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Metabolic Syndrome: from the clinical to the molecular

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ABSTRACT

Metabolic syndrome prevalence has increased worldwide. In Mexico, it is 46% according to the criteria of the American Heart Association/ National Heart Blood and Lung Institute, but varies depending on the criteria used for its diagnosis. This syndrome is a well-documented cause of increased cardiovascular diseases like coronary heart disease or stroke as well as cardiovascular mortality. The main physiopathological mechanism involved in its development is the insulin resistance, whose definition seems to be simple, but it implies molecular changes at receptor, pre-receptor and post-receptor levels, caused by certain molecules as free fatty acids or cytokines secreted by adiposse tissue in obese patients. The purpose of this review is to describe current definitions of metabolic syndrome as well as novel aspects of insulin secretion and theories about physiopathologic mechanisms for insulin resistance with special emphasis in molecular aspects. (REV MEX ENDOCRINOL METAB NUTR. 2018;1:21-32)

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Key words: Metabolic syndrome. Obesity. Resistance to insulin.

RESUMEN

La prevalencia del síndrome metabólico ha aumentado en todo el mundo. En México, su prevalencia es del 46% según los criterios de la *American Heart Association/National Heart Blood and Lung Institute*, pero varía según los criterios utilizados para su diagnóstico. Este síndrome es una causa bien documentada de aumento de las enfermedades cardiovasculares como la enfermedad coronaria o el accidente cerebrovascular, así como de la mortalidad cardiovascular. El principal mecanismo fisiopatológico involucrado en su desarrollo es la resistencia a la insulina, cuya definición parece ser simple, pero implica cambios moleculares en los niveles de receptor, prerreceptor y posreceptor causados por ciertas moléculas como ácidos grasos libres o citocinas secretadas por el tejido adiposo en pacientes obesos. El objetivo de esta revisión es describir las definiciones actuales del síndrome metabólico, así como aspectos novedosos de la secreción de insulina y las teorías sobre los mecanismos fisiopatológicos de la resistencia a la insulina, con especial énfasis en los aspectos moleculares.

Palabras clave: Síndrome metabólico. Obesidad. Resistencia a la insulina.

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INTRODUCTION

In the past 30 years, the incidence of cardiovascular diseases such as ischemic heart disease (IHD) and stroke has increased worldwide. In the U.S.A, they constitute the first and third causes of death in general population[1], and have been associated with a greater prevalence of type 2 diabetes mellitus (T2D), hypertension (HTN) and obesity. In fact, approximately 30% of the population has HTN, 28% lives with prediabetes, 12% has T2D, 34% has overweight, and 33.9% some degree of obesity. In that country, 174 billions dollars were assigned to diabetes-attributed health costs in 2010[2].

In Mexico, according to the National Health and Nutrition Survey 2012 (Encuesta Nacional de Salud y Nutrición 2012), overweight and obesity affect 71% of the population between ages of 20 to 59 years. According to the survey, overweight is more prevalent in men (42.6% vs. 35.5%) meanwhile obesity is more prevalent in women (37.5% vs. 26.9%). Additionally, the prevalence of T2D by previous diagnosis was 9.2% and the prevalence of HTN among those reporting diabetes was 46.9%. Therefore, there are 26 million of people with overweight, 22 million with obesity, 6.4 million with diabetes and 14 million with dyslipidemia[3,4]. This situation is reflected throughout Latin America, where chronic degenerative diseases cause 75% of deaths.

These alarming data are very similar in worldwide population, and this situation has allowed numerous groups of researchers to investigate the association between those cardiovascular risk factors grouped under the term of metabolic syndrome (MS), that share the common physiopathology mechanism of insulin resistance. Because of the high prevalence of this syndrome and its components among general population, first-contact physicians and specialists other than endocrinologist, must be familiarized with its current definition and physiopathology, in order to give prompt diagnosis and reference to integrative treatment by a multidisciplinary team. The purpose of this manuscript is to review some clinical and molecular characteristics of MS with a special emphasis on its inflammatory component.

DEFINITION OF METABOLIC SYNDROME

The MS is a complex entity defined as the association of factors that increase cardiovascular risk: HTN, dyslipidemia (hypertriglyceridemia, high lipoproteins that contain apolipoprotein B [apoB], and hypoalphalipoproteinemia), alteration of glucose metabolism (prediabetes or T2D), and central obesity. Other conditions such as a proinflammatory state, prothrombotic state, non-alcoholic steatohepatitis, hyperuricemia, and obstructive sleep apnea/hypopnea syndrome (OSAHS) have been associated with MS, nevertheless this is not universally accepted. There are different classifications and diagnostic criteria that have been proposed and modified over the years.

In 1988, during a meeting of the American Diabetes Association (ADA), Reaven proposed that glucose intolerance, hyperinsulinemia, an increase of triglycerides in very-low density lipoproteins (VLDL), decrease of cholesterol associated to high-density lipoproteins (HDL-c), and HTN, shared the insulin resistance as physiopathology mechanism, and grouped them under the term of "X Syndrome"[5], and years later substituted this term for MS. Since then, it has been named in many different ways: the "death quartet", insulin resistance syndrome, and dysmetabolic syndrome; also different definitions have been performed by groups such as the World Health Organization (WHO)[6], the European Group for the Study of Insulin Resistance (EGIR)[7], the National Cholesterol Education Program (NCEP)[8], the American Association of Clinical Endocrinology (AACE)[9], the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI)[10], and the International Diabetes Federation (IDF)[11]

One of the first attempts to define MS was done by the WHO in 1998[6]. This organization proposed as central point for its diagnosis the presence of diabetes, glucose intolerance, altered fasting glucose or insulin resistance (defined as glucose uptake under the lower quartile for the population in study, with conditions of hyperinsulinemia and euglycemia) in addition to two of the following criteria:

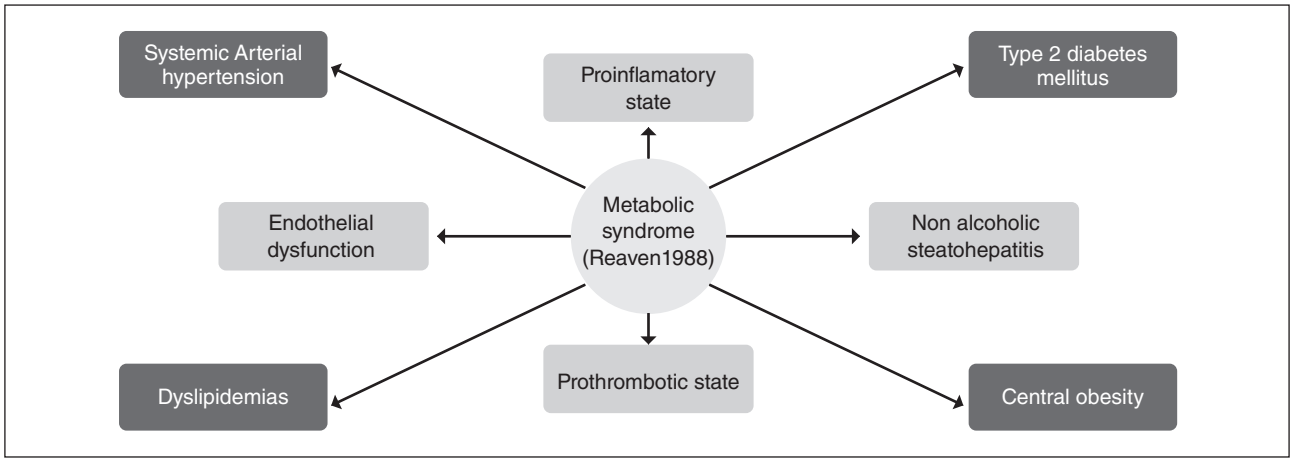

Figure 1. The Metabolic syndrome. In darker gray, the four diseases that serve as the criterion for MS. In light gray, some associated diseases that are not part of the diagnosis.

1) Waist to hip circumference relationship >0.90 in males and >0.85 in females.
2) Serum triglycerides (STG) >1.7 mmol/L (>150 mg/dl) or HDL-c <0.9 mmol/L in males (<35 mg/dl) and <1.0 mmol/L in females (<40 mg/dl).
3) HTN defined as a blood pressure (BP) >140/90 mmHg.
4) Urinary albumin rate >20 µg/min or albumin: creatinine ratio >30 mg/g.

One year later, the EGIR establishes that the main point for diagnosis was fasting hyperinsulinemia (defined as a percentile higher than 75%) and two of the following[7]:

a) Fasting glucose >110 mg/dl, excluding diabetes (<126 mg/dl).
b) BP ≥140/90 mmHg or with treatment for HTN.
c) STG >2 mmol/L or HDL-c <1.0 mmol/L or treatment for dyslipidemia.
d) Waist circumference ≥94 cm in males and ≥80 cm in females.

Those definitions require the determination of insulin resistance or hyperinsulinemia, and that is difficult to evaluate in the first-contact health centers due to the need of a standardized laboratory to determine serum insulin concentration. Considering this situation, in 2001 the NCEP in their Third Report of the Experts Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATPIII]), proposed that MS could be defined with at least three of the following components[8]:

1) Waist circumference >102 cm in males and >88 cm in females.
2) Plasma STG ≥150 mg/dl.
3) HDL-c <40 mg/dl in males and <50 mg/dl in females.
4) BP: systolic ≥130 mmHg or diastolic ≥85 mmHg.
5) Fasting plasma glucose ≥110 mg/d.

In 2003, other pathologies were associated with insulin resistance in an attempt to incorporate them, the AACE proposed a new definition that included the following factors: diagnosis of coronary arterial disease (CAD); HTN; polycystic ovary syndrome (PCOS); non-alcoholic steatohepatitis (NASH); acanthosis nigricans; familial history of DM2; history of gestational diabetes or impaired glucose tolerance (IGT); non-Caucasian ethnicity; sedentary lifestyle; body mass index (BMI) >25 kg/m^2 and/or waist circumference >40 inches (101.6 cm) in males and >35 inches (88.9 cm) in females; age >40 years, in addition to two of the following: STG >150 mg/dl; HDL-c <40 mg/dl in males and <50 mg/dl in females; BP >130/85 mmHg and impaired fasting glucose (IFG) or IGT[9]).

In 2004, AHA/NHLBI define MS in the simplest way and proposed the following criteria[10]:

1) Waist circumference ≥102 cm in males or ≥88 cm in females.
2) STG ≥150 mg/dl.
3) HDL-c <40 mg/dl in males or <50 mg/dl in females.
4) BP >130/85 mmHg.
5) Fasting glucose ≥100 mg/dl.

Later, in 2005 the IDF aims to focus in central obesity, defined with a waist circumference of >94 cm in males and >80 cm in females for groups of Europeans, sub-Saharan Africans, Mediterranean populations, and Arab populations; and a waist circumference of >90 cm in males and >80 cm in females for South Asians, Chinese, Japanese, and Central and South American populations[11]. This parameter has been discussed because of the overdiagnosis generated by these data in countries like Mexico. At this point, Alonso, et al. analyzed 1,036 healthy Mexicans and found a weak association between the waist circumference cut-off points established by IDF and AHA/NHLBI criteria with other metabolic-syndrome risk factors, and established that greater specificity and sensitivity were determined employing measures of >98 cm for males and >84 cm for females, which in turn modified the prevalence of MS[12]). This has been considered and in 2009 the AHA/NHLBI and the IDF published "the Joint Statement criteria", establishing that cut-off points for diagnostic of central obesity with waist circumference, should be based on the epidemiological studies from each country[13].

Rojas, et al. explored the prevalence of MS in Mexican adults older than 20 years using data from ENSANUT 2006 (n = 6021). Using definition of NCEP: ATPIII, AHA/NHLBI and IDF, the prevalence reported was 36.8%, 41.6% and 49.8%, respectively[14]. Additionally, Rojas-Martínez, et al. observed that AHA/NHLBI classification allowed greater diagnosis of MS in comparison with IDF classification (OR 15.0, IC 95%: 11.37-19.92 vs. OR 13.71, IC95%: 9.0-28, respectively)[15].

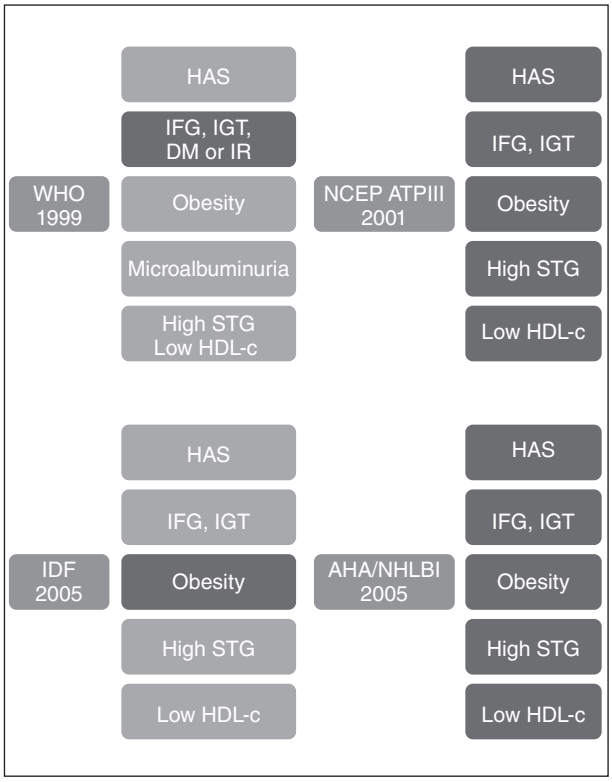

Figure 2. Diagnostic criteria of Metabolic Syndrome. In dark gray its shown the parameter most frequently associated with metabolic syndrome (MS) for each classification. Those classifications represented in dark gray, it implies equal importance for each parameter *(adapted from Romero)*[16].

INSULIN SECRETION AND ACTION

Pancreas is an organ with multiple functions, works as an exocrine gland through secretion of enzymes implicated in digestive processes, as endocrine organ through the secretion of hormones by Langerhans islets and as paracrine and autocrine tissue through regulation of those hormones between them. These islets include alpha cells involved in glucagon secretion, beta cells involved in insulin secretion, delta cells responsible of somatostatin secretion, as well as cells involved with secretion of gastrin, vasoactive intestinal peptide (VIP), and neuropeptide Y.

Insulin is initially synthesized as a preproinsulin (110 amino acids), which enters to endoplasmic reticulum through peptide-conducting channel and is cleaved by signal peptidase, eliminating its amino terminal signal peptide and generating proinsulin.

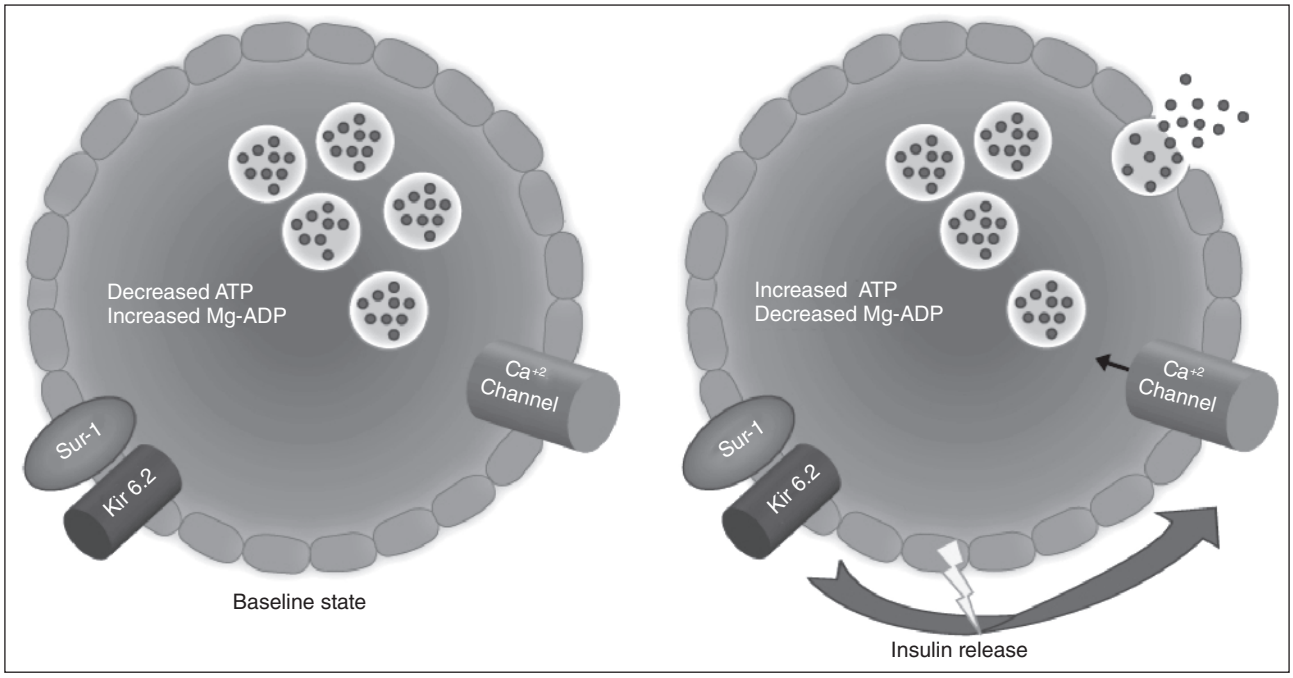

Figure 3. Insulin secretion mechanism. The entry of glucose into the beta cell causes an increase in the numbers of ATP units that inhibit potassium channel activity, induces calcium entry and insulin secretion from the preformed granules. The potassium channel is a hetero-octameric complex of two subunit types: the Sulphonylurea receptor type 1 (SUR-1), and the potassium rectification channel (Kir 6.2). Mutations that cause gain-of-function of SUR-1 result in the lack of closing of the ATP-dependent potassium channel, causing the cell to persist as hyperpolarized and maintaining the calcium channels closed, impeding insulin secretion *(adapted from Aittoniemi, et al.)*[22].

Proinsulin is a molecule structurally homologous to Insulin-like growth factors I and II (IGF-1 and IGF-2), which can bind weakly to the insulin receptor. In a first stage, proinsulin acquires a three dimensional structure subsequent to formation of disulfide bonds. Then, it is transported to Golgi apparatus where is cleaved to form insulin and C-peptide, that are stored together and simultaneously segregated from the secretory granules of beta cells[17]. These granules also store islet amyloid polypeptide (amylin)[18].

Mature insulin is released from β–cells granules in form of hexamers that are dissociated in dimers and then in monomers, which is the active form of insulin. The monomeric form of insulin consists in 51 amino acids grouped in two chains "A" and "B". There are two disulfide linkages between A and B chains and one whiting A chain. The A chain has special importance for receptor binding and it has been proved that certain amino acid residues (specially those disposed in amino terminus) affect affinity for insulin receptor. Meanwhile B chains play an important role in maintaining insulin structure and its carboxy terminus has highly conserved residues related to receptor binding[19].

Glucose is the essential regulator of insulin secretion, although amino acids (as glutamine and leucine)[20], ketones, diverse nutrients, gastrointestinal peptides (as gastric inhibitory polypepetide, GIP or glucaglon-like peptide, GLP-1)[21], and hormones (as estrogens) also exert a secretory influence. Glucose concentrations >70 mg/dl intensify insulin translation and processing. Glucose enters into the beta cell through glucose transporter type 2 (GLUT-2) and it is phosphorylated by glucokinase (an hexokinase) to form glucose 6- phosphate, whose later metabolism by glycolysis pathway generates adenosine triphosphate (ATP) that also close ATP-sensitive potassium channels. The ATP-sensitive potassium channel mainly consists of two proteins: the Sulphonylurea Receptor type 1 subunit (SUR-1), and the structural protein of the K+ rectifying channel (Kir 6.2). Closure of this channel induces depolarization of the membrane which in turn opens

voltage-dependent calcium channels, allowing the entrance of calcium (Ca^{+2}) toward the cell, inducing insulin secretion[22,23].

Insulin is released in two phases. The first or early phase begins at the first minute after glucose stimulus, has a maximum peak of 3-5 minutes, a maximum duration of 10 minutes, and represents the insulin stored in beta-cell granules. The second or late phase initiates at 10 minutes, it has a 4-hour duration and induces a continuous insulin production, in a plateau-like manner with a slow decline. This second phase represents insulin synthesis and release[24].

Physiological secretion of insulin has two main components: 1) baseline secretion, during post-absorptive periods and 2) pulsating secretion stimulated by the food ingestion. The main purpose of this process is utilization and storage of the nutrients acquired from food, as well as the production of energy through ATP synthesis[25]. Baseline secretion of insulin occurs in the absence of exogenous stimulus; it is a pulsating secretion that occurs every 5 to 8 minutes and every 90 to 150 minutes (superimposed ultradiane pulses). Insulin levels range between 0.75 and 1.5 IU/hour (18-36 IU/24 hours), representing 50% of the total insulin secreted in 24 hours[26].

Insulin secretion depends of preformatted granules on β–cells. The 99% of them are from "reserve pool" and only 1% is available for immediate release, and is called "readily releasable pool, RRP". First phase of insulin secretion depends of RRP previously docked to plasma membrane meanwhile second phase depends of recruitment of granules from RP. For this purpose, granules must be modified and translocate through plasma membrane[19]. However, Seino, et al. have proposed that both phases consist on insulin granules located far away from plasma membrane that are recruited after glucose stimulus. Under this new model, RRP granules are rapidly recruited after stimulus meanwhile RP is recruited after modifications on actin network regulated by glucose-evoked signals. This response depends on cAMP and in the case of first phase of secretion it involves two related proteins: Epac2A and Rap1 (in a PKA-independent mechanism), and in case of the second phase depends on PKA. It has also been observed that Epac2A is a direct intracellular target of sulfonylureas[27].

Once in the bloodstream, insulin has two main target organs: adipose and muscle tissue. The insulin receptor is codified in the short arm of chromosome 19, contains two α subunits capable of binding to insulin, and two β subunits that possess tyrosine kinase activity. These units are joined covalently to form a heterotetrameter complex, with a total molecular weight of approximately 460 kDa[28]. Once the insulin binds to the α-subunit, it produces a configurational change in the receptor that causes the auto-phosphorylation of tyrosine residues in the intracellular region of the β–subunits through trans-phosphorylation[29]. In an inactive state, the catalytic site of this kinase is blocked to prevent the access of ATP and other substrates. Once activated, phosphorylation of tyrosine residues at positions 1158, 1162, and 1163 induces a conformational change that allows the ATP to reach the catalytic site. Then, begins the recruitment of intracellular signaling molecules such as Insulin Receptor Substrate 1 and 2 (IRS-1 and -2). IRS-1 is a cytoplasmic protein with a molecular weight of 131 kDa. These substrates are the first proteins that are modified due phosphorylation of insulin receptor and play a critical role in intracellular communication signals. Once IRS-1 is phosphorylated, it binds to Src Homology 2 (SH2) domains in diverse proteins that include Phosphatidyl inositol 3-kinase (PI-3K), Growth factor Receptor binding protein 2 (GRb2), CRk, Nck, and Fyn[30-32].

Grb2 is an adaptor molecule flanked by two Src-Homology 3 (SH3) domains that bind to the sequences that contain proline in mSos, which activates the G protein termed Ras, by means of the phosphorylation of Guanosine triphosphate (GTP)-mediated guanosine. This triggers the activation of a serine-threonine kinase cascade that in turn activates Raf, Mitogen-activated protein/Extracellular signal-regulated Kinase (MEK-1 and -2), and Mitogen-Activated Protein Kinase (MAPK), proteins involved in the cellular growth and regulation of the expression of several genes[33-35].

On the other hand, activation of PI3K (through the phosphorylation of subunits p85 and p110) activates Phosphatidyl inositol-Dependent Kinases 1 and 2 (PDK 1 and -2) that in turn phosphorylate multiple proteins including Akt/Protein kinase B, atypical isoforms of Protein Kinase C (PKC), and Serum/glucocorticoid-activated protein kinases (Sgk). Akt has been associated with the activation of proteins involved in the synthesis of lipids, glycogen, in pathways involved in apoptosis and it appears to be a determinant in the development of insulin resistance. In fact, Akt phosphorylates the Forkhead box O1 (FOXO1), a transcription factor that causes expression of genes related to gluconeogenesis, activates sterol regulatory element binding protein 1C (SREBP-1c), and induces transcription of proliferator-actived receptor gamma (PPARγ), which in turn is responsible for the transcription of Glucose transporter 4 (GLUT-4) that mediates glucose entry into the cell[33,36,37].

INSULIN RESISTANCE

Insulin resistance can be defined as the decreased response to insulin in muscle, hepatic, and adipose tissue. There are several theories that have attempted to explain the mechanism by which insulin resistance is produced. These theories include defects in insulin receptor signaling and mechanisms involving pro-inflammatory cytokines (there are three levels of resistance: at insulin receptor, pre-receptor and post-receptor)[38].

Some studies have proposed that insulin resistance has genetic causes. Thus, certain mutations have been observed in genes that codify for insulin receptor or some of their signaling molecules. In fact, it has been proposed that defects in IRS-2 induce insulin resistance in liver and disturb pancreatic beta cells growth; meanwhile defects in IRS-1 induce muscle-tissue resistance. Alternatively, there are certain cases of insulin resistance due defects in the Akt signaling molecule[39,35].

Other theories propose that insulin resistance is induced by a phosphorylation of serine or threonine residues in the insulin receptor, instead of the habitual phosphorylation of tyrosine residues[40]. Therefore, TNF-α and free fatty acids (FFA) appear to participate in this mechanism of resistance. Consequently, the high amount of fat tissue in people with obesity increases the release of FFA that also induces formation of metabolites such as diacylglycerol, who intervenes through PKC in the phosphorylation of serine and threonine residues.

Toward the end of the 1960s, Randle and collaborators described a cycle that appeared to join the lipids and glucose metabolism[41]. Those authors observed that high serum concentrations of free fatty acids inhibited glucose uptake in rat muscle. They proposed that an increase in free fatty acid oxidation enhance acetyl coenzyme A (acetyl-CoA) concentration and with this, the formation of citrate. A high intracellular citrate concentration also diminishes the activity of the enzymes involved in glycolysis (pyruvate dehydrogenase, phosphofructokinase, and glucokinase), decreasing the need for glucose uptake[35,42,43].

Nowadays, is proposed an "unifying theory", that involves glucose-induced increase in malonyl CoA levels, which serves both as the immediate precursor of the *de novo* lipogenesis, and as an important allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT1), the rate limiting enzyme for transport of long chain acyl CoAs (LC-CoA) into the mitochondria matrix for Beta oxidation[44]. Evidence supports that the impairment of mitochondrial fatty acid oxidation induces accumulation of lipid-derived species into cytosol and this generates hepatic insulin resistance via serine and threonine phosphorylation, as previously mentioned. This phenomena is also observed in muscular tissue, where the oversupply of lipids results in an increased generation and release of LC-CoA species into cytosol with subsequent production of triglycerides, diagylglicerol and ceramide; and in an enhanced fatty acid oxidation owing to transcriptional regulation and increased substrate supply. In the absence of exercise, fatty acid oxidation is not balanced with an increase in tricarboxylic acid cycle activity, and that results in accumulation of lipid derived intermediates in mitochondria, generating mitochondrial stress and finally insulin resistance[44].

INSULIN RESISTANCE AND BETA CELL DYSFUNCTION

Chronic exposure of pancreatic islets to elevated levels of nutrients induces beta cell dysfunction and ultimately triggers its death. It has been observed that exposure of isolated rodent islets to hyperglycemia for several days, initially raises basal insulin secretion, but later abolishes response to glucose. Furthermore, exposure to elevated levels of fatty acids does not impair glucose stimulated insulin secretion unless the islets are cultured above a threshold concentration of glucose (usually 8 mM). These and other findings have led to the concept of beta cell functional impairment as a consequence of "glucolipotoxicity", rather than as a consequence of exposure to every single nutrient[44].

There are a lot of mechanisms that associate glucolipotoxicity with final β–cell damage. In first place, the increased glucose entry to β–cell related with insulin resistance, induces generation of reactive oxygen species (ROS) as well as advanced glycation end products (AGEs), that itself can induce cellular damage. In normal conditions, ROS are cleaved through the action of antioxidant enzymes like superoxide dismutase (SOD), catalase and the glutathione system (peroxidase/reductase), meanwhile AGEs are inhibited by aminoguanidine. However, those mechanisms could be overloaded depending on glucose concentration and once this happens, ROS could affect insulin-receptor signaling cascade that is involved in islet differentiation and β–cell survival. In second place, the increase in glucose entry to β–cell also enhances cytosolic calcium. This metabolite is involved in apoptosis (trough FAS system) and also in signaling cascades related to proinflammatory processes[19]. Finally, high glucose levels in β–cell induce disruption of normal insulin transcription as well as in key enzymes as glucokinase. The role of FFA in insulin resistance has been explained above.

Another mechanism responsible of β–cell failure is the formation of amylin fibrils. As previous mentioned amylin is also synthetized with insulin and C-peptide. Chronic overnutrition increases insulin secretion, but also induce overexpression of human amyilin. It seems that deposition of amyloid fibers is associated with increased rates of beta cell apoptosis and diminished first phase of insulin secretion, ultimately resulting in the onset of glucose intolerance and finally diabetes[44].

ADIPOSE TISSUE, INFLAMMATION AND INSULIN RESISTANCE

Some years ago, adipose tissue was considered only involved in energy storage. Nowadays, it is known that it participates actively in the pathogenesis of insulin resistance as well as in metabolic syndrome-associated comorbidities through the secretion of molecules such as resistin, leptin, proinflammatory cytokines, vasculogenesis modulators, glucocorticoids, etc.

It has been observed that obese subjects with a greater amount of adipose tissue (predominantly in waist) have greater activity of cytokines related with energy homeostasis. These cytokines, grouped under the name of adipokines or adipocytokines, possess autocrine, paracrine, and endocrine effects directly implicated in the physiology of the metabolic syndrome[45-48]. The most important adipokines are:

a) Adiponectin. It is also known as adipoQ, Adypocyte complement-related protein 30 (Acrp30), or apM1. It is a protein exclusively secreted by adipose tissue, with a serum concentration of 5-10 micrograms per mL (10-30 nM). Initially, researches found diminished Acrp30 levels in rats and humans with obesity and diabetes. Later, it was observed that hypoadiponectinemia presented prior to insulin resistance and in animal models, restitution of its levels decreased insulin resistance. Furthermore, it possess anti-inflammatory and antiapoptotic properties[47,45,49].

b) Resistin. It is a protein discovered by Steppan, et al., codified in chromosome 19 and it's a member of the family of Resistin-like molecules (RELM). . Although their functions are not yet precisely understood, its increased concentration in patients with T2D and obesity has been related with insulin resistance in liver and seems to play a role as an immune system regulator[50,51].

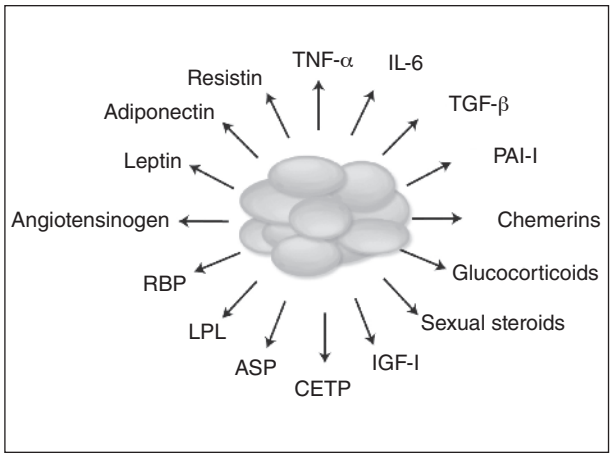

Figure 4. Adipose tissue will no longer been considered as a fat reservoir. Tumor necrosis factor alpha (TNF-α), Interleukin 6 (IL-6), Transforming growth factor beta (TGF-β), Plasminogen activator inhibitor 1 (PAI-1), Insulin-like growth factor 1 (IGF-1), Cholesteryl ester transfer protein (CETP), Lipoprotein lipase (LPL), Retinol Binding Protein (RBP).

c) Tumor necrosis factor-alpha (TNF-α). Initially, TNF-α was described as a proinflammatory cytokine released by macrophages and monocytes as part of the immune response. Soon, its expression and release in the adipocytes was discovered and linked with insulin resistance in rodents and humans with obesity. Previous studies had demonstrated that TNF-α is implicated in several insulin resistance-related processes such as the following[52-54]:

   1) Reduces *GLUT4* gene expression and consequently decrease glucose entry into the cell.

   2) Induces serine phosphorylation in insulin receptor, interrupting normal signaling cascades

   3) Induces down-regulation of IRS-1, altering insulin signaling.

   4) Increases IL-6 and leptin expression and decreases adiponectin expression.

   5) It's involved in adipocyte apoptosis, increasing the concentration of free fatty acids.

d) Interleukin 6 (IL-6). Like TNF-α, IL-6 was initially characterized as a proinflammatory cytokine and its secretion by adipose tissue was not discovered until later. It has been linked with down-regulation of IRS-1, alteration of tyrosine-kinase phosphorylation, and decrease of *GLUT4* expression, as well as increase of other cytokines such as resistin and decrease of adiponectin[55-57].

e) Leptin. It is the product of the *ob* gene expressed principally in subcutaneous adipose tissue and is an important mediator of appetite at hypothalamic level, regulates metabolism, and constitutes a permissive factor for the initiation of puberty. It is associated with insulin resistance, increasing the fat content in insulin-sensitive tissues[58-60].

f) Retinol Binding Protein. Secreted by adipose tissue and the liver, Retinol binding protein 4 (RBP 4) is related with the initial development of insulin resistance. However, there is controversy concerning its association due its levels appear to vary according to race, gender, the presence of renal damage, the iron sufficiency state, and adequate levels of retinol[61,62].

g) Angiotensinogen/PAI 1. Angiotensinogen/Plasma activator inhibitor 1 (PAI 1) regulate blood support to adipose tissue and are associated with the development of endothelial and pro-coagulant alterations in patients with obesity[63,64].

h) Chemerins. Initially, chemerin was known as a chemoattractant factor. Later, its immunomodulator effect was found and it is present at high concentrations in patients with obesity because is involved in adipocyte differentiation. It also acts as an angiogenesis regulator and some investigators suggest that is the link between obesity and the development of cancer[65-67].

i) Other products of adipose tissue. Other products secreted by adipose tissue that intervene in the development of insulin resistance are 11-β–hydroxy steroid dehydrogenase type 1[68] whose expression is increased in certain hyperinsulinemic states and is involved in the activation of cortisone into cortisol with subsequently accumulation of fat at central distribution; enzymes such as Lipoprotein lipase (LPL) and the Cholesterol ester transfer protein (CETP), both involved in lipid metabolism, and glucocorticoids associated with the alteration of carbohydrate metabolism in patients with obesity.

ENDOTHELIAL FUNCTION IN INSULIN RESISTANCE

Endothelial dysfunction is included as one of the factors related with the physiopathology of insulin resistance. The vascular endothelium is a metabolic and endocrine organ that is intensely active through the production of vasoactive hormonal peptides, growth factors, cytokines, and other factors; it regulates the equilibrium between vasoconstriction/vasodilatation, coagulation/fibrinolysis, proliferation/apoptosis, and leukocyte adhesion/diapedesis. It is known that MS is associated with changes in the proliferation of smooth muscle cells and endothelial dysfunction; in addition, hyperinsulinemia alters endothelium-dependent vasodilatation in large arteries, probably because of the increase of oxidative stress[63,69].

There is a clear relationship between insulin sensitivity and endothelial function in normal subjects, subjects with obesity, and in patients with T2D. Studies carried out *in vivo* and *in vitro* have demonstrated that insulin directly possess physiological effects on the vascular endothelium. In this tissue, insulin stimulates the generation of NO in a dose-dependent manner by means of the Phosphatidyl inositol-3 kinase (PI-3K)/Protein kinase B (PKB) pathway. Thus, activation of NOS by insulin in the arteriolar muscle cells depends on an intact PI-3K/Protein kinase B pathway and insulin resistance at this level disrupts insulin-mediated vasodilatation, resulting in endothelial dysfunction. Additionally, it has been found that despite the severe defect of PI-3K/Protein kinase B activation, activation of MAPK or mitogenic pathway by insulin is intact. The stimulation of this pathway results in the proliferation of smooth muscle cells and increased release of growth factors and proinflammatory cytokines as Endothelin 1, critical in the development of atherosclerosis.

The mechanism responsible for the increase of insulin-stimulated NOS activity is the phosphorylation of a serine residue in NOS isoform located at muscle vessels (eNOS), which causes 2-4-times increase in the NO synthesis rate. Hyperglycemia inhibits serine phosphorylation in residue 1177 of eNOS and interferes with insulin signaling by the PI-3K/AKT and IRS pathways.

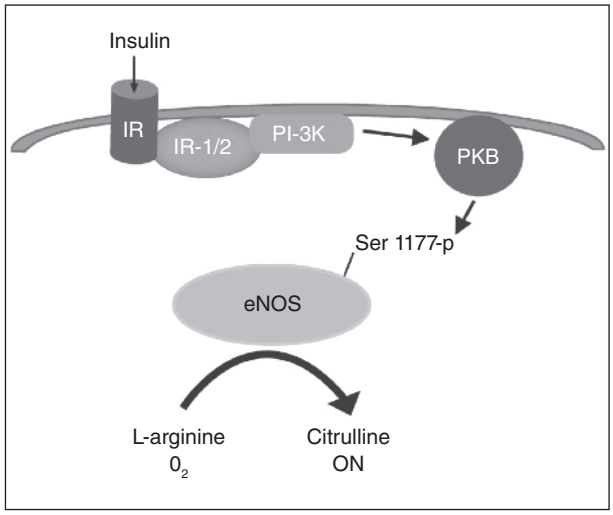

Figure 5. Mechanism of production of insulin-stimulated NO. *(adapted from Ritchie, et al.)*[63] IR: Insulin receptor, IRS-1/2: Insulin receptor substrate 1 or 2, PI-3K: Phosphatidylinositol 3-kinase, PKB: Protein kinase B, eNOS: entohelial nitric oxide synthase.

In addition, evidence has been found that chronic hyperglycemia and alteration of insulin action, leads to an increase in the production of Intercellular adhesion molecules/Vascular cell adhesion molecules (ICAM/VCAM), which contributes to the development of accelerated atherosclerosis.

Another mechanism implicated in the vascular damage and high blood pressure present in individuals with insulin resistance is related with Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS that has been found to be high in the plasma of vascular disease-associated clinical syndromes and could contribute to endothelial dysfunction in patients with insulin resistance.

DEFECTS ON INSULIN SECRETION IN OBESITY AND T2D

We have shown that insulin resistance is the main factor associated with hyperinsulinemia observed in obesity and T2D. However, due to the recent advances in insulin secretion dynamics, it has been

proven that those diseases are also accompanied with alterations in insulin secretion[27]. In fact, a study of patients with moderate obesity observed a decrease in first phase of insulin secretion according with the decrease in glucose tolerance. In obesity, the temporal pattern of insulin secretion and the secretory pulses that occur every 1.5 to 2 hours are preserved, but phases of secretion are enhanced due to an increase in β cell mass. Meanwhile in impaired glucose tolerance, the first phase is affected due to decrease in the size of RRP and defects of the exocytotic process of this granules, and a moderate reduction on granules from RP. Finally in T2D, the first phase of secretion is abolished due to complete loss of RRP and completely impairment of exocytotic process of these granules, meanwhile second phase is also affected due to reduction of RP granules and disturbance of cortical actin network[27].

CONCLUSION

The MS represents a group of diseases mainly characterized by insulin resistance. This syndrome confers an increased cardiovascular risk and therefore must be early identified in order to receive promptly treatment. There are a lot of classifications, but it seems that AHA/NHLBI criteria (also known as Joint Statement Criteria) allowed great diagnosis in Mexican population. However, even those criteria may lead to overdiagnosis due to the lack of cut-off points for waist circumference, which must be obtained from national epidemiological studies.

In this review, we described the physiological secretion of insulin with special emphasis in insulin granules on β–cells. We also describe how certain stimuli affect those granules and alter insulin secretion, a condition widely known as "lose of the first phase". Additionally, we present different models for insulin resistance and how it is caused by cytokines and free fatty acids due to obesity. Finally, we explained the proposed mechanism for hypertension and endothelial dysfunction induced by insulin resistance.

The understanding of these models and mechanisms will lead to further investigation towards prevention and resolution of metabolic syndrome and its comorbidities in our population.

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