

Postprandial hyperglycemia and hyperlipidemia-generated glycoxidative stress: its contribution to the pathogenesis of diabetes complications

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Abstract. – Postprandial glucose and triglyceride increments after a mixed meal are more prolonged in people with type 1 and 2 diabetes or with impaired glucose tolerance than in normal individuals. Evidence in the literature suggests that these transient increases represent an additional and independent risk for chronic hyperglycemia to induce endothelial dysfunction, an important fact for the development of diabetic vascular complications. This article presents the more relevant mechanisms by which acute postprandial hyperglycemia and hyperlipidemia have been proved to determine the risk of reactive oxygen species overproduction, an increased synthesis of non enzymatic early-glycated and nitrated proteins, and a more atherogenic lipoprotein profile. Recent recommendations suggest that care for this transient glycoxidative stress should be associated with fasting glucose or HbA_{1c} care, to reduce the risk of macro- and microvascular complications in people with diabetes.

Key Words:

Postprandial state, Oxidative stress, Diabetic complications, Postprandial lipoproteins, Hyperglycemia.

Characteristics, Evaluation and Effects of Postprandial Hyperglycemia

The rise and duration of hyperglycemia after a meal depends of its composition, i.e., quantity and quality of carbohydrate content, presence of other nutrients, time of digestion and absorption, secretion of gastrointestinal hormones, insulin, glucagon and other hormones, and time of the day of food intake¹. Therefore, the definition of postprandial glycemia is much debated, and the plasma glucose concentration 2-hour post food intake is a reasonable assessment, as proposed by the American Diabetes Association (ADA)². Based on this 2-hour post food intake, people can be classified as normal, with impaired glucose tolerance (IGT) or with diabetes³.

Glycated hemoglobin (HbA_{1c}) values in people with type 2 diabetes show a positive correlation with preprandial and fasting glucose levels; however, such correlation is low with respect to the level of postprandial glycaemic excursions. Therefore, monitoring of fasting glucose and/or HbA_{1c}, as well as of glycaemic values measured at different times of the day (postprandial periods in particular), is essential for a correct evaluation of daily glucose homeostasis³.

People with type 1 diabetes lack endogenous insulin, and the increase in postprandial glucose depends on the manner, quantity, time, and type of insulin administered. In people with type 2 diabetes and subjects with IGT, those increases depend on the level of impaired insulin, glucagon, GLP-1 and GIP secretion, on glucose uptake by the liver and

Introduction

Diabetic hyperglycemia is the result of an increase in daily glycaemic profiles as compared with that observed in persons with normal carbohydrate metabolism. In both cases, glycaemic profiles are related with postprandial and fasting periods, originating two well-differentiated components: intermeal (fasting) and postprandial glycemias (Figure 1).

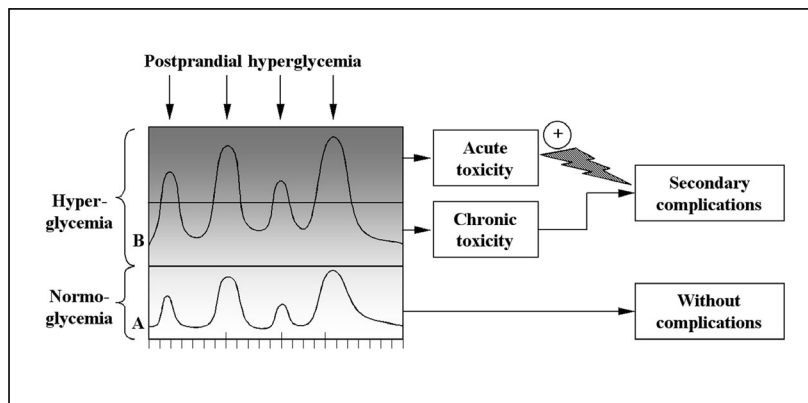


Figure 1. Glycemic profiles in normal (A) and diabetic (B) subjects. In the latter, secondary complications develop as a result of chronic hyperglycemia; although transitory, postprandial hyperglycemia represent an additional toxic risk.

peripheral tissues, and on insulin inhibition of the endogenous glucose production by the liver. In people with IGT, the progression towards type 2 diabetes is particularly associated with a gradual disappearance of the first phase of insulin secretion and concomitant alterations in the pulsatile secretion of the hormone. Therefore, postprandial glucose increments are more prolonged in people with IGT and/or type 1 and 2 diabetes than in normal individuals.

It is widely accepted that chronic hyperglycemia causes the development of micro- and macroangiopathic complications in diabetes mellitus. Prospective studies performed in numerous populations, namely the DCCT⁴ in people with type 1 diabetes and the UKPDS⁵ and Steno-2⁶ in people with type 2 diabetes showed that intensive treatments aimed at controlling hyperglycemia are associated with a decreased frequency of appearance of chronic complications.

The effect of chronic hyperglycemia is concomitant with the additional risk caused by the intensity and duration of postprandial hyperglycemia, as suggested by DCCT experts and other observations². Taking the recent ADA and American College of Endocrinology observations about the effect of postprandial hyperglycemia as a starting point, a panel of experts concluded that postprandial hyperglycemia is a risk indicator for the development of micro- and macrovascular complications in people with type 2 diabetes and IGT⁷. The experts also recommended to monitor chronic and acute glycemia fluctuations to prevent and control the development of type 2 diabetes microvascular complications, and

suggested that the postprandial rise of triglycerides (TG) and other components of the metabolic syndrome should be also controlled to decrease the risk of cardiovascular disease and the consequent mortality.

Table I summarizes some of the changes induced by postprandial hyperglycemia and hypertriglyceridemia on the homeostatic mechanisms of different organs and tissues^{8,24}. Some of these effects were also observed in non-diabetic people.

Hyperglycemic Toxicity

Understanding of the mechanisms by which alterations in lipid, protein and carbohydrate metabolism as a result of insulin resistance associated with decreased pancreatic beta cell insulin secretion, has lead to accept that hyperglycemia produces a complex stress with toxic effects (Figure 2). This stress comprises various processes:

- generation of an oxidative stress with increased reactive oxygen species (ROS);
- alteration in the activity of several enzymatic systems and metabolic pathways;
- changes in the structure and properties of proteins, lipoproteins and DNA by glycation.

The presence of the above mentioned does not involve a cause-effect relation; rather, a feedback and interaction among the three processes resulting in the so called “glycoxidative-carbonylic stress”.

Table I. Effects of postprandial glucose and triglyceride increases.

Organ, system or process	Effect	Outcome
Retina	↑ Perfusion	↑ Development of retinopathy in type 2 diabetes ⁸
Kidney	Glomerular hyperfiltration ↑ mesangium collagen synthesis	↑ Risk of nephropathy ⁹ Alterations in basal membrane structure ¹⁰
Sensory and motor nerves	Impaired conduction velocity	Impaired pain threshold ¹¹ Hyperglycemic clamp impairs nerve function in normal individuals ¹²
Cardiovascular system	↑ More atherogenic plasma TG and LDL Endothelial dysfunction	↑ Risk of CVD and stroke in diabetic and normal people ^{13,14} ↑ Adhesion molecules (ICAM-1, VCAM-1 and selectin E) ¹⁵ ↓ Vascular reactivity ¹⁶⁻¹⁹
Coagulation ECG	Activation of Factor VII Prolonged (QT)c interval	Hypercoagulability ²⁰ ↑ Sympathetic tone? ^{21,22} Impaired $[K_e^+]$ ²³ ↓ NO ²⁴

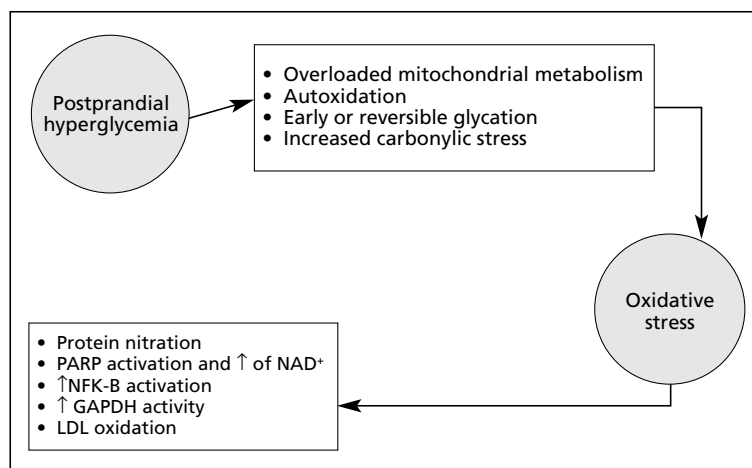
This review will not focus primarily on the effects of stress on organs or specific tissues, but on the mechanisms generating that stress. All three processes are present in people with normal carbohydrate metabolism and in insulin resistant or diabetic people; the difference is in the intensity and velocity with which they occur in each case.

There is no clear definition for the “toxic threshold of glycemia”. Although glycemia is a continuous variable, studies on the possibility of developing microangiopathic complications identified a threshold HbA_{1c} value of

8%, equivalent to daily glycemic mean values close to 150-160 mg/dL^{25,26}. However, studies in people with fasting glycemia or normal glucose tolerance showed that those in the upper quartile had higher morbidity and mortality risk for cardiovascular disease^{27,28}, suggesting that different tissues or systems would have a different glucose sensitivity for the development of complications.

The duration of hyperglycemia conditions the characteristics of its toxic effect. Transitory hyperglycemias produce reversible alterations; in chronic hyperglycemia, however,

Figure 2. Processes resulting from postprandial hyperglycemia. Postprandial hyperglycemia generates oxidative stress through different mechanisms, which in turn produce alterations. Chronic hyperglycemia adds AGE synthesis to the mentioned processes.



changes produced by the incipient addition of glucose to protein molecules (*see Initial or early glycosylation products or early glycation*) will form complex advanced glycoxidation products, known as *Advanced Glycation End Products* (AGE), that are no longer reversible.

The presence of both types of hyperglycemia has therefore additive effects (Figure 1), so that treatment of people with diabetes or IGT should tend to reduce hyperglycemia and postprandial hyperglycemic excursions.

Hyperglycemia-Induced Mechanisms

As already mentioned, oxidative stress production, changes in metabolic pathways, and hyperglycemia-induced non-enzymatic glycosylation of proteins are simultaneous and interdependent mechanisms; each will be considered separately and then integrated to the other ones.

Hyperglycemia and Oxidative Stress

Oxidative stress is the imbalance between the production of oxidative products and antioxidant defenses. Highly reactive and oxidative substances derived from oxygen are called reactive oxygen species (ROS). However, oxidative stress is also produced by nitrogen-(nitric oxide [NO] and peroxynitrites) and chloride-(hypochloric acid and atomic chloride) derived products, being the latter characteristic of the immune response.

In the presence of an excess production of ROS, oxidative stress is related with the progression or development of different pathologic processes such as cancer, rheumatoid arthritis, Alzheimer disease and atherosclerosis.

Recent clinical and experimental studies have shown that chronic hyperglycemia alters the oxidative balance increasing ROS production, which play a key role in the initiation, development and perpetuation of diabetes complications²⁹⁻³¹. Since oxidative stress appears at early stages of the disease before the onset of complications, it would be the cause rather than a consequence of complications. The actual consensus is that hyperglycemia – either mild, chronic or fluctuating

– is a pro-oxidative condition. However, its effect does not manifest in the same manner or intensity at different organs and tissues, probably due to a different sensitivity or defense capacity. The individual genetic load would also act as a conditioning factor of the response to ROS injury.

Free Radicals and Oxidative Stress

Free radicals are molecules or atoms with an unpaired electron at their outer electron layer; consequently, they have a high tendency to interact with other molecules to obtain the missing electron, thus resulting in electron-acceptor oxidative molecules. In the reaction, the free radical disappears and a new molecule is generated – another free radical – giving rise to a dangerous chain reaction. In cells, polyunsaturated fatty acids (PUFAs) of cell membranes or lipoproteins, proteins or nucleic acids are target molecules of these oxidative reactions. These alterations produce structural damage, genetic alterations, and even cellular death.

In the process of respiration of aerobic cells, oxygen is reduced by the combination with hydrogen ions and electrons, forming water. However, this process may also derive in the production of intermediate ROS: the superoxide ($O_2^{\bullet-}$) and peroxide anion (these form hydrogen peroxide), and the hydroxyl radical.

In normal cell metabolism, a certain amount of ROS is produced. Superoxide anion and hydrogen peroxide are formed by enzymatic processes in the mitochondria and peroxisomes from cells involved in the inflammatory response, such as neutrophils, eosinophils, and macrophages. They are also produced by reactions catalyzed by xanthine oxidase, cyclooxygenase, and NADPH oxidase, among others.

The action and level of ROS in cells and tissues are controlled by an antioxidant system formed by specific enzymes and antioxidant products. The enzymatic system includes superoxide-dismutases (SODs), catalase and the reduced glutathione – glutathione peroxidase system (GSH-GP). SODs are metalloenzymes that eliminate superoxides converting them into hydrogen peroxide and molecular oxygen. Catalase destroys hydrogen peroxide and the (GSH-GP) system detoxifies hydrogen peroxides and organic peroxides.

Vitamins C, A, E, K and reducing agents such as cystein, glutathione, methionine, uric acid, bilirubin, ubiquinone and some aminoacids are antioxidant endproducts that in general destroy ROS and are destroyed in the neutralization process.

Oxidative damage modifies the structure and function of proteins through a process associated to non-enzymatic glycation. Unsaturated fatty acids (UFAs) of different lipids are transformed into peroxides, and bases of nucleic acids are modified by a reaction with the hydroxyl radical. For instance, the existence of increased guanosine oxidative products in urine correlating with HbA_{1c} levels have been reported in people with type 2 diabetes.

Oxidative stress can neutralize the vasodilator effect of NO produced by endothelial cells. This gas, which is also a free radical, rapidly reacts with the superoxide radical to produce the peroxynitrite anion (ONOO⁻). ONOO⁻ produces nitrated compounds reacting with tyrosine residues from proteins, modifying their structure and function. It has been shown that early stages of vascular damage are related with the increased adhesion of monocytes to vascular endothelium and their trans-endothelial migration produced by ROS.

In diabetes, there is not only oxidative damage as a result of increased ROS, but also decreased antioxidant defenses³⁰.

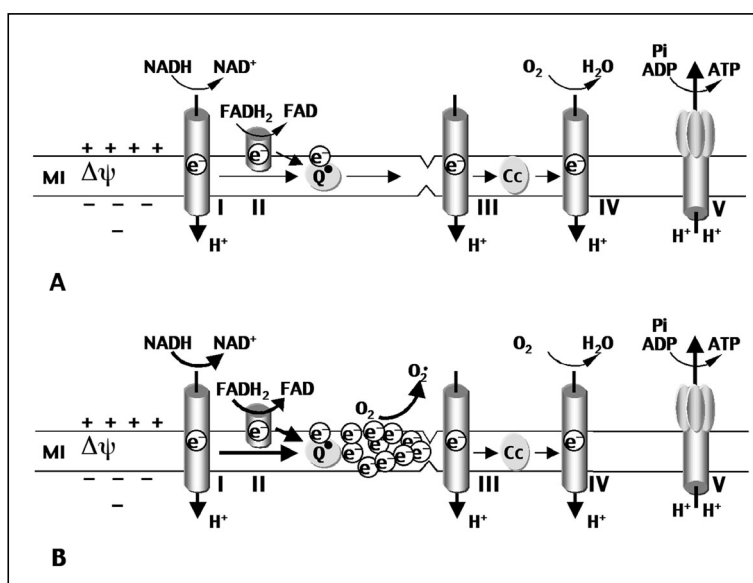
Overload of Mitochondrial Metabolism

One of the mechanisms of hyperglycemia-induced oxidative stress takes place in the mitochondria during cellular respiration. As shown in Figure 3, the final oxidation of reduced coenzymes NADH and FADH₂ produced in the cytosol and in the tricarboxylic acid cycle (Krebs cycle) occurs in mitochondria from different energetic substrates (mainly glucose and fatty acids), and is aimed at storing the energy contained in chemical links as ATP.

The oxidative process is produced by four main membrane-fixed enzymatic processes and the movable intermediary ubiquinone (coenzyme Q); it consists in reoxidizing coenzymes to reutilize them, and transporting H⁺ and electrons towards O₂ to form H₂O. The intensity in the transference of electrons to O₂ through this respiratory chain depends on the electric potential generated by the transport of protons from inside to outside the mitochondrial membrane. This difference in potential provides energy to generate ATP.

When there is a higher affluence of metabolic substrates (glucose and/or free fatty acids [FFA]), the difference in potential between both sides of the membrane increases as a result of the higher availability of reduced coenzymes. Thus, as shown in Figure 3, when a critical value is surpassed, the transport of electrons is partially inhibited at the level of complex III, causing its accumulation

Figure 3. A, Normal condition where electrons provided by metabolized substrates are transported through the respiratory chain to O₂; the simultaneous transport of protons outside the mitochondria inner membrane causes a difference in the potential; this energetic gradient is used by the complex V (ATP sintase) to store energy in the form of ATP. B, Excess reduced coenzymes (NADH and FADH₂) lead to accumulation of electrons in ubiquinone (Q), increasing the transference of one electron at a time to O₂ with the consequent synthesis of superoxide radical (O₂⁻).



in coenzyme Q. The excess electrons are transferred to O_2 , increasing the synthesis of the superoxide radical ($O_2^{\bullet}$).

It has been recently suggested that glucotoxicity and lipotoxicity would be mediated by oxidative stress generated at mitochondrial level³²⁻³⁴; therefore, the pharmacological inhibition of the overproduction of $O_2^{\bullet}$ radicals in the mitochondria would decrease them^{15,35}. On the other hand, $O_2^{\bullet}$ overproduction could represent the common and integrative process to explain most hyperglycemic harmful effects on micro- and macrovasculature.

Hyperglycemia and Impaired Metabolic Pathways

Production of peroxynitrite-nitration of proteins: Figure 4 shows a series of reactions in the production of the vasodilator NO, activated by the hyperglycemia-generated superoxide radical, which mainly impact on the function of vascular endothelia:

- Whereas NO production is decreased, inhibiting endothelium sintase (eNOS), it is increased indirectly through the activation of nuclear factor kappa B (NF- κ B),

triggering the activity of inducible nitric oxide sintase (i-NOS). The result is a net increase of NO. NF- κ B also increases oxidative stress through the activation of i-NOS and NADPH oxidase³⁵⁻³⁷.

- NO contributes to re-feed its synthesis by activating protein kinase C isoforms (PKC), leading to higher synthesis and activity of NADPH oxidase³⁸.

The final balance of the mentioned processes is the simultaneous increase of NO and radical $O_2^{\bullet}$. NO oxidation by radical $O_2^{\bullet}$ creates a new free radical, the ion peroxynitrite ($ONOO^{\bullet}$). This highly oxidative end-product has cytotoxic effects, namely, it gives rise to lipid peroxidation, oxidizes sulphhydryl groups of proteins, and produces the nitration of tyrosine residues from proteins, generating nitrotyrosines. The increase of these nitrated proteins, and therefore of the peroxynitrite precursor, is associated with hyperglycemic states.

Increased nitrotyrosines during the hyperglycemic clamp have been observed in normal subjects, in people with uncompensated type 2 diabetes, and in diabetic patients during postprandial hyperglycemia and hypertriglyceridemia^{36,39,40}.

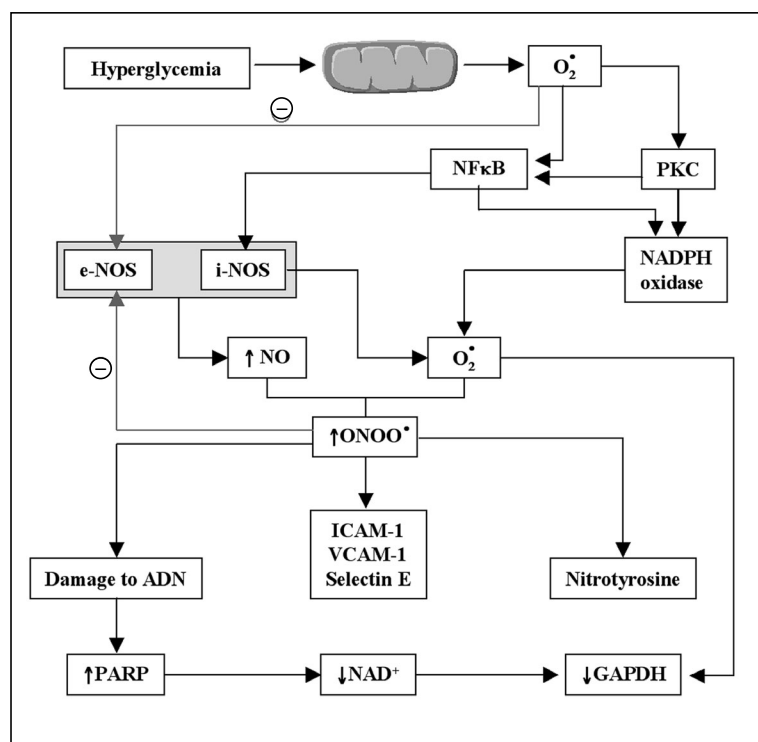


Figure 4. Hyperglycemia leads to the overproduction of superoxide radical ($O_2^{\bullet}$), which is further increased by activation of nuclear factor (NF- κ B), protein kinase C (PKC) and inducible nitric oxide sintetase (i-NOS). Even with the partial inhibition of e-NOS, NO increases and is rapidly transformed into peroxynitrite ($ONOO^{\bullet}$). The latter produces oxidative damage to DNA, generates nitrated proteins and induces leukocyte migration through the vascular wall and vasoconstriction. The symbol  means inhibition.

In people with diabetes, it has been specifically shown that postprandial hyperglycemia and hypertriglyceridemia increase the plasma levels of nitrotyrosine, selectin E and intracellular adhesion molecule (ICAM-1) via an independent and cumulative effect¹⁵. These observations associate the oxidative stress produced by postprandial hyperglycemia with endothelial dysfunction and higher risk for atherosclerosis, both present in people with diabetes.

Activation of poly(ADP-ribose)polymerase and decrease of cellular NAD^+ . Another well-known factor associated with hyperglycemia is the activation of poly (ADP-ribose) polymerase (PARP) by the free radicals superoxide and peroxynitrite as well as their pathogenic role in the development of endothelial dysfunction in diabetes⁴¹⁻⁴³.

PARP is a nuclear enzyme in charge of repairing oxidative damage in the structure of the DNA molecule. Coenzyme NAD^+ is used as a cofactor for this process, thus lowering its cellular content. Consequently, NAD^+ -dependent stages, such as cell respiration, ATP production and glycolysis are weakened, with a concomitant accumulation of glyceralde-

hyde 3-phosphate (GAD-3-P) and other upstream intermediaries of this last metabolic pathway.

Therefore, postprandial hyperglycemia would contribute to stimulate PARP activity in diabetes due to the generation of oxidative stress.

In diabetic animal models with a marked endothelial dysfunction, the pharmacological inhibition of PARP rapidly reverts such dysfunction while restoring ATP, NAD^+ and NADPH values to normal in vascular tissues⁴².

Reversible alterations produced by the partial inhibition of glyceraldehyde-phosphate dehydrogenase (GAPDH). As depicted in Figure 5, intracellular oxidation of glucose starts at the cytoplasm, generating NADH and pyruvate. Cytoplasmic NADH yields reducing equivalents to the mitochondria electron-transporting chain through two launching systems, or is used to reduce pyruvate to lactate coming out of the cell as substrate for hepatic gluconeogenesis. Pyruvate also enters the mitochondria where it is oxidized in the Krebs cycle, producing NADH and $FADH_2$. These two cofactors provide energy for ATP pro-

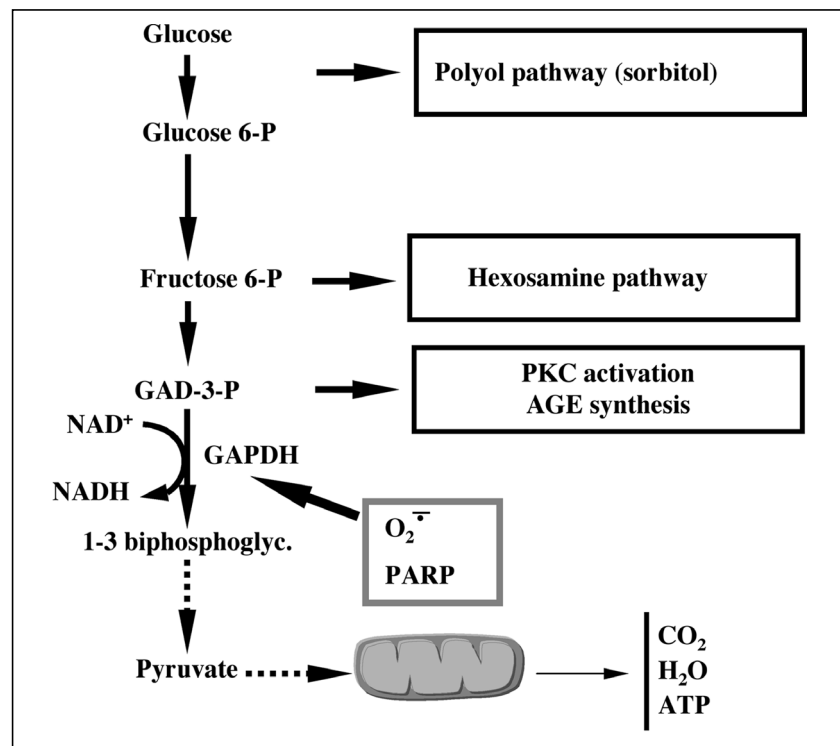


Figure 5. Alteration of metabolic fluxes produced by hyperglycemia.

duction when they enter to the electron-transporting chain through oxidative phosphorylation.

In tissues where glucose enters freely (retina, cells from the glomerular mesangium and the vascular endothelium, peripheral nerves and crystalline), hyperglycemia stimulates the mentioned pathways, increases the production of superoxide ion in the mitochondria, and produces a deficit in NAD^+ . The increase of free radicals causes a concomitant PARP activation, as already reported⁴². Both effects inhibit by 60% the activity of GAPDH, NAD^+ -dependent enzyme, thus arresting glycolysis^{32,44}. This produces an excess accumulation of glycolysis intermediaries up to the stage of GAD-3-P formation. Alternative pathways are increased to consume the excess intracellular glucose (Figure 5), namely:

- *Polyol pathway:*

The increased activity of the metabolic polyol pathway or sorbitol-fructose pathway alters the balance of the NADPH-NADH/NAD^+ system, favoring the production of ROS.

During hyperglycemia, there is a higher activity of the polyol pathway in non-insulin dependent tissues such as the crystalline, the retina and peripheral nerves, thus allowing tissues to use the excess intracellular glucose when the maximal phosphorylating capacity of glucokinase is surpassed. The process is initiated by the action of aldose reductase, that reduces glucose to sorbitol which is later converted to fructose *via* sorbitol deshydrogenase. However, the pathway simultaneously originates oxidative stress, consuming NADPH and reduced glutathione (GSH). This is a potent antioxidant which cannot be regenerated by glutathione reductase, that needs NADPH as cofactor. Since the antioxidant capacity is reduced, the accumulation of ROS is favored.

NADPH deficit also decreases NO synthesis by nitric oxide synthase, resulting in a deficient relaxation of vascular smooth muscle.

On the other hand, since aldose reductase belongs to the family of aldo-ketoreductases, it can reduce the aldehyde and carbonyl groups of glyoxal and methylglyoxal, thus decreasing carbonylic stress. This beneficial effect would be important because in the post-prandial periods dicarbonylic compounds

(with high capacity to glycate proteins) increase in parallel with hyperglycemic excursions⁴⁵.

Recent experimental evidence supports the effects of the polyol pathway during post-prandial hyperglycemic periods. It has been reported that in an animal model with type 2 diabetes, pharmacological control of post-prandial hyperglycemias attenuated the neuropathic manifestations by decreasing the sorbitol and 3-desoxyglucosone content in the sciatic nerve⁴⁶.

- *Hexosamine pathway:*

Excess fructose-6-phosphate (Figure 5) is derived to the hexosamine pathway and promotes a decrease in fibrinolysis by increasing the synthesis of the inhibitor of plasminogen activator (PAI-1). It also increases the production of transforming factors $\text{TGF-}\alpha$ and $\text{TGF-}\beta$ ^{32,44}. The increase of these cytokines stimulates the synthesis of mesangium matrix components, such as heparan sulphate-proteoglycans and fibronectin. On the other hand, the hexosamine pathway plays a key role in the development of hyperglycemia- or FFA-induced insulin resistance since its activation impairs the entrance of glucose to cells and its phosphorylation. This fact was demonstrated in adipocytes which recover insulin sensitivity when the pathway is inhibited. Infusion of FFA to rats induces insulin resistance in muscle due to an increase in the flux of fructose-6-P to the hexosamine pathway. Increments in this pathway also decrease e-NOS activity, thus reducing NO synthesis. Therefore, activation of the hexosamine pathway by hyperglycemia would induce marked changes both in gene expression and in protein function, contributing in this way to the development of complications.

Accumulation of triosphosphates (GAD-3-P and di-hydroxy acetone phosphate [DHAP]) also favors the development of complications. The increment of DHAP increases the synthesis of diacylglycerol (DAG) which in turn stimulates the translocation and activation of PKC isoforms. PKC activation contributes to a higher synthesis of fibronectin and Type IV collagen, increasing the thickness and therefore modifying the function of basal membranes^{32,47,48}.

The increment of GAD-3-P increases the glycation of intracellular proteins.

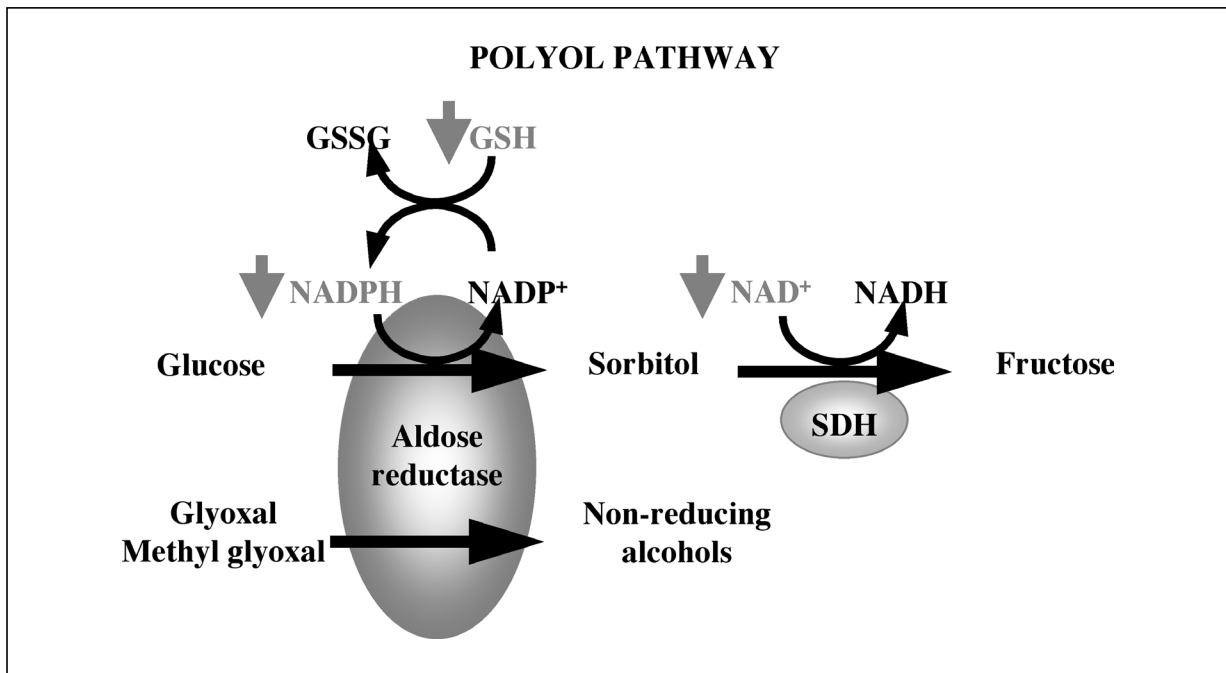


Figure 6. Effects of the polyol pathway (sorbitol). A) production of oxidative stress to consume GSH and NADPH. B) decrease of carbonylic stress to metabolize glycating dicarbonylic compounds such as glyoxal and methyl glyoxal. SDH: sorbitol dehydrogenase.

Early Glycation, Autoxidation and Carbonylic Stress

One of the main pathophysiological consequences of hyperglycemia is the increased interaction of glucose with proteins. This process – “non enzymatic glycation” – takes place without the participation of enzymes and has different stages. Its development is exclusively conditioned by the protein, the

concentration of carbohydrate, and the time of contact between them; the latter conditions the endproducts of the process^{49,50}. Protein glycation is a complex and important pathophysiological process since it modifies the structure, function and biological activity of proteins. It can be divided into two main steps: initiation, an early and reversible stage, and an advanced irreversible one (Figure 7).

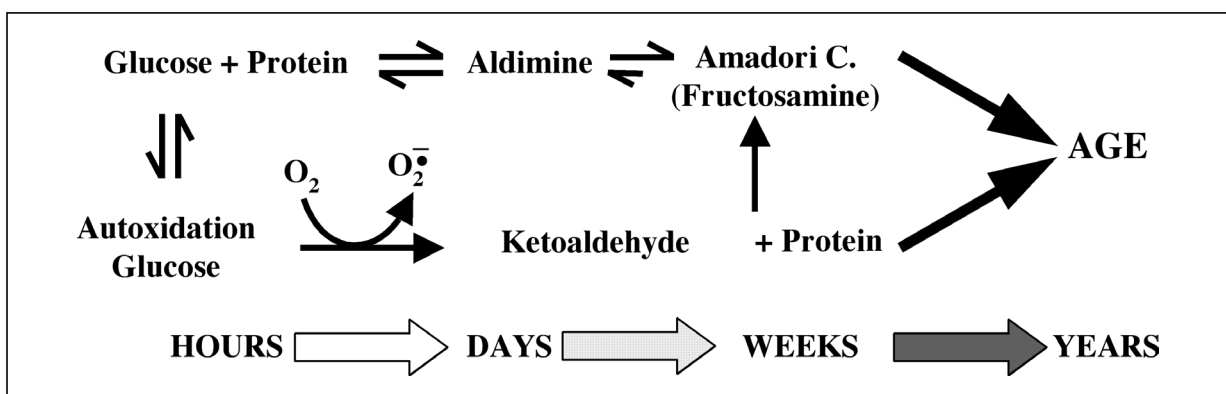


Figure 7. Mechanism of formation of glycated proteins: aldimines and Amadori compounds (also called fructosamines) are early glycation products. Complex transformations (Maillard reactions) of the latter lead to the synthesis of AGE. The example is given with glucose, but the reaction can be initiated with different sugars or products derived from its intracellular metabolism (see text for a more detailed description).

Non enzymatic glycation reactions begin with a condensation between the aldehyde or carbonyl group of a monosaccharide (aldose or ketose) and free amine groups of proteins, lipoprotein phospholipids or nucleic acid bases. This bimolecular condensation constitutes a mechanism by which the protein or the nucleic acid suffer a post-ribosomal modification without the involvement of enzymes, thus giving the name to the *reaction*.

The loss of a water molecule originates a labile aldimine or Schiff base product. This unstable intermediate aldimine turns into a stable ketoamine by an internal rearrangement of the molecule, called Amadori reaction. Ketoamines are generically known as Amadori compounds (AC) or fructosamines. The aldimines thus formed are labile and disappear when the glucose concentration decreases; however, they progress to fructosamines with temporary or chronic hyperglycemias since the balance of the reaction – which is established in about 4 weeks – is shifted to form AC.

Glycation can also be initiated by ketoaldehydes originated by glucose autooxidation and catalyzed *in vivo* by small amounts of metals such as iron and copper, which are present in body fluids. At the same time, O_2 radicals are generated^{51,52}. Ketoaldehydes have two carbonyl groups in their structure ($C=O$) in adjacent position in the molecule, thus conferring them a great reaction capacity with protein's free amine groups. Glyoxal and methyl glyoxal are similar compounds originated from trioses in intracellular fluids and 3-desoxyglucosone, formed by AC decomposition. All these constitute the so called "carbonylic stress" produced by hyperglycemia⁵³; the name refers to the high glycation capacity with the simultaneous production of oxidative stress⁵². In brief, all these products participate in the "glycooxidation" process, involving the simultaneous development of metabolic and oxidative stress.

On the other hand, peroxidation of lipoprotein-PUFAs also produces carbonylic compounds that increase the mentioned stress (see below).

Not all free amine groups of a given protein present the same reactivity to sugars, which have a different glycation capacity depending on their being aldoses or ketoses and on the proportion of open or ring molecular

forms. The velocity with which glucose forms aldimines is lower than that of other sugars due to the low proportion of glucose as free aldehyde form; however, endproducts produced by intracellular glucose utilization, such as GAD-3-P, have a 200-fold higher glycation capacity than glucose. Although extracellular glycation is glucose-dependent, intracellular glycation produced by compounds formed during glucose metabolism is gaining increasing importance.

The amount of glycated hemoglobin (AC of hemoglobin) in blood allows to estimate the retrospective and overall glycemic levels for a 6-8 week-period, and provides clinicians a reliable and useful parameter to evaluate the degree of metabolic control achieved. However, while its value correlates with daily glycemic values, it does not strictly reflect acute changes, such as postprandial glycemic excursions, as already mentioned.

In summary, and according to the kinetics of the non enzymatic glycation process, the formation of early glycation endproducts and the development of oxidative stress are more relevant during postprandial hyperglycemia.

Toxicity of Postprandial Dyslipidemia

In people with diabetes or insulin resistance, the incidence of dyslipidemia is high and lipoprotein abnormalities play a key role in the development of atherosclerotic vascular complications.

Most studies reporting lipoprotein abnormalities and cardiovascular risk have been performed under fasting conditions. Under such circumstances, type 2 diabetes and insulin resistance states are characterized by high levels of plasma TG and very low density lipoproteins (VLDL), decreased levels of cholesterol (Chol) of high density lipoproteins (HDL-chol), and predominance of small, dense low-density lipoproteins (small, dense LDL)⁵⁴⁻⁵⁶.

However, considering a person's normal eating habits, a great part of the day is in the postprandial state that, according to available evidence, would contribute to the development of atherosclerosis⁵⁷⁻⁵⁹. In people with diabetes, the concomitant increase of postprandial glucose and TG magnifies the phenome-

nom⁶⁰. The mechanisms through which excess postprandial levels of lipoproteins and glucose favor the development of vascular disease are diverse and complex, and their effects are combined. We will refer to these mechanisms with special reference to type 2 diabetes, due to the higher incidence of dyslipidemias and macrovascular complications in this type of diabetes.

Postprandial Lipoprotein Metabolism in Diabetes

Increased postprandial lipidemia is a characteristic aspect of diabetic dyslipidemia; it has a high prevalence among people with diabetes even when they have normal fasting TG levels. In the postprandial state, the metabolism of TG-rich lipoproteins is highly altered. There is a significant increase in the concentration and time of permanence of chylomicron remnants, VLDL and VLDL remnants (also called IDL) in plasma, as well as of TG. Therefore, the metabolism of the remaining lipoproteins (LDL and HDL) is also affected due to their interrelation, which involves a permanent lipid and apoprotein interchange between them. Measurement of postprandial lipidemia rather than of fasting plasma TG would be a good atherogenic indicator, since postprandial lipoprotein abnormalities are more evident and occur earlier than those in the fasting state. During this phase, and as a result of the massive entrance of nutrients, enzymatic systems are subjected to a higher demand. This occurs together with structural changes produced in lipoproteins by glycoxidative-carbonylic stress, characteristic of the diabetic postprandial state. Another important conditioning element of those alterations is the presence of insulin resistance and its relationship with insulin circulating levels. Insulin plays a key role in the control of lipid and lipoprotein metabolism because of its effect on enzyme and lipoprotein receptor activity⁶¹⁻⁶³. According to the magnitude and duration of postprandial abnormalities, their effects are prolonged in time up to the fasting state, and are detected in laboratory tests performed in that period.

VLDL is the lipoprotein fraction most affected by diabetes in the postprandial period since a greater hepatic synthesis of VLDL is produced to transport endogenous TG to adipose tissue. VLDL plasmatic level depends

on two processes: hepatic synthesis and extrahepatic clearance, mainly in adipose tissue. In the diabetes state, both processes appear altered^{61,64,65}.

On the one hand, there is an increase in hepatic VLDL production with respect to normal hepatic production; it manifests mainly at the VLDL₁ fraction (larger and with higher TG content) related to a higher provision of substrates, FFA and glucose, consecutive to alterations in insulin secretion and action (Figure 8). The early loss of the first phase of insulin secretion in people with diabetes and the presence of insulin resistance, reduce the hormone suppression of hepatic glucose production and its peripheral utilization, and also decrease its inhibitory effect on FFA release by adipose tissue. Consequently, there is a simultaneous higher provision of glucose and FFA to the liver capable of originating FFA by lipogenesis, a process that in obese patients is favored by an increased caloric intake.

On the other hand, extrahepatic VLDL clearance is decreased. Plasma removal of TG-lipoproteins depends on the action of lipoprotein lipase (LPL) of adipose tissue, an insulin-dependent enzyme that degrades chylomicrons and VLDL by hydrolysis of most TG, turning them into remnants, Chol-rich particles of smaller size. Both chylomicrons and VLDL compete for this common removal mechanism. In normal people, LPL reaches its maximal activity in the postprandial period, when the sudden increase of chylomicrons and VLDL saturates its lipolytic capacity for hours. In people with type 2 diabetes, the activity of the enzyme is diminished and the clearance of TG-lipoproteins is reduced. The greater affinity of LPL for chylomicrons and the hepatic overproduction of VLDL, result in a hyperlipidemia with a higher proportion of VLDL.

In addition to these lipoproteins, there is an increased amount of circulating remnants. The contribution of remnants to postprandial hyperlipidemia is partly due to alterations in their interaction with hepatic receptors involved in their catabolism, related to changes in ligand composition. As opposed to what occurs with chylomicrons and large VLDL, chylomicron remnants and VLDL remnants (IDL) can enter into arterial tissue and accumulate in the subendothelial space^{66,67}.

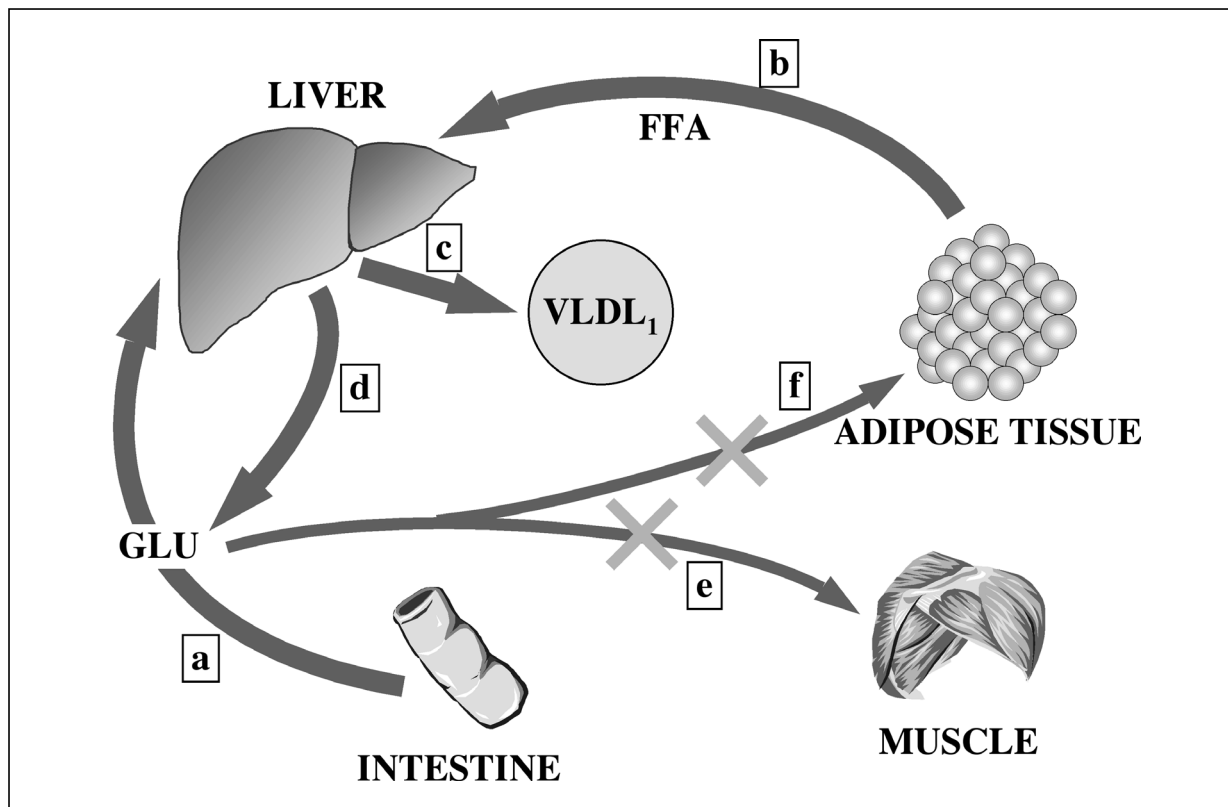


Figure 8. Processes inducing a higher release of VLDL₁ by liver (c) in the diabetic postprandial period or insulin resistant state. (a) ↑ caloric intake (b) ↑ FFA release by adipose tissue (d) ↑ hepatic glucose production (e) and (f) ↓ glucose uptake by muscle and adipose tissue.

Another important aspect in the postprandial state is the interchange of neutral lipids that depends on the cholesteryl-ester transfer protein (CETP). This is one of the factors conditioning postprandial lipoprotein composition, an effect which is later reflected in the fasting state (Figure 9)^{65,68,69}. CETP is involved in the molecular interchange of cholesterol esters (CE) by TG between Chol-rich lipoproteins (mainly HDL and LDL) and TG-lipoproteins (mainly VLDL and chylomicrons). Under normal conditions, food intake favors such interchange in relation with the massive increment of TG-lipoproteins. In type 2 diabetes and insulin-resistant states with postprandial hypertriglyceridemia, this process is increased and there is a marked transference of CE from HDL and LDL to VLDL and TG in the reverse way. This fact affects adversely the lipoprotein metabolism because it favors the formation of more atherogenic particles and reduces Chol reverse transport: (a) VLDL_{rem} and chylomicron remnants increase their CE content, de-

crease their affinity for hepatic receptors, and become more atherogenic; (b) TG transferred to LDL make them more susceptible to hepatic lipase-mediated hydrolysis, which reduce their size leading to the formation of small, dense LDL; (c) through a similar mechanism, the proportion of HDL₂ – in charge of delivering CE to the liver – is decreased and the proportion of smaller and denser HDL₃ is increased, with the subsequent reduction of Chol reverse transport.

Postprandial Lipoproteins and Atherogenesis

Postprandial hyperlipidemia exerts a relevant effect on the development of diabetic macrovascular complications since the excessive increment of TG and their persistence in the circulation is accompanied by deviations towards a more atherogenic metabolism, with accumulation of remnants and small, dense

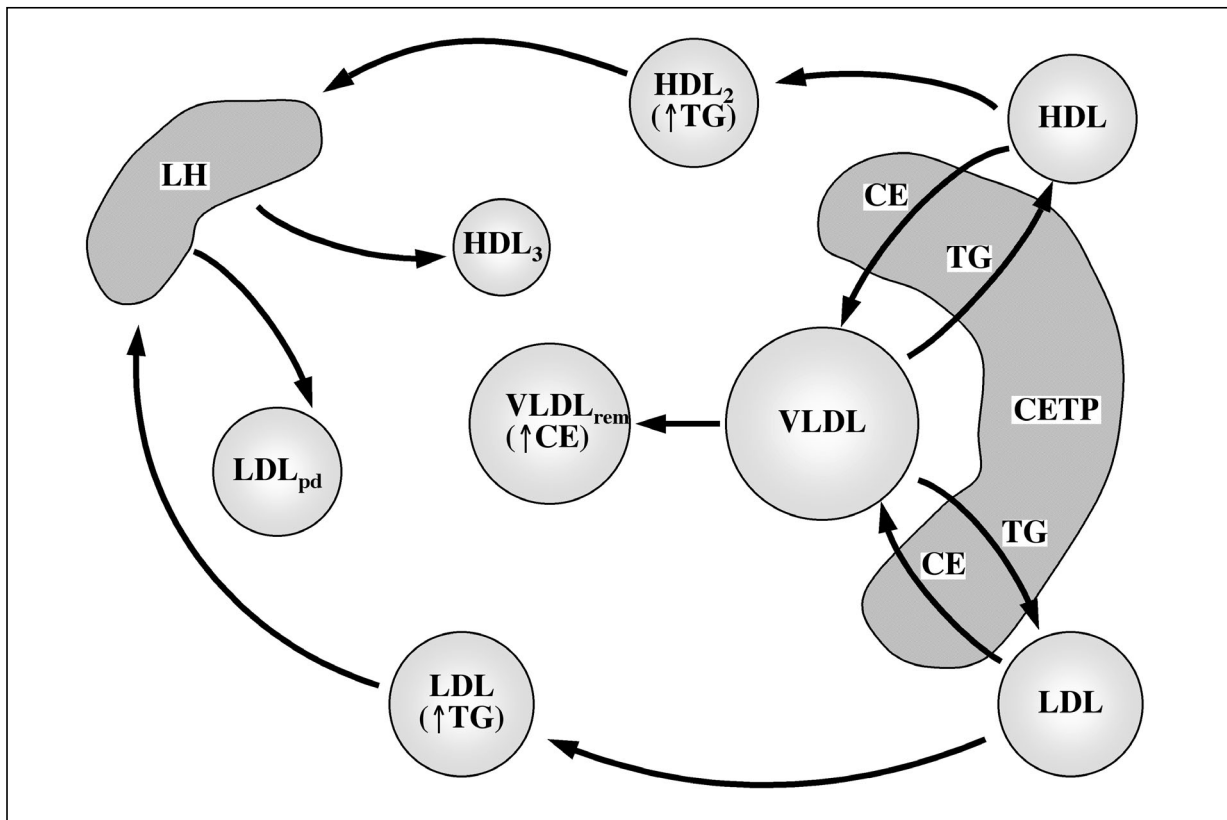


Figure 9. Interchange of neutral lipids in the postprandial diabetic state.

LDL. These are more easily oxidized than large buoyant LDL. Therefore, their predominance is highly atherogenic despite the total LDL level is within the normal range, as it occurs in type 2 diabetes. On the other hand, CE-rich and small chylomicron remnants and VLDL remnants (IDL) can also be trapped in the arterial intima, oxidized and taken up by macrophages, thus initiating the atherosclerotic mechanism.

The atherogenic power of lipoproteins depends on oxidative changes occurring in the arterial intima, in microdomains isolated from plasma antioxidants, and when the lipophylic antioxidants that they transport have been depleted. Lipid oxidation is a key factor in the pathogenesis of atherosclerosis⁷⁰⁻⁷².

Although lipoproteins in plasma are protected against oxidation, there are always small amounts of circulating oxidized lipoproteins containing small amounts of oxidized PUFAs coming from food intake and/or cell membranes or from some inflammatory site of the body. This previous “seed-

ing” stage would facilitate arterial oxidation. The action of intracellular arterial lipoxigenases also enriches with hydroperoxides those LDL or other lipoproteins trapped at the extracellular matrix of proteoglycans from the arterial intima⁷³.

Once lipoproteins have reached a critical level of ROS and the antioxidants they transport have been consumed, oxidation of the particle intrinsic lipids begins. The process occurs through a mechanism of extracellular non enzymatic peroxidation by means of rapid autopropagating reactions. Peroxidation starts in the surface-phospholipids and depends on the type of component fatty acids. The higher the proportion of PUFAs with respect to monounsaturated fatty acids (MUFAs) the easier the oxidation. PUFAs, mainly linoleic and arachidonic acids, are the primary targets of oxidative attack. Oxidation turns PUFAs into free radicals that expand the reaction in an uncontrolled manner. Peroxidation expands rapidly affecting surface phospholipid PUFAs first and then the parti-

cle-core neutral lipid PUFAs, mainly CE. The break of the hydrocarbon chain of phospholipidic hydroperoxides thus formed gives rise to a family of oxidized lipoproteins as well as short-chain carbonylic compounds.

The metabolic effects of oxidized lipoproteins are different from those of original lipoproteins. Many of them act as potent biological mediators capable of inducing gene expression and start an inflammatory response in endothelial cells leading to recruiting, adhesion and differentiation of monocytes to macrophages. Likewise, they stimulate the proliferation of arterial smooth muscle. Initially, the main role granted to LDL in the development of atherosclerosis was foam cell formation, as a result of Chol deposits in macrophages. However, the generation of biologically active molecules derived from oxidized lipoproteins and CE – with proatherogenic properties due to their action on the main cellular types of arterial tissue – has presently gained great importance⁷⁴.

On the other hand, the carbonylic compounds produced (aldehydes and ketones) can form lipoxidation products as a result of their reaction with apoprotein free amine groups. In this stage, apart from lipid peroxidation, profound alterations in the protein structure are produced due to the generated carbonylic stress. ApoB of LDL is no longer

recognized by its LDL-apoprotein B classical receptor (Rec B/E) and is in turn taken up with high affinity by *scavenger* receptors of arterial macrophages, which recognize the new epitopes. At this stage the production of great amounts of ROS by macrophages increases the oxidative power^{70,71}.

Contribution of Postprandial Hyperglycemia to Lipoprotein Peroxidation

In people with diabetes, the already described lipoprotein peroxidation sums to the effect of hyperglycemia. As already mentioned, postprandial hyperglycemia increases the early glycation of proteins and at the same time produces oxidative stress that reduces the antioxidant defenses. The combination of these processes produces changes in lipoprotein structure and metabolism that increase their susceptibility to oxidation. Therefore, the effect of postprandial hyperglycemia together with that of hypertriglyceridemia, favor atherogenesis^{7,15,75-78}.

Lipoprotein glycation is particularly important because it affects not only the protein component (apoprotein) but the lipid component as well. Particularly, lipoproteins containing phosphatidylethanolamine have free amine groups that can react with glucose to form CA and eventually AGE, as opposed to phosphatidylcholine lipoproteins whose qua-

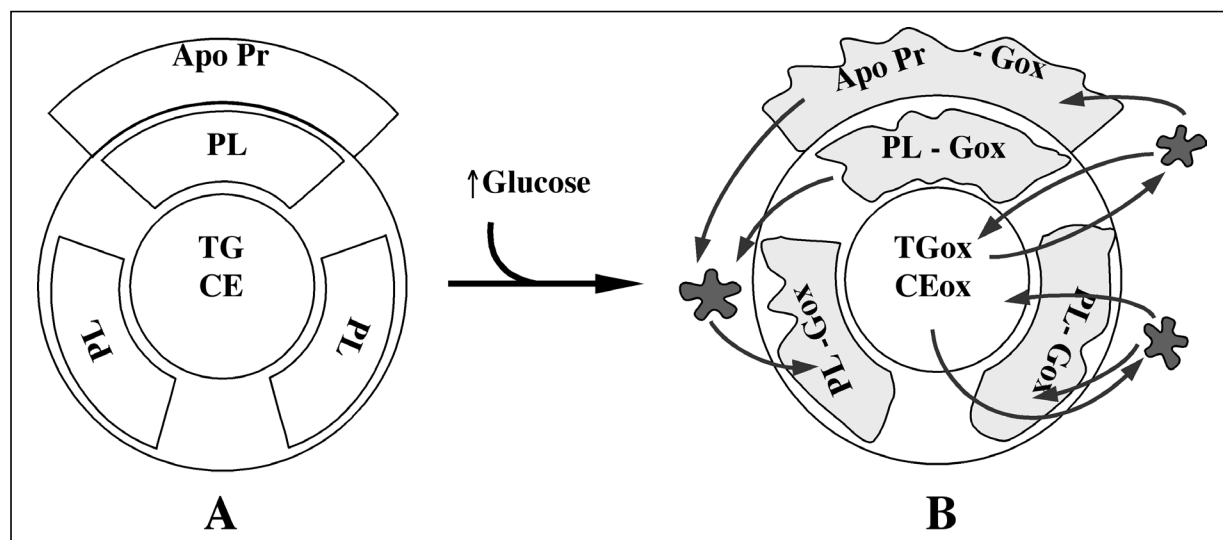


Figure 10. Contribution of hyperglycemia to lipoprotein peroxidation. *A*, Native lipoprotein. *B*, Glycoxidation-modified lipoprotein; ApoPr, apoprotein; PHL, phospholipids of phosphatidylethanolamine; – Gox, glycoxidation-induced changes; ★, ROS

ternary ammonium group cannot form the initial aldimine. In parallel with phosphatidylethanolamine lipoprotein glycation, PUFAs peroxidation is stimulated by the action of ROS produced in glycation reactions. Glycation would facilitate the access of free radicals to the surface adjacent PUFAs, then extending the reaction to the PUFAs inside the particle core^{79,80}.

In summary, as shown in Figure 10, glycoxidation of lipoproteins in diabetes, that occurs on apoproteins and lipoproteins, is interrelated with and stimulates the concomitant lipid peroxidation. The interaction of these mechanisms is facilitated by the coexistence of apoproteins and lipids in the lipoprotein structure. Both processes potentiate each other; ROS generate in increased amounts that enlarge the oxidative damage; carbonylic derivatives that fix to apoproteins are also produced. As a result of glycoxidative-carbonylic stress, the lipoprotein structure suffers profound alterations that modify its behavior and favor a more atherogenic metabolism^{53,81}. Recognition of LDL by their specific receptor (Rec B/E) decreases proportionally to the level of glycation. The interaction with arterial proteoglycans is stimulated, increasing their retention and uptake by macrophages with accumulation of CE in them^{78,81}. Sakata et al.⁷⁸ showed the colocalization of glycoxidation and lipoxidation products in human atherosclerotic lesions and proposed that the synergic action of glycoxidation and peroxidation would promote the development of atherosclerotic lesions. On the other hand, HDL glycoxidation alters its behavior in Chol reverse transport.

Conclusions

Postprandial hyperglycemia and dyslipidemia would synergistically act with chronic hyperglycemia as risk factors for the development of diabetic micro- and macroangiopathic complications. Prospective intervention studies are necessary to prove the possible cause-effect relationship⁷.

In diabetes, enhanced postprandial hyperglycemia and lipoprotein alterations combine their effects to generate a complex glycoxidative-carbonylic stress.

After a mixed food intake, the development in time of hyperglycemia and hyperlipidemia occur sequentially. Postprandial hyperglycemia is a short-term and early phenomenon in which reversible or early glycation prevail and is accompanied by the development of an oxidative stress. These changes are followed by a more prolonged period of hypertriglyceridemia in which lipoprotein dysmetabolism leads to the formation of atherogenic lipoproteins⁷.

Therefore, there is strong evidence to support that control of postprandial hyperglycemia and dyslipidemia as well as control of fasting glycemia and HbA_{1c}, should be considered important treatment targets for the prevention and management of diabetes complications⁸².

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