4 (GLUT4) per cell surface area, or a change in the three-dimensional structure of GLUT4, which affects the rate of glucose transport across the cell membrane. The latter is more likely, because the etiology of type 2 diabetes does not include any significant deficiency of GLUT4mRNA or GLUT4 protein compared to healthy controls [5].

To appreciate the change in the three-dimensional structure of GLUT4, we first must briefly examine the biochemistry of the proteins facilitating the net movement of glucose, and process of GLUT molecules insertion across the phospholipid membrane.

Glucose transporter proteins are integral membrane proteins containing 12 membrane-spanning helices. The glucose channel comprises eight helices that are immersed in a box formed by the remaining four helices [6]. The cross-section of this box has a mean surface area of 1,100 $Å^2$, which covers an area of about 17 molecules of a phosphatidylcholine bilayer with saturated fatty acyl chains. Thus, the insertion of a GLUT molecule across a phospholipid membrane requires flexibility of the bilayer membrane.

Glycerophospholipids are the major class of naturally occurring membrane phospholipids in eukaryotes. The two fatty acyl chains yield a roughly cylindrical molecule (the hydrocarbon region) that can easily pack in parallel to form extended membrane sheets (Figure 1). The area (A) of a lipid molecule is the surface area of the cross-section of this cylindrical part of the phospholipid molecule. Experimental values of A have been obtained by using electron density profiles for various samples of fully hydrated fluid-phase artificial phosphatidylcholine lipid bilayers, which have been demonstrated that unsaturation of the fatty acyl chains clearly leads to a larger value of A, compared to saturation (Table 2) [7]. This means that a reduction in the number of cis carbon-carbon double bonds in a fatty acyl chain reduces the distance between two fatty acyl chains, and thereby increases the interaction energy between the two chains, resulting in reduced membrane flexibility.

Table 2. Experimental data of fully hydrated fluid phase phosphatidylcholine lipid bilayers [7].

	DMPC	DPPC	DOPC	PDPC
Fatty acid structure	$[C14:0]_2$	$[C16:0]_2$	$[C18:1]_2$	C16:0; C22:6
Temperature (°C)	30	50	30	30
Area A per lipid molecule $(Å)^2$	60.6	64.0	72.5	74.8
Carbon interchain distance (Å)	4.39	4.51	4.80	4.88
Mean Interaction energy U (kJ/mol)		–0.61		–0.38
UI	0	0	100	300

Note: DMPC: Dimyristoylphophatidylcholine; DPPC: Dipalmitoylphosphatidylcholine; DOPC: Dioleoylphosphatidylcholine; PDPC: Palmitoyl-docosahexaenoicphosphatidylcholine; UI: Unsaturation index.

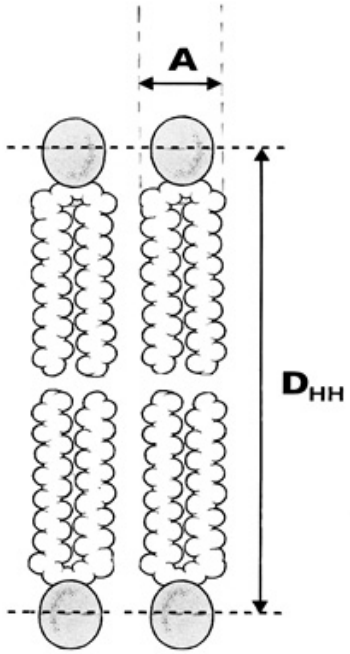

Figure 1. The most basic structural result obtained from x-ray scattering from oriented bilayers in model phospholipid membrane systems is the area A per lipid molecule (the cross-sectional area of the cylindrical part of the phospholipid). Membrane bilayer thickness (D_{HH}).

Variations in the acyl composition of cell membranes can strongly influence the function of proteins embedded therein, i.e., small changes in the pressure profile of a bilayer membrane can induce a shift in the protein conformational distribution [8]. The biochemical and physical background of this mechanism in type 2 diabetes is a reduction in the unsaturation index, which lowers the area (A) of lipid molecules. The reduction in A translates into increased attractive forces between the mutual phospholipid acyl chains, which leads to redistribution of the lateral pressure in cell membranes. Finally, this redistribution induces the cross-sectional contraction of all class I glucose transporter proteins, thereby reducing the rate of glucose transport across the cell membrane, initiating the onset of type 2 diabetes.

The unsaturation index is a useful parameter for describing the flexibility of a biological membrane, which is calculated by multiplying the mean number of cis double bonds per fatty acid residue by 100, as observed in erythrocytes [9]. The membrane phospholipid acyl chains consist of saturated, mono-unsaturated, or polyunsaturated hydrocarbon chains that normally vary from 14 to 22 carbons in length, and with a relative distribution between saturated and unsaturated fatty acids following a ratio of 40:60 (Table 3) [10]. At a body temperature around 37 degrees Celsius and a constant phospholipid tail length, the membrane flexibility of a phospholipid bilayer is mainly determined by the unsaturation index [11,12]. One of the important functions of cholesterol is its biological function as a spacer in bilayer phospholipid membranes [13]. As a clear trend, the fully saturated 1,2-dipalmitoyl-sn-glycero-3-phosphocholines have the strongest interactions with cholesterol. The effect of cholesterol on monounsaturated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine bilayer is weaker than on a fully saturated 1,2-dimyristoyl-sn-glycero-3-

phosphocholine bilayer. This is also similar in the case of di-unsaturated 1,2-dioleoyl-sn-glycero-3-phosphocholines on which the effect is weaker than on the fully saturated 1,2-distearoyl-sn-glycero-phosphocholines. Thus, in case of type 2 diabetes or its prediabetic phases, the effect of cholesterol on a bilayer depends on the value of the bilayer unsaturation-index.

Table 3. Erythrocyte acyl chain composition of phospholipids and unsaturation index of control individuals, people with type 2 diabetes without retinopathy, and people with type 2 diabetes with retinopathy[1].

Biochemical characteristics	Control individuals (n = 18)	Individuals with type 2 diabetes without retinopathy (n = 14)	Individuals with type 2 diabetes with retinopathy (n = 46)
Total SFAs (%)	42.0	44.2	46.6
Total MUFAs (%)	18.8	21.7	21.3
Total PUFAs (%)	38.0	31.9	29.5
UI	155.4	134.3	123.3

Note: [1] Ex-post calculations performed by the author are based on the original data listed by Koehrer et al. [14]. SFA: Saturated fatty acid; MUFA: Mono-unsaturated fatty acid; PUFA: Poly-unsaturated fatty acid; UI: Unsaturated index.

Recalculation of the data from a French study on diabetic retinopathy development—together with the categorization of phospholipid fatty acyl chains into three groups (saturated fatty acids, mono-unsaturated fatty acids, and poly-cis-unsaturated fatty acids)—revealed substantially different unsaturation index values in controls (155.4), and in type 2 diabetes patients without retinopathy (134.3) and with retinopathy (123.4) (Table 3) [14]. Thus, the reduction in the unsaturation index is caused by an essential increase in the number of saturated acyl chains and, simultaneously, an essential reduction in the number of poly-unsaturated acyl chains. In a previous paper, using data reported by Min et al., we calculated the unsaturation index of phosphatidylcholine and phosphatidylethanolamine fatty acyl chains, and found a substantial decrease in the unsaturation index (16.3%) of women with gestational diabetes mellitus (an early stage of type 2 diabetes) compared with control individuals [7]. Thus, we propose that a decreased unsaturation index in type 2 diabetes, and in the prediabetic phase, results in a marked reduction in membrane flexibility, which affects the rate of transmembrane glucose transport, and thereby leads to an increased plasma glucose concentration. This is confirmed by findings that the percentages of docosahexaenoic acid (C22:6) and arachidonic acid (C20:4) are decreased by approximately 110-fold and 9-fold, respectively, in the released free fatty acid pool of human white cells, compared to the human serum pool, and that the unsaturation index of released free fatty acids from human white fat cells (85.5) is substantially lower than the unsaturation index of serum free fatty acids in healthy controls (191.9) [2].

Contrary to these studies, one study reported higher levels of highly unsaturated fatty acids in 21 type 2 diabetic man, whose condition was diagnosed within 1 year since screening, who were less than 45 years of age, and treated with a diet [15]. Achieved results indicated that a key feature in the type 2 diabetic individuals is a significant increase, compared to healthy controls, in the amount of arachidonic acid (C20:4n-6), i.e., 14.3% versus 11.2%, which is 47% of the carbon-carbon double bonds of the polyunsaturated fatty acid fraction. The authors suggest the increase in arachidonic acid may be diet-induced or imply an increased incidence of atherosclerotic complications. It will be

interesting to know whether supplementation of polyunsaturated fatty acids during pregnancy and early childhood can prevent adult onset of type 2 diabetes [16].

A pivotal moment in the history of type 2 diabetes is a recently published study that performed RNA sequencing to compare the genome-wide changes of gene expression in skin between patients with type 2 diabetes and non-diabetic subjects, which yielded the identification of two significantly downregulated genes: NKX2-1 and TPD52L3 [17].

The most downregulated gene in the gene regulation category is NKX-1 [17]. Defective NKX2-1 production is related to an essential reduction in mitochondrial respiratory chain complex activity, which reduces ATP production. This idea is supported by the data of a study suggesting that dysregulation of intramyocellular fatty acid metabolism in offspring of patients with type 2 diabetes was associated with an inherited defect in mitochondrial oxidative phosphorylation [18]. To restore ATP production, the β-oxidation of fatty acids provides assistance by increasing the levels of plasma free fatty acids via hydrolysis. Calculation of the unsaturation indexes of FFAs released from human white fat cells and human plasma free fatty acids in healthy controls reveals that the index of the former is substantially lower (85.5 and 191.9, respectively) [2]. Thus, we can conclude that an increase in release of free fatty acids into the blood circulation due to an essential reduction in the activity of the mitochondrial respiratory chain complex leads to a marked reduction in the unsaturation index of human plasma free fatty acids. Notably, reduced mitochondrial activity is one of the characteristics of type 2 diabetes [19].

The most downregulated gene in the metabolism category is tumor protein D52-like family of proteins (TPD52L3) [14]. In brown adipose tissue, the mitochondrial population exists as two subclasses: cytoplasmic mitochondria that do not adhere to lipid droplets and mitochondria that do adhere to lipid droplets [18]. Lipid droplets are cytosolic storage organelles consisting mostly of neutral lipids and enclosed by a phospholipid monolayer membrane [20]. This monolayer has persistent surface packing defects, whereby neutral lipids are accessible to the aqueous cytoplasm and the blood circulation. Based on the 3D-structure of protein TPD52L3, the idea is that TPD52L3 covers these defects of the monolayer in healthy individuals. Thus, it seems likely that significant downregulation of TPD52L3 causes an increase in free fatty acids in the blood circulation and lowers the unsaturation index.

I will argue that downregulation of NKX2-1 and TPD52L3 triggers an increase in release of free fatty acids into the blood circulation. The unsaturation index of free fatty acids from human white fat cells is substantially lower than the unsaturation index of serum free fatty acids in healthy controls. This deficiency leads to an essential shift from unsaturated to saturated acyl chains in phospholipids of both erythrocyte and vascular membranes, which finally reduces the pore diameter of all class I glucose transporter proteins. These phenomena lead to a marked reduction in transmembrane glucose transport, and initiate impaired glucose tolerance, gestational diabetes mellitus, and type 2 diabetes [7].

β-cells play an important role in unraveling the Gordian knot of insulin resistance in type 2 diabetes. Firstly, a detailed working hypothesis proposes that one key feature of the etiology of type 2 diabetes, which appears in the prediabetic phase, is a reduction in the unsaturation index compared to healthy controls, which leads to a lower rate of transmembrane glucose transport, and consequently causes reduced glucose effectiveness. Thus, the quantitative deficiency in plasma glucose entering the β-cell via GLUT2 reduces insulin production, leading to reduced insulin sensitivity. Secondly, after synthesis of monomer insulin, six monomer insulin molecules can join together, forming stable 36,000 molecular weight hexamer in the presence of zinc ions [21]. The mature hexamers are packed inside

mature intracellular vesicles that are transported to the β-cell plasma membrane. Finally, fusion of the intracellular vesicle membrane with the β-cell plasma membrane creates a fusion pore that allows expulsion of the monomer insulin molecules into the blood circulation. The large dimensions of the monomer insulin molecule (30 Å wide and 35 Å high) require substantial flexibility of both the vesicle membrane and the β-cell plasma membrane. Reduction in the unsaturation indexes leads to a lower rate of insulin transport into the blood circulation, which results in further decrease of insulin sensitivity.

An important observation in the presented data is that, compared to healthy controls, patients with type 2 diabetes show a reduction in insulin sensitivity (57.9%) that is essentially greater than the reduction in glucose effectiveness (26.2%) (Table 1). This may be explained by the dual-participation of the β-cell plasma membrane during insulin production, i.e., the plasma glucose entering the β-cell plasma membrane via GLUT2, and the transport of synthesized insulin into the blood circulation.

Thus, the final plasma insulin concentration is determined by reductions in both glucose effectiveness and insulin sensitivity, due to the cross-sectional contraction of all class I glucose transporter proteins. This contraction reduces the rate of glucose transport across cell membranes, initiating the onset of type 2 diabetes.

The above-described mechanism is quite different from the general understanding of "insulin resistance" as a term describing the relative ineffectiveness of insulin [1]. I should stress that this term ignores the essential reduction, compared to the plasma glucose level, in the amount of glucose entering the β-cell via GLUT2, resulting in reduced insulin production.

An increase in glucose effectiveness, powered by an increased unsaturation index, reframes fundamentally the mechanisms that participate in the glucose homeostasis during type 2 diabetes. This pivotal difference may largely explain the findings of a study by the Diabetes Prevention Program Research group, that among high-risk individuals the incidence of type 2 diabetes development was reduced by 50% with lifestyle intervention, and by 31% with metformin, as compared with placebo [22]. In other words, we can make a difference in improving the quality of type 2 diabetes treatment. The key is that regular physical activity reduces human brown adipose tissue, and thereby induces a phospholipid shift from saturation to unsaturation [23], which promotes membrane flexibility, and thus increases both glucose effectiveness and insulin sensitivity. This realization finally brings closure to MacBryde's comment on "insulin resistance" in 1933: "There is as yet no general agreement as to its definition" [24].

Acknowledgments

None.

Conflict of interest

The author declares no conflicts of interest.

References

1. Falta W, Boller R (1931) Insulärer und insulinresistenter diabetes. *Klin Wochenschr* 10: 438–443. https://doi.org/10.1007/BF01736348

2. Weijers RNM (2015) Membrane flexibility, free fatty acids, and the onset of vascular and neurological lesions in type 2 diabetes. *J Diabetes Metab Disord* 15: 13. https://doi.org/10.1186/s40200-016-0235-9
3. Shulman GI, Rothman DL, Jue T, et al. (1990) Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by ^{13}C nuclear magnetic resonance spectroscopy. *N Engl J Med* 322: 223–228. https://doi.org/10.1056/NEJM199001253220403
4. Roden M, Price TB, Perseghin G, et al. (1996) Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97: 2859–2865. https://doi.org/10.1172/JCI118742
5. Pedersen O, Bak JF, Andersen PH, et al. (1990) Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. *Diabetes* 39: 865–870. https://doi.org/10.2337/diab.39.7.865
6. Salas-Burgos A, Iserovich P, Zuniga F, et al. (2004) Predicting the three-dimensional structure of the human facilitative glucose transporter Glut1 by a novel evolutionary homology strategy: insights on molecular mechanisms of substrate migration, and binding sites of glucose and inhibitory molecules. *Biophys J* 87: 2990–2999. https://doi.org/10.1529/biophysj.104.047886
7. Weijers RN (2020) Fundamentals about onset and progressive disease character of type 2 diabetes mellitus. *World J Diabetes* 11: 165–181. https://doi.org/10.4239/wjd.v11.i5.165
8. Cantor RC (1997) Lateral pressures in cell membranes: a mechanism for modulating of protein function. *J Phys Chem* 101: 1723–1725. https://doi.org/10.1021/jp963911x
9. Weijers RNM (2015) Unsaturation index and type 2 diabetes: unknown, unloved. *World J Meta-Anal* 3: 89–92. https://dx.doi.org/10.13105/wjma.v3.i2.89
10. Naudi A, Jové M, Ayala V, et al. (2013) Membrane lipid unsaturation as physiological adaptation to animal longevity. *Front Physiol* 4: 372. https://doi.org/10.3389/fphys.2013.00372
11. Blog, What is the phospholipid bilayer and what determines its fluidity? Available from: https://blog.cambridgecoaching.com/what-is-the-phospholipid-bilayer-and-what-determines-its-fluidity
12. de Meyer F, Smit B (2009) Effect of cholesterol on the structure of a phospholipid bilayer. *Proc Natl Acad Sci U S A* 106: 3654–3658. https://doi.org/10.1073/pnas.0809959106
13. Róg T, Pasenkiewicz-Gierula M, Vattulainen I, et al. (2009) Ordering effects of cholesterol and its analoges. *Biochim Biophys Acta* 1788: 97–121. https://doi.org/10.1016/j.bbamem.2008.08.022
14. Koehrer P, Saap S, Berdeaux O, et al. (2014) Erythrocyte phospholipid and polyunsaturated fatty acid composition in diabetic retinopathy. *Plos One* 9: e106912. https://doi.org/10.1371/journal.pone0106912
15. Pelikánová T, Kohout M, Válek J, et al. (1991) Fatty acid composition of serum lipids and erythrocyte membranes in type 2 (non-insulin-dependent) diabetic men. *Metabolism* 40: 175–180. https://doi.org/10.1016/0026-0495(91)90170-2
16. Das UN (2018) Arachidonic acid in health and disease with focus on hypertension and diabetes mellitus: A review. *J Adv Res* 11: 43–55. https://doi.org/10.1016/j.jare.2018.01.002
17. Takematsu E, Spencer A, Auster J, et al. (2020) Genome wide analysis of gene expression changes in skin from patients with type 2 diabetes. *Plos One* 15: e0225267. https://doi.org/10.1371/journal.pone.0225267

18. Petersen KF, Dufour S, Befroy D, et al. (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 350: 664–671. https://doi.org/10.1056/NEJMoa031314
19. Rovira-Llopis S, Bañuls C, Diaz-Morales N, et al. (2017) Mitochondrial dynamics in type 2 diabetes: Pathophysiological implications. *Redox Biol* 11: 637–645. https://doi.org/10.1016/j.redox.2017.01.013
20. Jarc E, Petan T (2019) Lipid droplets and the management of cellular stress. *Yale J Biol Med* 92: 435–452.
21. Blundell TL, Cutfield JF, Cutfield SM, et al. (1972) Three-dimensional atomic structure of insulin and its relationship to activity. *Diabetes* 21: 492–505. https://doi.org/10.2337/diab.21.2.S492
22. Diabetes Prevention Program Research Group (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346: 393–403. https://doi.org/10.1056/NEJMoa012512
23. Weijers RNM (2014) Membrane flexibility and cellular energy management in type 2 diabetes, gestational diabetes, and obesity. *EMJ Diabet* 2: 65–72. https://doi.org/10.13140/2.1.1027.6803
24. MacBryde CM (1933) Insulin resistance in diabetes mellitus. *Arch Intern Med (Chic)* 52: 932–944. https://doi.org/10.1001/archinte.1933.00160060106008

AIMS Press