The Battle between Free Radicals and Anti-Oxidants: How Glutathione Can Prevent Diabetes and its Complications

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YOU ARE A BUNDLE OF TRILLION CELLS

The journey of every human life begins with just one cell. However, as an adult, a person is worth more than 70 trillion cells! In fact, it is correct to say that every human being is just an assembly of trillions of cells. However, the cells are not all the same in terms of their structures and functions. For instance, the liver cells are different from kidney cells, from heart cells, brain cells, lung cells, skeletal muscle cells, *etc*.

Whatever happens to a person ultimately happens at cellular level. Heart failure, for instance, results from injury to the cardiomyocytes (that is, cells of the heart muscle, the myocardium) which might have resulted from a variety of causes such as ischemia, hypertension, and diabetes. If it involves just one cell or very few cells a person may not have conscious perception of it. However, when many cells are affected it will, in most cases, evokes conscious perception such as pain and/or difficulty in carrying out certain activities or functions.

A CELL IS AS COMPLEX AS A CITY

Human cells are not visible to the unaided eye. However, each of the cells in human body can be likened to a big city in terms of activities, complexity and organization. For instance, there will be in a large city a power source, industrial manufacturing, warehousing, waste generation, waste management, traffic within the city, export and import across the city boundaries, regulations, law enforcement agents, custom, border control, diplomatic centers, constitution of the city and a government house where the affair of the city is being controlled based on the constitution. Biological types of these activities are going on in nearly every single healthy cell of the over 70 trillion cells in your body right now. In fact, it is correct to summarily say that the activities going on inside and around a cell is by far more complex than can be found in a city like Lagos or New York.

CELLS ALSO BURN FUEL TO GENERATE ENERGY

In the cell, the power house is the mitochondria that generate energy from end products of the food we consume. The endoplasmic reticulum is the industrial site for manufacturing of different molecules while the Golgi apparatus is the warehouse. Mitochondria produce a lot of waste by using oxygen to burn glucose in order to produce energy. Some of the wastes are evacuated from the cell by simple diffusion while some stay within the cell and require special handling. These two categories of wastes may be compared with waste products from fossil fuel which is majorly CO_2 that is released into the atmosphere and the other type of waste from nuclear power plant, requiring delicate and complicated handling.

Our cells produce CO_2 also, but the more dangerous wastes they produce are called free radicals. These are more or less the nuclear-type waste that if not handled properly and promptly will damage the cell that produced them and some of its immediate and distant neighbors beyond repair. Excessive production of these waste (that is, free radicals) and/or its inadequate handling has been convincingly shown to be behind nearly all the sicknesses and diseases that afflict human beings. These free radicals at certain amount in the cell will damage the cell that produced them and some of the neighboring cells. Such damages may include destroying the cell membrane, cell organelles and DNA.

The production of these free radicals (which can be described as cellular hooligans, or robbers) in human bodies will increase dangerously under certain conditions, which include stress, sickness, living on poor diet, exposure to radiation, smoking, alcoholism, drug abuse/misuse.

If one thinks about the amount of stress that human being is exposed to in the modern world and then factor in the unrestrained lifestyle of living on poor and high calorie diet, then it will be very easy to understand why the human body is producing more free radicals now than at any other time point in human history. In a nutshell, all the factors that favor excessive production of free radicals in human body have risen to an all-time high in our generation than previous generations.

It is, therefore, correct to say that human cells are in deep crises of oxidative stress. The battle between free radicals and antioxidants is currently at its fiercest level since the birth of mankind. The battle is going on inside every living human being! Who will win depends upon one factor: the amount of glutathione in the cells!

TYPES OF FREE RADICALS

Free radicals are reactive chemical entities that are short lived species containing one or more

unpaired electrons. Free radicals can be classified into following three types:

1. Reactive oxygen species (ROS), the most familiar and most studied. 2. Reactive nitrogen species (RNS). 3. Reactive chlorine species (RCS).

Oxidative stress is a state of excess free radicals in the cell or tissue beyond physiologic requirement. This occurs principally when cellular antioxidant defense capacity is overwhelmed by the level of pro-oxidants. The steps leading to this involves either excessive generation or inefficient removal of pro-oxidants due to antioxidant deficit.

THE BASIS OF MOST SICKNESSES

For emphasis, the pathogenesis and progression of nearly all sicknesses have some connections with rising level of free radicals within the cells. A case in point is type 2 diabetes, which is fast becoming a global epidemic. So, how does type 2 diabetes develop?

To provide a bit of insight into this question, it necessary to give a brief recap of what happens in a person's body who is not diabetic. When a person eats carbohydrate food, his gut breaks it down to glucose and the glucose is absorbed into his/her blood and travels to his/her liver and from there to the heart and then to all parts of the body. As the glucose molecules travel in the blood within the blood vessels, they leave the blood vessels when they get to the very small vessels. Having come out of these vessels, the glucose molecules move into the interstitial fluid in which the cells are bathed. Being in this fluid allows the glucose molecules to be very close and to even touch the cell membrane (the cell covering) in anticipation of entering the cell where glucose will be used to produce ATP (adenosine triphosphate), the form of energy that cells use to perform their functions.

HIGH BLOOD SUGAR IN TYPE 2 DIABETES RESULTS FROM INABILITY OF SUGAR TO ENTER SOME CELLS

However, glucose does not just enter all cells alike. Glucose cannot on its own cross the cell membrane of many cells, especially cells of skeletal muscles. In most cells, there are transporters that take the glucose molecules that surround the cells and move them into the interior of the cells. Just as the name implies, these transporters are the molecules that ferry glucose across cell membrane into cell interior. In the skeletal muscle, the largest consumer of glucose, there are mainly two different transporters that perform this function of moving glucose molecules into the cell's interior. The transporters are called GLUT1 and GLUT4. GLUT1 is located on the surface of cell membrane but is less abundant. The more abundant GLUT4 is located in the cytosol (that

is, the fluid in the cell's interior). For GLUT4 to perform its function, it must be translated into the surface of the cell membrane. This translocation occurs in response to exercise, hypoxia, (inadequate oxygen in body tissue) and/or insulin.

Let's now examine how type 2 diabetes develops. Type 2 diabetes develops majorly when GLUT4 fail to respond to insulin. That is, when cells resist insulin attempt to translocate GLUT4 to cell surface. In fact, this is why type 2 diabetes is also called insulin-resistant diabetes. What is insulin by the way?

Insulin is a chemical secreted in the pancreas and it is released following the absorption of glucose from the gut. The goal of insulin is to stimulate the cells especially, cells of skeletal muscles to take up glucose. Insulin does not directly effect the movement of glucose into cells. The way it causes glucose to enter the cell is to orchestrate cellular events that will immediately translocate GLUT4 into the surface of cell membrane.

At the cell surface, GLUT4 permits the facilitated diffusion of circulating glucose down its concentration gradient into muscle and fat cells. Once within cells, glucose is rapidly phosphorylated by glucokinase in the liver and hexokinase in other tissues to form glucose-6-phosphate, which then enters glycolysis (process of producing energy from breakdown of glucose) or is polymerized into glycogen. Glucose-6-phosphate cannot diffuse back out of cells, which also serves to maintain the concentration gradient for glucose to passively enter cells.

However, in some people, GLUT4 just stops responding to insulin's call. This means only GLUT1 is left to perform this function and unfortunately, GLUT1 alone cannot do the job effectively. When this happens, the blood glucose (sugar) level will keep rising because the glucose molecules in the interstitial fluid have nowhere to go and they just keep loitering around the cells and in the blood. Eventually, to get rid of the excess glucose, the kidney will allow some of to pass through to the urine.

LOW LEVEL OF GLUTATHIONE IN THE CELLS IMPAIRS THE FUNCTIONING OF GLUCOSE TRANSPORT

But why will GLUT4 refuse to respond to insulin? Well, it must be understood that cellular events are complex and complicated and many happen in microseconds. Never forget to compare a cell to a large city! There is what is called pre-diabetes stage, which is a period when there is elevated blood glucose but is not high enough to be called diabetes. Several studies have discovered one common disorder at pre-diabetes stage— high level of free radicals and low level of glutathione. Without going into the complicated molecular analysis of translocalization of GLUT4 unto cell

surface, it is sufficing to note that glutathione is involved in the process. Now, the riddle is partially solved: low level of glutathione will result in reduced or zero GLUT4 response to insulin and lead to high level of blood sugar and eventually full blown diabetes.

However, glutathione does not just suddenly get reduced or become zero. Glutathione is found in every single cell in the body. This is a hint about the importance of glutathione, which will be examined later in this write up.

WHY GLUTATHIONE LEVEL MAY BECOME LOW

If glutathione is so valuable in the cell why can't human cell always produce it in abundance?

Glutathione is produced from three amino acids namely glutamine, cysteine and glycine.

Glutamine and glycine are readily available in the cell but cysteine is not so abundant. Therefore, the amount of glutathione that the cell can make is proportional to the amount of cysteine that is available in the cell. Here is the bad news: Only very few foods are rich in cysteine. Now it gets worst, the gut enzymes largely destroy cysteine in food. That is the main problem and the very reason why there has been very limited success at boosting cellular level of glutathione. In addition, as you age, your body's ability to produce glutathione decreases.

Although our understanding of how hyperglycemia-induced oxidative stress ultimately leads to tissue damage has advanced considerably in recent years, effective therapeutic strategies to prevent or delay the development of these damages remain limited. Thus, further investigation of therapeutic interventions to prevent or delay the progression of diabetic vascular complications is needed.

PATHOGENESIS OF DIABETIC COMPLICATIONS

There is substantial evidence that hyperglycemia, the main defining feature of diabetes, results in increased production of reactive oxygen species (ROS) and consequently leads to oxidative stress. This hyperglycemia-induced oxidative stress represents a major pathophysiological mechanism by which diabetic complications develop. The oxidation of glucose generates hydrogen peroxide and reactive intermediates. Several lines of evidence indicate that free radicals play an important role in tissue damage. The cellular damage induced by free radicals falls into three major categories: lipid peroxidation, DNA-modification and reaction with thiol-groups.

Here are three pathways involved in the pathogenesis of diabetic complications; and each has direct association with free radicals and low glutathione level.

A. Pathogenesis Involving Polyol Pathway

Elevated glucose level lightens up the polyol pathway leading to the production of sorbitol and subsequently fructose by aldose reductase and sorbitol dehydrogenase, respectively. Aldose reductase-mediated reduction of glucose to sorbitol is associated with consumption of NADPH, and because NADPH is required for regeneration of glutathione, it then means that the activation of this pathway will considerably limit the amount of glutathione that is available within the cell. Consequently, a state of oxidative stress develops.

B. Pathogenesis Involving Advanced Glycation End Products (AGEs)

Hyperglycemia accelerates the generation of AGEs via attachment of reactive carbohydrate groups to proteins, nucleic acids, or lipids. These groups tend to damage the biological task of proteins, which as a result affects cellular function. Extracellular AGEs also bind to the receptor of AGE (RAGE) and initiate inflammatory flows, activate NADPH oxidases, and generate oxidative stress. The AGE and polyol pathways directly alter the redox capacity of the cell either through depletion of necessary components of glutathione recycling or by direct formation of ROS.

C. Pathogenesis Involving Protein Kinase C (PKC)

Hyperglycemia often stimulates the formation of diacylglycerol, which then activates PKC. Following, PKC triggers stress genes that phosphorylate transcription factors and thus alter the balance of gene expression resulting in oxidative stress.

These pathophysiological mechanisms are similar in all diabetic complications as explained below using diabetic retinopathy as an example.

DIABETIC RETINOPATHY

One of the commonest microvascular complications of diabetes is diabetic retinopathy, a disease of the retina, which if left untreated can lead to acquired blindness. Basically, the microvasculature of the retina is damaged involving swelling of the blood vessels and leaking of fluid. Subsequently, if left untreated, new vessels start to grow, and ultimately lead to the detachment of the retina.

The underlying mechanisms by which hyperglycemia induces diabetic vascular damage in the retina are beginning to emerge. These include five major mechanisms: increased formation of advanced glycation end products (AGEs), polyol pathway flux, increased expression of the receptor for advanced glycation end products and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine pathway. The most important piece of evidence is

that fact that each of these five mechanisms is directly associated with overproduction of reactive oxygen species and depletion of antioxidants especially glutathione in diabetic retinopathy. The structure of the retina makes a perfect site for oxidative stress during metabolic stress, a condition created by high glucose level in diabetes. One, the retina has high content of polyunsaturated fatty acids. Two, the retina has the highest oxygen uptake and glucose oxidation relative to any other tissue. Consequently, it is summarily correct to infer that ROS is the major pathophysiological mechanism by which diabetic retinopathy progresses and by which diabetes damages retinal cells.

Studies have shown that oxidative stress contributes not only to the development of diabetic retinopathy but also to the resistance of retinopathy to reverse after good glycemic control is reinstituted. That is, diabetic retinopathy does not immediately improve following normalization of blood glucose level because the oxidative stress created persists and there is accumulation of damaged molecules and ROS even after good glycemic control is reestablished.

BOOSTING GLUTATHIONE IN HUMAN CELLS TO PREVENT DIABETES COMPLICATIONS

Glutathione plays a critical role in a multitude of cellular processes, including cellular transportation, cell differentiation, proliferation, apoptosis and scavenging. It is therefore, understandable why perturbation of glutathione homeostasis is implicated in the etiology, pathogenesis and/or progression of many diseases such as cancer, diseases of aging, cystic fibrosis, and cardiovascular, inflammatory, immune, metabolic, and neurodegenerative diseases.

The amount of glutathione the cells make when a person is young and in perfect health is usually sufficient to combat the free radicals that are being generated as result of normal cellular respiration. However, the ability of human cells to synthesize glutathione begins to slowly decrease beginning from early twenties. This slow decrease will be accelerated by age, diseases and exposure to radiations. This means that everybody has the responsibility to help his/her cells boost glutathione production in order to stay healthy.

The best way to prevent diabetes complications is to prevent diabetes by combating free radicals before and during pre-diabetes stage. Of course, this should be done in addition to healthy lifestyle. However, if full blown diabetes has set in, the next stage is to manage glucose level and keep it from rising too high. But even with the best glucose level management, the risk of generation of free radicals is ever real. Consequently, the critical measure that will prevent

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diabetic complications is dealing with free radicals more effectively. This requires boosting the antioxidant defense system so that the free radicals are efficiently neutralized and rendered harmless. The most important antioxidant that can do this is glutathione. Boosting its level in the cells represents the smartest way to prevent the onset, slow the progression and effectively manage diabetes complications.

Bibliography

1. Anwar M.M., and Meki, A.R. (2003). Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative PhysiologyI, 135:539–547.

2. Raza H., Prabu S.K., Robin M.A., and Avadhani N.G. (2004). Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-induced diabetic rats: tissue-specific variations and roles in oxidative stress. Diabetes, 53:185– 194.

3. Bianconi E.1., Piovesan A., Facchin F., Beraudi A., Casadei R., Frabetti F., Vitale L., Pelleri M.C., Tassani S, Piva F, Perez-Amodio S., Strippoli P., and Canaider S. (2013). An estimation of the number of cells in the human body. Ann Hum Biol, 40(6):471.

4. Frank R.N. (2004). Diabetic retinopathy. New England Journal of Medicine, 350(1):48– 58.

5. Aylward G.W. (2005). Progressive changes in diabetics and their management. Eye, 19(10):1115–1118.

6. Kempen J.H., O'Colmain B.J., Leske M.C., Haffner S.M., Klein R., and Moss S.E. (2004). Eye Diseases Prevalence Research Group: The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol, 122:552–563.

7. Saaddine J.B., Honeycutt A.A., Narayan K.M., Zhang X., Klein R., and Boyle J.P. (2008). Projection of diabetic retinopathy and other major eye diseases among people with diabetes mellitus: United States, 2005-2050. Arch Ophthalmol, 126:1740–1747.

8. Watson R.T., Kanzaki M., and Pessin J.E. (2004). Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. Endocrine Reviews, 25(2):177– 04. DOI:10.1210/er.2003-0011.