Beyond Insulin Resistance and Syndrome X: The Oxidative-Dysoxygenative Insulin Dysfunction (ODID) Model – Part I

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Abstract

Oxidative-dysoxygenative insulin dysfunction (ODID) is defined as impairment of any or all aspects of insulin production and metabolism caused by oxidative injury to any or all molecular pathways in which insulin serves any pathophysiologic roles. This definition reaches beyond the prevailing concepts of insulin resistance, syndrome X, and diabetes mellitus. Specifically, it integrates into a global view of insulin dysfunction, myriad molecular interrelationships of insulin pathways to those of exercise, nitric oxide, NF-κB, TNFα, leptin, peroxisome proliferator-activated receptor-γ (PPARγ), resistin, IGF-1, IGF-2, and glutamic acid decarboxylase (GAD). Equally important are the diverse counterregulatory signaling pathways involving glucagon, adrenal hormones, hypothalamic factor(s) and related molecular species that contribute to glucose/lipid homeostasis in health and disruptions of that in pathophysiologic states. Beyond that, the ODID model covers many "non-insulin-glucoregulatory" phenomena in the bowel, blood, and liver ecosystems that significantly contribute to epidemics of insulin resistance, syndrome X, and diabetes mellitus and yet are seldom, if ever, included in discussions of those disorders. Furthermore, the ODID model addresses the core issues of epidemics of rapid hyperglycemic-hypoglycemic shifts and brisk glucose-insulin-adrenergic responses in persons with chronic disorders characterized by accelerated oxidative molecular injury, such as chronic fatigue syndrome, fibromyalgia, multiple chemical sensitivity syndrome, Gulf War syndrome, and related autoimmune disorders.

Dysoxygenosis (dysfunctional oxygen metabolism) is defined as a state of sustained impairment of cellular enzymatic functions involved with oxygen metabolism. The ODID model is based on the following fundamental aspects of glucose and insulin pathophysiology: (1) essential oxidative nature of glucose metabolism (oxidative phosphorylation and oxidation of hydrogen atoms released during glucose degradation); (2) incremental hyperglycemic oxidative stress (oxidosis) in the circulating blood caused by chronic and cumulative sugar overload, oxidative endproducts of glycation, and sensitivity of antioxidant enzyme systems to incremental oxidosis; (3) vulnerability to oxidosis of insulin receptors and other proteins involved in insulin signaling; (4) direct cellular glucose toxicity associated with cumulative intracellular glucose burden; (5) spreading epidemics of obesity-associated type 2 diabetes mellitus in the Western countries and low-body-weight-associated type 2 diabetes in the Orient; (6) association of derangements of glucose and insulin metabolism in clinical states characterized by accelerated oxidative molecular injury; and (7) reversibility of ODID state with measures that control oxidosis and dysoxygenosis.

The ODID model offers a unifying concept for disparate biochemical, genetic, and clinical observations concerning hyperinsulinemia, rapid hyperglycemic-hypoglycemic shifts, insulin resistance, syndrome X, and diabetes mellitus. Beyond that, it encompasses myriad "non-glucoregulatory" aspects of insulin pathophysiology, such as overproduction of androgens in women with polycystic ovaries as well as interactions of insulin pathways with major mediators of the inflammatory and immune responses. This model also has a strong explanatory power for normalization of insulin functions with "non-insulin therapies" that primarily address issues of the bowel, blood, and liver ecosystems.

Introduction

In 1983, in a monograph entitled *Spontaneity of Oxidation in Nature and Aging*,[1] I put forth a hypothesis that spontaneity of oxidation in nature provides the primary drive for all metabolic pathways in human biology and serves as the core mechanism for initiating, amplifying, and perpetuating molecular and cellular injury in *all* disease processes. In a series of follow-up publications,[2-14] I described many clinical, biochemical, and morphologic observations to support that hypothesis including: (1) oxidative coagulopathy,[9,10] (2) oxidative lymphopathy,[11] (3) oxidative dysautonomia,[12] (4) oxidative regression to primordial cellular ecology,[13] (5) primacy of the erythrocyte in vascular ecology,[14] and (6) oxidative dynamics of apoptosis.[15] My colleagues and I also firmly established the central role of those oxidative phenomena in a host of clinicopathologic entities including coronary heart disease,[9,16] chronic fatigue syndrome,[17] fibromyalgia,[18] environmental sensitivity syndrome,[19] allergic diathesis,[20] autoimmune disorders,[21] asthma,[22] cancer,[23] oligomenorrhea and amenorrhea,[24] arrested growth in children,[25] and a host of other disorders.[26-28]

A large number of recent reports of oxidative phenomena involving reactive oxygen species, reactive nitrogen species, oxidant products of glycation, and other types of prooxidant molecules have elucidated the essential oxidative nature of myriad pathophysiologic changes involving carbohydrate and insulin metabolism.[29-41] Those observations provide additional strong support for the oxidative insulin dysfunction. Specifically, antioxidant enzymes are proteins and, like other functional proteins, are vulnerable to oxidative injury or destruction leading to loss of their enzymatic functions.[42]

I presented the model of dysoxygenosis and marshalled extensive clinical and biochemical evidence for that model in a series of articles[42-44] and in a book titled *Oxygen and Aging*.[45] I marshall some additional evidence for that model in the forthcoming book titled *The Principles and Practice of Integrative Medicine. Volume I: Nature's Preoccupation With Complementarity and Contrariety*.[46] In this article, a large body of clinical, biochemical, and genetic knowledge concerning the pathophysiology of carbohydrate and insulin metabolism is reviewed to propose a unifying model of oxidative-dysoxygenative insulin dysfunction.

The Epidemic of Oxidative-Dysoxygenative Insulin Dysfunction

There is a global epidemic of oxidative-dysoxygenative insulin dysfunction. Insulin resistance and type 2 diabetes mellitus are recognized as spreading epidemics by the

* Recent guidelines for diagnosis and classification of diabetes issued by the American Diabetes Association include the following four categories: (1) Type 1 caused by cell-mediated immune destruction of pancreas, often presenting with ketoacidosis; (2) Type 2 caused by insulin resistance; (3) Third category including a host of specific types characterized by genetic defects involving insulin actions, B-cell function, endocrinopathies, and chemically induced pancreatic injury; and (4) gestational diabetes.

medical communities in all countries.[47-54] In the United States, 15.7 million people suffer from this type 2 diabetes and nearly 200,000 of them die every year of complications of diabetes. The disease affects over 250 million people worldwide and is the leading cause of blindness, kidney failure, and amputation among adults.[55-63]

The defining features of type 2 diabetes include defects in: (1) insulin-stimulated peripheral glucose uptake and disposal (insulin resistance); (2) suppression of hepatic glucose production; and (3) insulin secretion. Insulin resistance is target-tissue resistance to insulin – a state in which glucose homeostasis cannot be maintained by ß-cell hypersecretory response. Type 2 diabetes and insulin resistance put an individual's lifespan in serious jeopardy. Both are strongly associated with obesity and lead to well-recognized complications of coronary heart disease, stroke, renal failure, peripheral vascular disease with threat of limb amputation, blinding retinopathy, and disabling neuropathy. The relationship between insulin resistance and type 2 diabetes has been elucidated with: (1) longitudinal studies that show insulin resistance generally precedes the onset of the disease by 10 to 20 years[64-66]; (2) cross-sectional studies that document the consistent presence of insulin resistance in the disease[67-69]; and (3) prospective studies that establish insulin resistance as the best predictor of the disease.[66-68,70,71]

Type 2 diabetes and insulin resistance are thought to develop insidiously in older persons with a family history of diabetes and who have normal or high blood insulin levels. That is a common mistake, because the increases in diabetes prevalence rates are most pronounced in children and adolescents.[47-49] Indeed, the presence of insulin resistance is seldom recognized and emphasized by pediatricians. In health, insulin is the primary hormone that facilitates entry of glucose into cells and its utilization there. Insulin resistance develops in younger individuals when the insulin receptors and signaling pathways on the cell membranes fail to respond to insulin blood sugar level rises. The pancreas produces more insulin to overcome the resistant insulin receptors, but to no avail.

Parallel to the epidemic of type 2 diabetes is the epidemic of obesity in the industrialized world. The two epidemics are clearly related to each other, with obesity being present in 60 to 80% of diabetics in the West.[72-74] However, the molecular links between the two are thought to be elusive. Obesity is associated with increased triglyceride storage in adipocytes. Triglyceride metabolism involves release of free acids from the fat cells to provide the energy needs of other cells. The blood levels of free fatty acids are higher in obese patients, and such acids are known to induce insulin resistance in adipose as well as other tissues.

Concurrent with the epidemic of obesity-associated type 2 diabetes in the West is the epidemic of low-body-weight-associated (LBW) type 2 diabetes in the Orient. Specifically, the incidence of this pattern of diabetes increased more than threefold in India during the last two decades.[51-54] In studies of hepatic glucokinase and microsomal enzymes (cytochrome P-450) employing antipyrine and lidocaine, *in vivo* probes showed hyperactivity with increased futile cycles of carbohydrate metabolism in LBW type 2 diabetes. The frequency and levels of islet cell antibodies and those with specificity for glutamic acid decarboxylase (GAD) in LBW type 2 diabetes are similar to that in the West.

There is another equally frightening epidemic of symptomatic hyperglycemic-hypoglycemic shifts and troublesome glucose-insulin-adrenergic responses of persons with chronic disorders characterized by unrelenting oxidosis, such as fibromyalgia, chronic fatigue syndrome, multiple chemical sensitivity syndrome, Gulf War syndrome, and related disorders. Nearly one in four patients visiting their primary care physicians report chronic fatigue.[75] According to a recent report by *The Wall Street Journal*, eight million Americans (mostly young) suffer from fibromyalgia.[54] The presence of abnormal glucose and insulin dynamics in such disorders generally goes unrecognized except in the hands of nutritionist-physicians and nutritionists.[76-77] Such an early form of oxidative insulin dysfunction in children and adolescents can be evaluated properly only by evaluating the oxygen metabolism, redox dynamics, and conducting timed studies of glucose and insulin kinetics. Such a work is seldom performed in those patient populations except by nutritionist-physicians. Amazingly, recent guidelines for diagnosis and classification of insulin disorders and diabetes issued by the American Diabetes Association do not include any reference to insulin dysfunctions associated with such syndrome.[78]

Insulin Dysfunction

Glucose Oxidation and Reactive Oxygen Species

Cellular glucose oxidation begins with cytosolic glycolysis that generates NADH and pyruvate. NADH so produced can either reduce pyruvate to lactate or donate reducing equivalents to the mitochondrial electron transport chain. NADH destined for mitochondria is transported through two shuttles: (1) the glycerol phosphate shuttle; and (2) the malate-aspartate shuttle.[79] Mitochondrial NADH (along with $FADH_2$) provide energy for ATP production by the electron transport chain through oxidative phosphorylation. Pyruvate reduced to lactate leaves the cells and is carried to the liver, where it is oxidized through the tricarboxylic acid cycle, to generate four molecules of NADH, one molecule of $FADH_2$, carbon dioxide, and water.

Pyruvate serves as the substrate for gluconeogenesis. Most of pyruvate generated in cytosol, however, is transported into mitochondria, here it is oxidized through the tricarboxylic acid cycle to four molecules of NADH, one molecule of $FADH_2$, carbon dioxide, and water. Transfer of cytosolic NADH into mitochondria is primarily conducted by the malate-aspartate shuttle.* Mitochondrial NADH and $FADH_2$ provide energy for ATP production by the electron transport chain through oxidative phosphorylation.

Electron flow in the mitochondrial electron transport chain involves the following five complexes:[80]

1. Complex I: NADH:ubiquinone complex;
2. Complex II: Succinate:ubiquinone oxidoreductase complex;
3. Complex III: ubiquinone:cytochrome c oxidoreductase complex;
4. Complex IV: cytochrome *c*:

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(*The malate shuttle involving the malate-citrate antiporter provides the mechanism by which 2-carbon units of substrate are transferred from the mitochondria to the cytosol for synthesis of fatty acids. Acetyl CoA is the primary molecule required for synthesis of fatty acids in the cytosol. It is a product of mitochondrial metabolism that does not traverse well the mitochondrial membrane, hence the value of the malate shuttle.)

cytochrome *c* oxidase ATP synthase; and

5. Complex V: ATP synthase.

The first four enzyme complexes are associated with the inner mitochondrial membranes. Cytochrome c and the mobile carrier ubiquinone are other important molecular species associated with that location.[80] Electron transport begins with donation of electrons by cytosolic and mitochondrial NADH to NADH: ubiquinone oxidoreductase (complex I). The complex next transfers its electrons to ubiquinone which also receives other electrons from a family of $FADH_2$-containing dehydrogenase, including succinate:ubiquinone oxidoreductase (complex II) and glycerol-3-phosphate dehydrogenase. Ubiquinone, in turn, donates its electrons to ubiquinone:cytochrome *c* oxidoreductase (complex III) by ubisemiquinone radical-generating Q cycle.[81] The next phase in electron transport involves cytochrome; cytochrome c oxidase (complex IV).

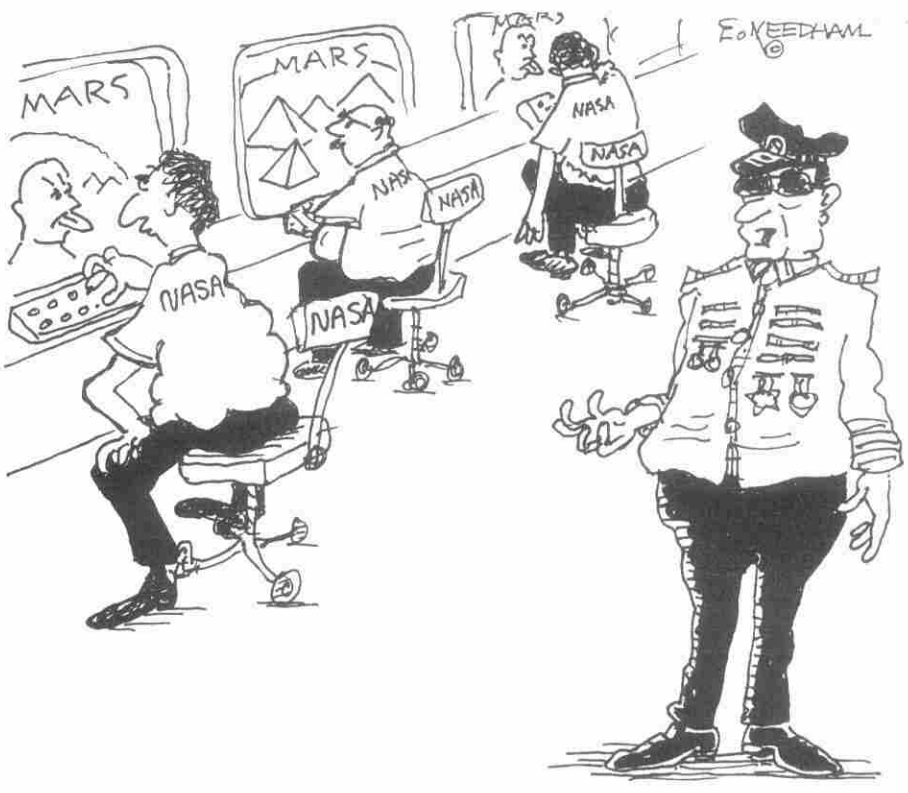
Superoxide generation occurs at two sites in the inner mitochondrial membrane: (1) NADH dehydrogenase at complex I; and (2) at the interface between ubiquinone and complex III.[82] A proton gradient is generated by electron transfer through complexes I, III, and IV that drives ATP synthase (complex V). As that gradient increases, there is a concomitant increase in the electrochemical potential difference produced by it. That potential, in turn, prolongs the life of ubiquinone and other superoxide-generating electron transport intermediates. Beyond a certain threshold, that potential markedly increases superoxide production. Thus, excess intracellular generation of reactive oxygen species (ROS) is driven by the proton electrochemical gradient generated by the mitochondrial electron transport chain. Overexpression of uncoupling protein-1 (a specific uncoupler of oxidative phosphorylation which is capable of collapsing the proton electrochemical gradient) reduces ROS production.[83] ROS generation is prevented by overexpression of manganese superoxidase dismutase (Mn-SOD), the major mitochondrial antioxidant enzyme system, in gene transfer experiments.[84]

Hyperglycemia causes excessive production of reactive oxygen species (ROS). The primary source of the substrate for increased ROS production in hyperglycemia is the tricarboxylic cycle.[85] The main mechanism of that is the proton electrochemical gradient generated by the mitochondrial electron transport chain described above. Those aspects of redox dynamics in hyperglycemia were first established by studies with bovine aortic endothelial cells. The blockade of the malate-aspartate shuttle in those cells with aminooxyacetate does not affect hyperglycemia-induced excess production of reactive oxygen species (ROS). However, blockade of glycolysis-derived pyruvate transported into mitochondria by 4-hydroxycyanocinnamic acid abolishes the generation of such ROS.

Advanced glycation end products (AGEs) are produced in excess in hyperglycemia.[86] That process is initiated and perpetuated by mitochondrial superoxide,[85] and involves increased production of AGE-forming methylglyoxal derived from fragmentation of glyceraldehyde-3-phosphate. The enzyme facilitating that reaction is glyceraldehyde-3-phosphate dehydrogenase. That enzyme is reversibly inhibited by ROS. Further evidence is drawn from the demonstration that excess production of methylglyoxal is blocked by Mn-SOD as well as by UCP1.[85]

In addition to glucose, other readily oxidizable substrates, such as Amadori adducts, reactive carbonyl and dicarbonyl compounds, and polyunsaturated fatty acids are increased in diabetes. Excess of these substrates leads to increased non-enzymatic oxidative pathways.[87] Those changes are generally associated with decreased levels of ascorbate and glutathione. Furthermore, other products of glycoxidation and lipoxidation are also increased.

The above brief review of glucose-related and glucose-triggered phenomena is presented to firmly establish the relatedness of glucose metabolism to the redox homeostasis. That relatedness, as becomes evident from later sections of this article, forms the core of the oxidative insulin dysfunction model. Those dynamics are the core issues of insulin pathophysiology in the context of the proposed model.



"Now, if you see any faces or pyramids report immediately to psychiatric...."

The Too-Much/Too-Little Sugar Dilemma

I first recognized the "too-much/too-little sugar dilemma" during my work with patients with fatigue/fibromyalgia complex and those with diabetes mellitus. On the too-little side, most patients with fatigue/fibromyalgia complex experienced troublesome symptoms of hypoglycemia. On the too-much glucose side, the potential of hyperglycemia to cause oxidative coagulopathy became clear to me during high-resolution (x15,000) phase-

contrast microscopy of freshly prepared peripheral blood smears of diabetic subjects with poor control of hypoglycemia.[88-90]

At a fundamental level, glucose is the principal source of energy for the human cell. The redox dynamics provide the pathways for extracting energy from glucose. From a teleologic perspective, glucose metabolism and redox equilibrium must be intricately related in *all* of their aspects. Glucose metabolism generates reactive oxygen species (ROS), reactive nitrogen species, and oxidative products of glycation.[50] Not unexpectedly, hyperglycemia is accompanied by excess ROS production. Not enough glucose means cellular starvation and consequent oxidosis. Too much glucose also causes oxidosis in many ways. Thus is created the too-much/too-little-glucose dilemma. Close regulation of glucose in the blood as well as the intracellular compartment is a metabolic high-wire balancing act. Oxidosis created by glucose dysregulation puts under increasing stress all hormonal pathways involved in glucose regulation. It also affects myriad molecular dynamics that regulate: (1) glucose traffic at cell membranes; (2) delivery to mitochondria of NADH and pyruvate produced by cytosolic glycolysis; and (3) the transport to the liver of reduced pyruvate (lactate) to serve as substrate for gluconeogenesis.

The teleologic consideration suggests that hyperglycemia-induced excess generation of ROS must influence – and be influenced by – endothelial nitric oxide dynamics. That, indeed, is true.[74] ROS lowers nitric oxide levels in diabetes. Seemingly, nitric oxide chemistry pitches in to counter regional oxidosis. Two recognized mechanisms are involved in the relationship between nitric oxide and hyperglycemia-induced generation of ROS. First, hyperglycemia-induced excess production of sorbitol (which is potentiated by nitric oxide) is blocked by manganese superoxide dismutase. Second, overproduction of mitochondrial superoxide enhances the activity of enzyme aldose reductase and stimulates the production of sorbitol by that enzyme. The activity of aldose reductase is reversibly downregulated by nitric oxide modification of a cysteine residue in the enzyme's active site.[92]

Adipocytes appear to autoregulate their responsiveness to insulin in many ways. Specifically, such cells secrete free fatty acids and a large number of metabolically active polypeptides, including leptin, adipsin, acrp30/adipoQ, and tumor necrosis factor-α (TNF-α) that affect insulin metabolism in different ways.[76-80] All those pathways also influence and, in turn, are influenced by local redox dynamics.

A clear understanding of the relationship between the symptoms of hypoglycemia and the rate of change in the blood sugar level is essential for understanding the glucose-insulin-adrenaline dynamics. Sharp rises in the blood sugar level evoke sharp responses from the beta cells of the pancreas that release insulin. Sudden bursts of insulin cause a sudden release of adrenaline and its cousins, the adrenergic molecules. What are generally considered to be symptoms of hypoglycemia are, by and large, symptoms of brisk adrenergic responses. A rapid insulin response induces a brisk adrenergic response. From a clinical standpoint, prevention of hypoglycemic symptoms requires a focus on the metabolic events that occur two to three hours before their development of symptoms.

Below, I reproduce some text from *The Butterfly and Life Span Nutrition*[101] to illustrate this sequence of events.

An eight-year-old girl has a blood sugar level of 100 mg/dl (or 1,000 mg in one liter of blood). Since she has a total circulating blood volume of about 5 liters, the total quantity of glucose in her circulating blood is 5000 mg or 5 grams. A teaspoonful of sugar holds 4 grams of sugar. Suppose this girl drinks, on an empty stomach, a can of soda containing eight to ten teaspoons of sugar. This means that girl pours six to eight times as much sugar into her blood as exists at any time. Such a large bolus of sugar creates a tide of glucose that evokes a brisk insulin response which, in turn, triggers an adrenergic surge. Similar molecular rollercoasters are caused when she drinks a 12-ounce glass of commercial orange juice. How is the sugar molecular rollercoaster initiated? With sugar overload. How is the sugar molecular rollercoaster perpetuated? By withdrawal symptoms. "Highs" in the blood sugar levels are followed by the "lows" that create biologic demands for yet more sugar. Sugar craving is another name for sugar addiction. An American child at the turn of the century consumed between five and ten pounds of sugar per year. His counterpart today ingests 150-175 pounds. How many thousands of molecular rollercoasters does that come to? The numbers add up. This is the essence of the hypoglycemia problem. How does our sugar industry respond to all this? They keep physicians on their payroll to publish absurd studies showing that our children are not hurt by sugar. This is the simple truth behind the hypoglycemia controversy.

Insulin triggers a cascade of myriad oxidative events. And so does adrenaline. Hypoglycemia is oxidizing, as is hyperglycemia. One of the mechanisms by which high intracellular concentration of glucose causes cellular toxicity is the hexosamine pathway, briefly described below.

Glucose Toxicity and Hexosamine Pathways

Glycolysis is the splitting of a molecule of glucose to form two molecules of pyruvic acid. It involves ten steps. The net reaction per molecule of glucose is expressed below:

$$\text{Glucose} + 2\text{ADP} + 2\text{PO4} \rightarrow 2 \text{ pyruvic acid} + 2\text{ATP} + 4\text{H}$$

In the above conversion, only two net moles of ATP are formed for each mole of glucose, producing 24,000 calories of stored

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energy. However during the ten successive steps of the above conversion, a total of 56,000 calories are lost as heat energy, giving an overall efficiency for ATP formation of 43%. It may be added here that only an equivalent number of ATP (two moles) are generated in the citric acid cycle. Clearly, the major portion of final ATP (nearly 90 percent) is generated during subsequent oxidation of hydrogen atoms released during earlier stages of glucose breakdown.

The concentration of intracellular glucose-6-phosphate is responsive to both glycogen synthesis and glucose transport since it is an intermediary between those two processes. Since the activity of glycogen synthase is decreased in type 2 diabetes, the intracellular concentration of glucose-6-phosphate is higher than in normal subjects. Furthermore, insulin-stimulated increase in the concentration of glucose-6-phosphate is blunted in the disease.[102] Similarly, intracellular glucose is a metabolic intermediary between glucose transport and hexokinase activity and its concentration is determined by the relative strengths of the processes. Metabolic studies of these aspects of glucose metabolism suggest that impaired insulin-stimulated glucose transport is responsible for the diminished rate of insulin-stimulated glycogen synthesis in the muscle tissue in type 2 diabetes.[102]

Some aspects of the complementarity and contrariety of influences of glucose on insulin function may be attributed to the hexosamine pathway.[103] This pathway provides an alternative to glycolysis at the level of fructose-6-phosphate and involves the enzyme fructose-6-phosphate amidotransferase and production of glucosamine-6-phosphate and other hexosamine products.[103] Glucosamine inhibits insulin-stimulated glucose uptake as well as GLUT-4 translocation.[104,105] Overexpression of glutamine:fructose-6-phosphate amidotransferase in transgenic mice results in insulin resistance (failure of insulin-induced glucose uptake in muscle).[106] Activity of glutamine:fructose-6-phosphate amidotransferase is also increased in the skeletal muscle of diabetic patients.[107]

Table 1. Clinicopathologic Entities Associated With ODID and Insulin Resistance

1. Chronic and cumulative sugar intake (severe)
2. Physiologic hyperinsulinemia (mild to moderate) Puberty/advanced age/pregnancy
3. Oxidative factors influencing cellular glucose utilization:
   - Cirrhosis
   - Diabetes
   - Fasting
   - Starvation
   - Fever
   - Stress (e.g., fever sepsis)
   - Hyperglycemia
   - Ketoacidosis
   - Obesity
   - Uremia
4. Specific hormonal or metabolic disorders*:
   - Acromegaly
   - Cushing's syndrome
   - Glucagonoma
   - Insulinoma
   - Pheochromocytoma
   - Thyrotoxicosis
5. Primary dysfunction of cellular glucose utilization
6. Oxidative-dysoxygenative enzyme dysfunction involving glucose-insulin dynamics
7. Neonatal hyperinsulinism:
   - Focal adenomatous islet-cell hyperplasia**
   - Diffuse hyperinsulinism***
8. Mutations of genes of:
   - Insulin-receptor and glucose transporters
   - Substrates for insulin-receptor kinase
   - Signaling intermediate
   - Cellular inhibitors of insulin-receptor kinase
9. Autoimmune dysfunctions
10. Disruptions of the bowel, blood, and liver ecosystems ****:
11. Miscellaneous:
    - Free fatty acids (nonesterified fatty acids)
    - Adenosine
    - Islet amyloid polypeptide (amylin)

*The human body has many levels of "glucose buffering" mechanisms.** Associated with a lost maternal allele from chromosome 11p15.*** A heterogeneous disorder involving gene encoding sulfonylurea receptor.**** See discussion below.

Insulin and Insulin Receptors

Human insulin is a small-sized protein (or a very large polypeptide) with a molecular weight of 5808. It is composed of two amino acid chains held together by disulfide linkages. To exert its myriad effects, insulin must first bind with a high affinity to a much larger membrane receptor protein called an insulin receptor with a molecular weight of about 300,000. The binding of insulin to an insulin receptor results in:

1. Very rapid increase in permeability of cell membranes for glucose involving about 80% of cells in the body. This effect is most pronounced in myocytes and adipocytes, but not in neurons.
2. Very rapid increase in membrane permeability for potassium and phosphate ions as well as many amino acids.
3. Delayed effects on functionality of myriad enzyme pathways involved in pathophysiology of insulin, carbohydrate, lipid, protein, and redox equilibrium.
4. Very delayed changes in translation of messenger RNAs at the ribosomes to form new proteins and yet more delayed effects on transcription of DNA in the cell nucleus.[108] (Samols)
5. Suppression of glucose production by inhibition of gluconeogenesis in the liver.
6. Inhibition of glycogenolysis in the liver.
7. Stimulation of glycolysis.
8. Enhancement of hepatic glycogen stores.
9. Induction of lipoprotein lipase which offloads triglycerides from very-low-density lipoprotiens and chylomicrons.
10. Stimulation of tryglyceride synthesis from glycerol and fatty acids in adipocyte.
11. Facilitation of cellular uptake of amino acids.

Each of those effects involve induction and/or suppression of a host of genes and expression of their glucoregulatory mediators. In the muscle and adipose tissue, insulin stimulates glucose uptake and disposal. The major part of the basal glucose uptake is thought to involve noninsulin-mediated pathways. In that view, the fasting levels of

insulin represent the suppressive effects of insulin on hepatic glucose output. The fact that insulin resistance (a decrease in biologic functions of insulin at a given insulin concentration) can be attributed to decreased sensitivity of cells to insulin or decreased responsiveness or both.

There are also differences in cellular responsiveness to insulin in various tissues and under pathophysiologic conditions. For example, sensitivity of ischemic myocardium to insulin is enhanced with an increase in glucose uptake and energetic metabolism through recruitment of the intrinsic myocardial insulin-response system.[109] Limited evidence suggests that, in contrast to coronary arteries and peripheral tissues, the myocardium of patients with NIDDM exhibits a competent glucoregulatory insulin response system.[110] The insulin receptor is a tyrosine kinase that adds phosphate groups to IRS-1 and IRS-2, its two substrate proteins. It is a transmembrane protein encoded by a gene composed of 22 exons located on chromosome 19.[111] A proreceptor precursor is synthesized from the mRNA that includes a 27-amino- acid signal peptide. The proreceptor is subsequently converted into a disulfide-linked tetramer composed of two α and two ß subunits by a process that includes removal of the signal peptide, glycosylation, cleavage into α and ß chains, and finally reassembly into the mature $\alpha_2\beta_2$ receptor. An extracellular segment of the α subunit provides insulin a high-affinity docking site. Insulin binding to subunit triggers rapid phosphorylation of the specific tyrosine residue of the intracellular portion of the ß subunit and activation of the tyrosine kinase intrinsic to that region.[112] A 23-residue hydrophobic domain of each ß subunit spans the plasma membrane and transduces the signal of high-affinity insulin binding to the extracellular α subunit.[113]

Following phosphorylation, IRS-1 and IRS-2 serve as docking sites for numerous intracellular proteins. When activated and assembled into a larger molecular complex, IRS-1 and IRS-2 turn on a multi- step signaling pathway that links insulin stimulation to at least two major intracellular signaling protein systems. The first turns on the enzyme phosphatydlinositol 3-kinase (PI 3-kinase), which in turn phosphorylates a host of proteins, including glucose transporter. The transporter ferries glucose into the cell. IRS-1 plays the dominant role in the glucose transport into mycocytes and adipocytes, whereas IRS-2 is the major player in transport into hepatocytes. Interestingly, IRS-2 also increases insulin production by the islet cells.

Another layer of complexity in the function of IRS proteins is added by the second pathway. The IRS-bound complex also activates a second pathway, called Ras pathway. Ras transcription activation complex leads to gene activations that have not been fully characterized. However, there is some evidence that Ras is involved with redox homeostasis and thus is likely to be proven to be an important link between oxidative insulin dysfunction and a wide array of molecular derangements that lead to incremental oxidosis. Pancreatic *b* cells respond to increase in ATP and changes in the ATP to ADP ratio.

C peptide is part of the proinsulin molecule which is released when that molecule matures into an insulin molecule in pancreatic beta cells. Like insulin, it stimulates glucose uptake in muscle and fat. However, it does so independent of insulin receptors. Thus, it creates a glucoregulatory mechanism that is distinct from insulin/insulin-receptor dynamics.[114,115] In many subjects with insulin resistance, serum concentrations of C peptide are also elevated and are seemingly insufficient for achieving glucose homeostasis.

Insulin Regulates Its Own Receptors

Binding of insulin to the insulin receptor also triggers endocytic internalization of the hormone-receptor complex.[116,117] Such endocytosis occurs only under two specific conditions: (1) when specific amino acids in the cytoplasmic segment of the ß subunit in close vicinity of the membrane are present; and (2) when functionally preserved receptor kinase is operant. Not surprisingly, the intact inside components of the ß subunit determine the functionality of the outside subunit. The internalized receptors may be degraded with the loss of the molecules or they may be recycled, externalized, and restored to the surface for ongoing use. Insulin affects the choice between degradation and recycling of its receptor (by decreasing the number of receptors) in a dose-dependent fashion. Thus, insulin downregulates its own receptors. Other physiologic factors, such as low-carbohydrate diet, exercise, and hormones also affect the number and function of insulin receptors.[118] This is of core importance to the proposed oxidative-dysoxygenative model of insulin dysfunction presented here.

Clinico-Pathologic Entities Associated with ODID and Insulin Resistance

The occurrence of hyperinsulinemic-hyperglycemic states (considered insulin resistance in the conventional sense) has been ➤

documented in a very large number of clinicopathologic, experimental, and genetic entities. The association of insulin resistance with a diverse group of endocrine and paracrine disorders has been exhaustively investigated and well-characterized. These subjects have been recently reviewed.[119-123] Table 1 gives a list of many such clinicopathologic entities.

Insulin failure should be defined as impaired pleiotropic biologic responses to insulin. This is rarely, if ever, the case since this term is employed almost exclusively for what is perceived to be hyperinsulinism in reference to blood glucose levels. For instance, the prevailing (and simplistic) notion of insulin resistance does not address the issue of increased ovarian production of androgens that occurs in women with hyperinsulinemia who do not show appropriate glucose-lowering response to injected insulin. For that and other reasons presented in this article, the concept of insulin resistance must be expanded in light of the proposed oxidative-dysoxygenative model.

More important from nutritional and ecologic perspectives, however, *the most common and important cause of insulin dysfunction is chronic and cumulative sugar overload* in subjects with accelerated oxidative molecular injury to various molecular pathways of the health/dis-ease/disease continuum. This pattern of oxidative insulin dysfunction is commonly encountered in patients with fibromyalgia, chronic fatigue syndrome, and a host of autoimmune disorders. This is not fully appreciated by many clinicians. Indeed, in a review article on the subject published in *The New England Journal of Medicine*, this important aspect of insulin dysfunction was not even mentioned in a long list of causes of insulin resistance.[61] Indeed, this is one of the major reasons for proposing the integrative model of oxidative insulin dysfunction.

Muscle and, to a lesser degree, fat are major sites of insulin-stimulated peripheral glucose disposal. Glucose transport is the rate-controlling step of glucose metabolism in skeletal muscle in normal subjects as well as in diabetic patients.[124] Failure of the stimulatory effect of insulin on glucose metabolism (insulin resistance) is the hallmark pathogenic feature in type 2 (non-insulin-dependent) diabetes, syndrome X, and obesity. Such insulin resistance also occurs, though to a much lesser degree, in autoimmune type 1 diabetes. Lesser degrees of insulin resistance also occur in non-diabetic relatives of type 2 patients.[125] This fact is often advanced as a reason for a genetic basis of diabetes. However, it clearly does not negate the presence of acquired risk of family members due to common sugar consumption habits. Clearly the pandemic of diabetes in the United States cannot be simply explained on genetic basis alone.

It is also a common mistake to establish a clear demarcation between the so-called insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) on the basis of presence or absence of insulin resistance. In fact, some impairment in cellular responsiveness to insulin is present in both types of insulin. There are documented cases of acute onset autoimmune type 2 diabetes in patients who were managed with insulin initially but whose blood glucose was well maintained without insulin with integrative protocols subsequently (personal unpublished data). In the past, results of studies on subjects with both types of diabetes were interpreted as indicating defects in glucose phosphorylation as well as glucose transport.[126-128] The recent trend has been to downplay the role of phosphorylation defects.[124]

Glucose Transporters

There are two distinct molecular families of cellular transporters for simple sugars (glucose, fructose, lactose, and others): a family of sodium-linked transporters and a family of GLUT proteins. The first family actively transports glucose against a glucose-concentration gradient and employs sodium cotransport as an energy source.[129] Since the intestines and kidneys are the two major sites of sharp glucose-concentration gradients, sodium-linked transporters are mostly active at these sites. The five members of the GLUT family, by contrast, facilitate glucose diffusion by down-regulating glucose-concentration gradient. The specificities, kinetics, and tissue distributions of five GLUT transmembrane proteins are given in Table 2.

GLUT 4 is regarded as the principal insulin-responsive glucose transporter. Mice with disruption of one allele of the GLUT-4 gene show a near 50% decrease in GLUT-4 concentration in the muscle and fat cells, severe insulin resistance, and frank diabetes in about half the animals.[130,131] About 90% of GLUT-4 is sequestered in vesicles in the absence of insulin stimulus. These vesicles also contain other proteins, such as insulin-responsive aminopeptidase, synaptobrevin (v-SNARE) and Rab-4 (a guanosine triphosphate-binding protein).

Insulin and exercise drive the GLUT-4 vesicles closer to the plasma membrane where the vesicles dock, form complexes with syntaxin-4 (t-SNARE) and synaptobrevin, fuse with the membrane, and increase glucose uptake. Insulin and exercise also drive Rab-4 into cytosol. When exercise ends or insulin is not available, vesicles are internalized and glucose uptake diminishes. In my view, reactive oxygen species produced during exercise provide the GLUT-4 tropic influence, though I am not aware of any direct experimental evidence for that.

Insulin resistance in skeletal muscle has been attributed to defects in translocation, fusion, and activation of GLUT-4 transporters. Some of those defects are thought to result from defects in intracellular signaling mediated by phosphoinositide-3 kinase. Specifically, activation of that enzyme by insulin is reduced in diabetics[132] and morbidly obese subjects with insulin resistance.[133] There are yet other molecular defects in addition to those involving intracellular signaling mediated by phosphoinositide-3 kinase. For example, concentrations of phosphorylated

Table 2. Characteristics of Glucose Transporters

Name	K_m for Glucose* mmo/liter	Distribution	Characteristics
GLUT-1	20	Large amounts in erythrocytes, brain, and endothelial cells	Constitutive glucose transporter
GLUT-2	42	Kidney, liver, epithelia, beta cells of pancreas, and intestine	Involved in glucose sensing in beta cells, low-affinity transporter
GLUT-3	10	Neurons, placenta	High-affinity transporter
GLUT-4	2-10	Myocytes and adipocyte	Insulin-receptor transporter
GLUT-5	NA	Intestine, brain, myocyte, sperm, kidney, brain, and adipocyte	High affinity for fructose and very low affinity for glucose

*K_m denotes Michaelis-Menten constant, and NA not applicable

insulin receptor and of IRS-1 are decreased in the muscle tissue of diabetics[128] and in severely obese subjects.[129] In a pathway involving insulin-stimulated tyrosine-phosphorylation, Cbl is recruited to the insulin receptor by the adaptor protein CAP.[134]

Glucose Supports Insulin, Glucose Opposes Insulin

For nearly three decades, I have been fascinated with nature's preoccupation with molecular complementarity and contrariety. Nothing in the human body, it seems to me, has fixed biologic roles.[46] From a teleologic perspective, one would expect that two molecules so intricately involved with each other as glucose and insulin must exert complementarian as well as contrarian influences on each other. Indeed, if that were not so, rising concentrations of insulin (in hyperinsulinemic states) would keep raising the intracellular glucose until incremental oxidosis caused by cumulative sugar toxicity would literally cause a cellular "burn-out." It may be pointed out here that exercise and appropriate dietary change restore glucose-insulin homeostasis in obesity, insulin resistance, and diabetes mellitus.

Glucose supports actions of insulin in some ways and opposes them in others. Glucose stimulates secretion and activities of insulin. Glucose also decreases insulin secretion and impairs its activities.[135] Insulin-stimulated uptake of glucose is diminished in muscle strips incubated with high concentrations of glucose.[115] This response is reversible and partly explains diminished insulin resistance observed with exercise and other aspects of integrative protocols that do not include insulin therapy.

Islet Cell Transplantation and Insulin Production by Stem Cells

In 1972, control of hyperglycemia in rats was first reported with islet transplantation.[136] In 1992, success with islet cell transplants in controlling hyperglycemia and obviating the need for insulin was reported in a few diabetic subjects.[137] However, by 1996, the Islet Transplant Registry could report successful results only in about six % of recipients.[138] By 2000, seven of seven transplant recipients who had received a minimum of 800,000 islets by direct injection into the portal vein had maintained normal blood glucose and glycosylated hemoglobin levels for an average of one year.[139]

Embryonic stem (ES) cells left to differentiate spontaneously usually produce cells resembling muscle, neuron, and sometimes intestine. Pancreatic cells appear only rarely under those conditions. However, some pancreatic cells express nestin, a protein typically produced by neural cells. That finding led to recent studies in which attempts were made to coax mouse ES cells to differentiate into insulin-producing pancreatic cells by culturing them under the following five conditions of cellular growth: (1) with leukemia inhibitory factor; (2) without leukemia inhibitory factor; (3) with serum-free medium; (4) with basic fibroblast growth factor; and (5) without basic fibroblast growth factor. Under those conditions, inner cells of the ES clusters produced insulin whereas outer cells produced glucagon and somatostatin. However, when implanted, ES-derived cells failed to normalize blood sugar, though the transplanted hyperglycemic mice lived longer than control hyperglycemic mice.[140]

Undoubtedly, progress in technology of islet transplantation and improved immunosuppression (such as regimens without glucocorticoids and low dose tacrolimus) will continue to improve the transplant results. However, the problems of supply and demand are daunting and certainly will remain so for decades to come.[141]

Free Fatty Acids and ODID

Energy is largely transferred from the adipose tissue to other sites in the body for metabolic needs as free fatty acids. Such acids are released into the bloodstream after being produced by breakdown of triglycerides, which constitute the main storage form of energy in tissues. The levels of free fatty acids are higher in the plasma of obese persons than in nonobese individuals. Excess free fatty acids diminish the sensitivity to insulin not only of adipocytes but also cells in other tissues.

Insulin Dysfunction

Elevated plasma levels of free fatty acids suppress myocyte and adipocytes glucose uptake and energetic metabolism.[142,143] One mechanism involved in the production of insulin resistance by free fatty acids involves interference with insulin substrate proteins. It is highly probable that excess intracellular free fatty acids (or their metabolites) interfere with glucose metabolism in mycocytes and hepatocytes by impairing or blocking both IRS-1 and IRS-2 pathways.[144,145] Indeed, even preliminary studies concerning those molecules have spurred considerable interest among pharmaceutical companies to develop drugs that block one or more enzymes to preserve or enhance functions of IRS-1 and IRS-2 proteins. For those reasons, for many years free fatty acids in excess were suspected to be the major cause of insulin resistance. However, it should be abundantly clear from the preceding discussion – as well as dynamics of tumor necrosis factor-α, PPARψ, resistin, and other factors presented later – that such a view is too simplistic to be given serious consideration.

The redox dynamics of fatty acid metabolism are being intensively investigated to elucidate the pathogenetic mechanisms of both type 1 and type 2 diabetes. Alpha-lipoate (lipoic acid, LA) reverses increased NADH/NAD+ (reductive stress) ratio in hyperglycemia and so enhances cellular glucose uptake. DHLA (dihydrolipoate) increases the synthesis and cellular levels of

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glutathione. LA and DHLA are involved in intracellular redox regulation, NF-kB activation, and gene expression.[146] Increased concentrations of plasma lipid hydroperoxides (ROOHs) and 8-epiDGF2 (an isoprostane) are increased in animal models of diabetes as well as in diabetic human subjects.

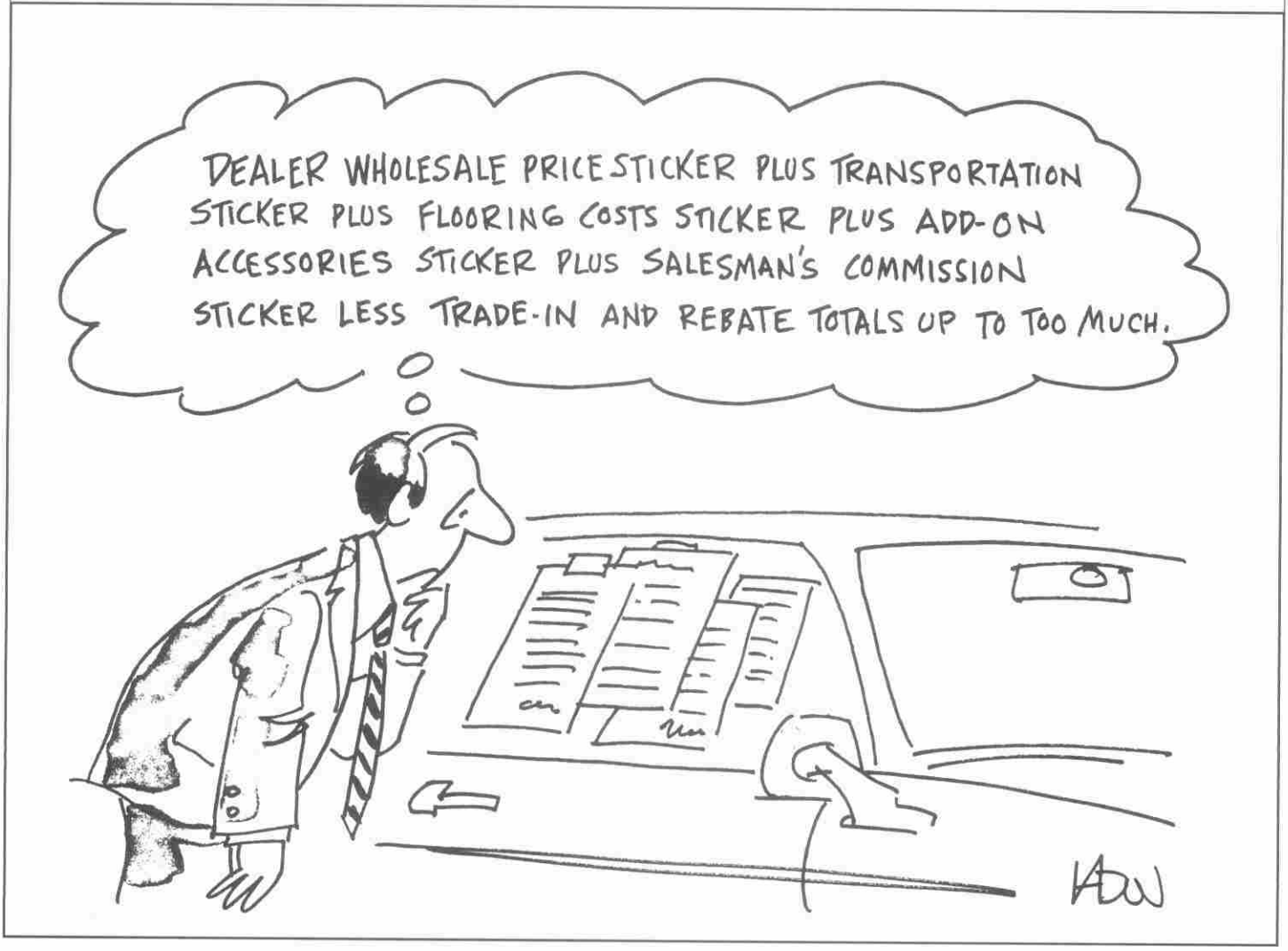
Under certain conditions, lipid-laden adipocytes release large amounts of free fatty acids that serve as substitutes for glucose as the metabolic substrate for energy pathways. Of course, fatty acid metabolism further adds to the stress of reactive oxygen species. Such dynamics are of special interest in the context of the oxidative insulin dysfunction model.

Counter-regulatory Hormones

In health, hypoglycemic effects of insulin are effectively managed by a host of counter-regulatory hormones. Hypoglycemia induces rapid release of glucagon from the pancreas, cortisol and adrenaline from adrenal glands, and growth hormone from the pituitary.

Glucagon is a single-chain, 29-amino acid peptide with a molecular weight of 3485. Typically, it is present in high concentrations during and for several hours after meals. By and large, glucagon focuses energetic metabolism on the endogenous production of glucose. Its primary role is to maintain blood glucose levels between meals by mobilization of fuel reserves of the body. Specifically, it inhibits glucose-utilizing pathways. In the liver, it both inhibits glycogen synthesis and simulates glycogenolysis. The first stage of glucagon action, as is the case with insulin, is its binding to its specific receptor. The signaling pathways triggered by the glucagon-receptor complex lead to binding of guanosine 5'-triphosphate (GTP) to a G protein complex and subsequent dissociation of G-protein subunits. One of those subunits (Gα) stimulates adenylate cyclase which converts ATP into a second messenger – cyclic AMP (cAMP) which, in turn, activates cAMP-dependent protein kinase. Next, that kinase phosphorylates regulatory enzymes that execute the metabolic effects of glucagon. Under physiologic conditions, however, the actions of glucagon are held in check by insulin-dependent activation of GTPase, phosphodiesterase, and phosphoprotein phosphatases. The major site of response to hyperglycemia in the central nervous system appears to be the ventromedial region of the hypothalamus. In rats, bilateral ablation of this region of hypothalamus, or perfusion of D-glucose into that region, blunts the hypoglycemia-induced release of catecholamines as well as glucagon.[147,148] Subjects with a combined deficiency of epinephrine and glucagon exhibit persistent hypoglycemia after ingestion of glucose.[149] Complete loss of counter-regulatory responses was recently reported in a patient with hypothalamic destruction by sarcoid infiltrate.[150]

The major counterregulatory influence, however, is exerted by the liver in two ways. First, glucose and other products of carbohydrate digestion are ferried from the intestine directly to the liver where glycogenesis is stimulated directly by rising concentrations of glucose in the portal circulation. Second, since most people do not engage in exercise during eating, glucose metabolism in the body is compartmentalized



for some time, with the muscle tissue not fully engaging in metabolic processes initiated by absorption of glucose from the intestine. I return to this important subject later in this article.

GAD and the GAD-less

Type 1 diabetes is caused by autodestructive T cells that infiltrate and destroy beta islet cells in pancreas and affects millions of people worldwide.[151,152] In contrast to type 2 diabetes that usually develops insidiously by insulin dysfunctions at several levels (some discussed later), type 1 diabetes is a severe acute-onset disorder that is often first diagnosed as a potentially fatal ketoacidosis accompanying pronounced hyperglycemia in young people. Recently it was claimed that a single protein expressed by beta islet cells, glutamic acid decarboxylate (GAD), is responsible for the development of type 1 diabetes in nonobese mice (an animal model considered good for human type 1 disease).

There are two types of GAD, GAD 65 and GAD 67. Their function in pancreatic islet beta cells remains to be elucidated. Several types of autoantibodies are detected in type 1 diabetes, including those against insulin. Of interest here is that some of the earliest autoantibodies detected in prediabetics have specificities for GAD.

In nonobese mice that efficiently express antisense transgene, there was no ß-islet cell GAD expression and the mice remained free of diabetes. In contrast, transgenic mice that expressed transgene with irrelevant information developed autoimmune diabetes. There was no anti-GAD T cell response in GAD-less mice. The injection of T cells from GAD-less animals failed to transfer disease. When transplanted into diabetic nonobese mice, GAD-less islets (but not normal cells) escaped autoimmune attack by T lymphocytes.

Of special interest to the oxidative insulin dysfunction model, both GAD 65 and GAD 67 are also expressed in brain cells, where they are involved in the production of neurotransmitter GABA.

Exercise and ODID

Abdominal obesity is estimated to account for up to 70% of non-insulin-dependent diabetes mellitus. The stage for this type of obesity is set by the lack of exercise in persons with chronically positive caloric intake. Exercise increases myocyte glucose uptake both directly and through its effects of insulin metabolism. Furthermore, physical exercise reduces the risk of developing diabetes in subjects at high risk for that.[153,154] Exercise also improves glucose tolerance in persons with diabetes.[153] Those observations point to the existence of its glucoregulatory effects that are independent of insulin.

Exercise, like insulin, facilitates glucose uptake in skeletal muscle by stimulating translocation of GLUT-4 to the plasma membrane.[153] However, it mediates its effects through signaling pathways that are different from those of insulin in that phosphoinositide -3-kinase is not involved.[155] Instead, 5'-AMP-activated kinase appears to be involved.[156] The initial steps in exercise-induced glucose uptake involve an increase in cytoplasmic calcium, subcontraction concentrations of which facilitate glucose transport.[153]

Exercise involves increased production of reactive oxygen and nitrogen species and clearly increases oxidative stress on insulin pathways. Thus, on the surface, the glucoregulatory role of exercise would be considered an argument against the oxidative insulin dysfunction model. However, increased generation of ROS and RNS invokes an upregulation of antioxidant defenses. The net effect of exercise is to strengthen the antioxidant arm of the redox equilibrium and reduction of oxidative stress in insulin pathways. This, of course, is also the explanation of other redox benefits of exercise.

Insulin Dysfunction

Obesity and type 2 diabetes are highly uncommon among Amish children as compared to other children in the United States. Amish children also do not take rides in automobiles and so walk and run much more frequently during ordinary household activities and chores. Is there a link between the two? My strong sense is that extra physical activity ensured by the lack of motorized transport among the Amish explains the low incidence of both obesity and type 2 diabetes among them.

Part II Next Month:
Nitric Oxide Dynamics and ODID

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