Nutrición Hospitalaria

Nutr Hosp. 2015;31(5):1947-1956
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Revisión

Hepatic inflammatory biomarkers and its link with obesity and chronic diseases

Ana Carolina Pinheiro Volp[1], Fernanda Cacilda Santos Silva[2] and Josefina Bressan[3]

[1]Department of Clinical and Social Nutrition, Federal University of Ouro Preto, Ouro Preto. [2]Department of Biological Sciences, Federal University of Ouro Preto, Ouro Preto. [3]Department of Nutrition and Health, Federal University of Viçosa, Viçosa, Brazil.

Abstract

Introduction: **The low-grade inflammation and insulin resistance are two events that could be present in varying degrees, on obesity and chronic diseases. The degree of subclinical inflammation can be gauged by measuring the concentrations of some inflammatory biomarkers, including the hepatic origin ones. Some of those biomarkers are sialic acid, α_1-antitrypsin and the C-terminal fragment of alpha1-antitrypsin, ceruloplasmin, fibrinogen, haptoglobin, homocystein and plasminogen activator inhibitor-1.**

Objectives: **To approach the relation between adiposity and hepatic inflammatory markers, and to assess the possible associations between hepatic inflammatory biomarkers and obesity, as well as their capacity of predicting chronic diseases such as type 2 diabetes and atherotrombotic cardiovascular diseases.**

Methods: **We used electronic scientific databases to select articles without restricting publication year.**

Results: **The sialic acid predicts the chance increase to become type2 diabetic independently of BMI. Moreover, the α_1-antitripsin, ceruloplasmin, fibrinogen and haptoglobulin biomarkers, seem predict the chance increase to become type2 diabetic, dependently, of BMI. So, this process could be aggravated by obesity. The concentrations of fibrinogen, homocystein and PAI-1 increase proportionally to insulin resistance, showing its relation with metabolic syndrome (insulin resistance state) and with type2 diabetes. In relation to cardiovascular diseases, every biomarkers reported in this review seem to increase the risk, becoming useful in add important prognostic.**

Correspondence: Ana Carolina Pinheiro Volp.
Department of Clinical and Social Nutrition – Nutrition School.
Federal University of Ouro Preto, Brazil. Campus Universitário.
Morro do Cruzeiro, s/no. Ouro Preto, Minas Gerais. Brasil.
CEP 35.400-000.
E-mail: anavolp@gmail.com

Recibido: 05-II-2015.
Aceptado: 10-III-2015.

BIOMARCADORES HEPÁTICOS DE INFLAMACIÓN Y SU VÍNCULO CON LA OBESIDAD Y LAS ENFERMEDADES CRÓNICAS

Resumen

Introdución: **El bajo grado de inflamación y la resistencia a la insulina son dos eventos que podrían estar presentes en mayor o menor grado, en la obesidad y las enfermedades crónicas. El grado de inflamación subclínica se puede evaluar por medición de las concentraciones de algunos biomarcadores inflamatorios, incluyendo los de origen hepático. Algunos de estos biomarcadores son el ácido siálico, α1-antitripsina y el fragmento C-terminal de la alfa 1-antitripsina, ceruloplasmina, fibrinógeno, haptoglobina, la homocisteína y el inhibidor-1 del activador del plasminógeno.**

Objetivos: **Evaluar la relación entre la obesidad y los marcadores de inflamación hepática, y las posibles asociaciones entre los biomarcadores inflamatorios hepáticos y la obesidad, así como su capacidad de predicción de las enfermedades crónicas como la diabetes tipo 2 y enfermedades cardiovasculares aterotromboticas.**

Métodos: **Se utilizaron bases científicas electrónicas para selección de artículos, sin límite de año de publicación.**

Resultados: **El ácido siálico predice el aumento de convertirse en diabéticos tipo 2 independientemente del IMC. Por otra parte, los biomarcadores α1-antitripsina, ceruloplasmina, fibrinógeno y haptoglobulina, parecen predecir el aumento de convertirse en diabético tipo 2, dependiente, de IMC. Por lo tanto, este proceso podría verse agravada por la obesidad. Las concentraciones de fibrinógeno, homocisteína y PAI-1 incrementam proporcionalmente a la insulinoresisténcia, mostrando su relación con el síndrome metabólico (estado de resistencia insulínica) y con la diabetes tipo 2. En relación con las enfermedades cardiovasculares, cada biomarcador informado en esta revisión parece aumentar el riesgo, llegando a ser muy útil en el complemento pronóstico.**

Conclusion: **This review integrates the knowledge concerning the possible interactions of inflammatory mediators, in isolation or in conjunction, with obesity and chronic diseases, since these biomarkers play different functions and follow diverse biochemical routes in human body metabolism.**

(*Nutr Hosp*. 2015;31:1947-1956)

DOI:10.3305/nh.2015.31.5.8525

Key words: *Obesity. Insulin resistance. Inflammation. Diabetes. Cardiovascular diseases.*

Conclusion: **Esta revisión se integra el conocimiento acerca de las posibles interacciones de los mediadores inflamatorios, en forma aislada o en combinación, con la obesidad y las enfermedades crónicas, ya que estos biomarcadores desempeñan funciones diferentes y siguen diversas rutas bioquímicas en el metabolismo del cuerpo humano.**

(*Nutr Hosp*. 2015;31:1947-1956)

DOI:10.3305/nh.2015.31.5.8525

Palabras clave: *Obesidad. Resistencia a la insulina. Inflamación. Diabetes. Enfermedades cardiovasculares.*

Abbreviations

AMI: acute myocardial infarction.
BMI: body mass index.
CRP: C-reactive protein.
FFA: free fatty acids.
FFA: free fatty acids.
HbA_{1c}: glycated hemoglobin.
HOMA-IR: homeostatic model assessment-insulin resistance.
HR: hazard ratio.
OR: *odds ratio*.
PAI-1: plasminogen activator inhibitor-1.
QUICKI: quantitative insulin check.
ROS: reactive oxigen species.
RR: relative risk.
S_I: insulinic sensitivity index.
tPA: tecidual plasminogen activator.

Introduction

The reaction of induced inflammation by factors of risk (abdominal obesity, hyperglycemia, dyslipidemias and systemic hypertension) and associated immune response are the principal events which conduct to aterogenetic process[1]. Individuals with these clinic manifestations, generally, show prothrombotic and pro-inflammatory states, characterizated by subclinic imflammatory condition[2-4], a process which could be aggravated by obesity[3,5,6].

Moreover, the insulin resistance has been associated with the increase of plasmatics proteins inflammation sensitive (inflammatory biomarkers)[2-6]. Prospective works corroborate these associations between many inflammatory biomarkers and the diabetes and atherothrombotics cardiovascular diseases incidence[5,6,9].

The adipose tissue has endocrine functions. Addtionally, it has been proposed that pro-inflammatory cytokines formed on it, increase the hepatic synthesis of acute phase protein[5,6,10,11]. However, it remains unknown how the inflammation of low intensity contributes to increase risk for cardiovascular diseases in overweight and obese individuals[12]. This risk could be very different for individuals with similar body mass. In fact, studies show that cardiovascular risk between obese individuals vary depending on the levels of others risk factors associated with obesity[13,14].

This review approaches the relation between adiposity and hepatic inflammatory markers. Moreover, it congregates the knowledge relating to possible interations of these inflammatory mediators with chronic diseases associated to obesity, as well as, demonstrates the capacity of them in predict the risk for diabetes and cardiovascular affections.

Methods

This review was conducted using electronic scientific databases, including Medline, PubMed and SciELO, using the following key words in English, Spanish and Portuguese: inflammation, obesity, cardiovascular diseases, type-2 diabetes, sialic acid, α_1-antitrypsin and the C-terminal fragment of alpha1-antitrypsin, ceruloplasmin, fibrinogen, haptoglobin, homocystein and plasminogen activator inhibitor-1. The articles were selected after reading the abstract and regardless of their year publication.

Hepatic Inflammation Markers

The accurate physiological events which conduct to beginning of inflammatory response in obesity are not totally known[11]. It is known that the mechanisms that obesity, specially central (visceral) obesity, associate with morbimortality include the increase in expression and release of adipose tissue cytokines and acute phase proteins; increase in activity of coagulation cascade (hypercoagulability) and decrease in activity of fibrinolytic cascade (pro-thrombotic process); increase in inflammatory process, oxidative stress and endothelial disfunction, besides disturbance of glucose and lipid metabolism (insulin resistance)[15].

The expansion of adipose tissue leads to adipocyte hypertrophy and hyperplasia and these big adipocytes decrease de local oxigen supply, causing cellular hypoxia and activation of cellular stress pathway (oxidatives and inflammatory), inducing a cellular autonomic inflammation (autocrine effect) and cytokines and release of others pro-inflammatory signals.

The adipocines (resistin, leptin and adiponectin), which are secreted by adipocytes, can also affect the inflammation and insulin resistance. As part of chronic and low intensity inflammatory process, chemokines locally secretaded attract pro- inflammatory macrophages for adipose tissue, which form a crown shape structure around the dead and/or sick big adipocites. Following, these macrophages stimulate the cytokines release, which will activate the inflammatory way in adipocytes and adjacent tissues (autocrine and paracrine effect), aggravating the inflammation and insulin resistance[4,10,11].

The hepatic inflammation can occur in obesity, because the activation of inflammatory pathways could be a steatosis result and/or increase in responses of hepatocytes stress pathways, which could result in hepatocytes autonomic inflammation (autocrine effect). The Kupffer cells (hepatic cells similar to macrophages) can also become activated, locally stimulating the cytokines release, which aggravated more the hepatic inflammation and insulin resistance. Morover, the caloric excess and obesity are, frequently, come along with the increase on tissue and tecidual circulant free fatty acids (FFA). They could activate directly the pro-inflammatory responses in vascular endothelial cells, adipocytes, myeloid derivatived cells[10,11]. The systemic inflammation developement is the result of these physiological events induced by obesity[11].

Amongst the hepatic biomarkes of inflammation related to the atherosclerose process are: sialic acid, α_1-antitrypsin and the C-terminal fragment of alpha1-antitrypsin, ceruloplasmin, fibrinogen, haptoglobin, homocystein and plasminogen activator inhibitor-1 (Fig. 1).

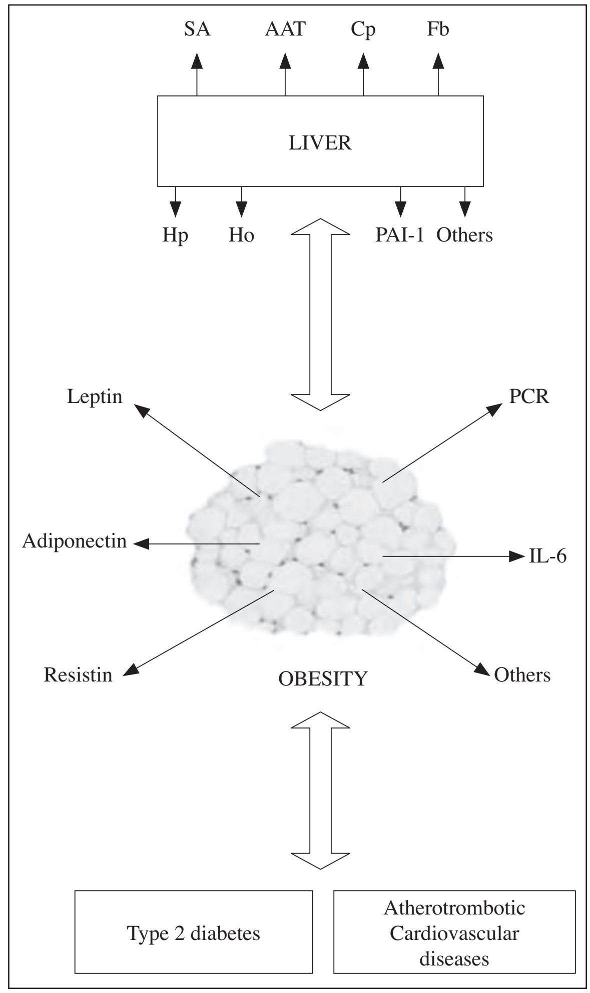

Fig. 1.–The adipose tissue product many inflammatory mediators which stimulate the hepatic tissue to product others inflammatory mediators. The process is bidirectional and increases proporcionaly to body fat quantity, enhancing the risk to develop type2 diabetes and atherothrombotic diseases. SA: sialic acid; AAT: α_1-antitripsin and C-36 antitripsin fragment; Cp: ceruloplasmin; Fb: fibrinogen; Hp: haptoglobuline; Ho: homocystein; PAI-1: plaminogen activator inhibitor-1.

Siliac acid

The siliac acid or N-acetylneuraminic is component of some acute phase protein terminal part, as alpha1- antichemotrypisin, alpha1-antitrypsin, haptoglobin and orosomucoid. These glucoproteins together explain 70% of sialic acid plasmatic concentration[16]. Its production in hepatocytes is stimulated in inflammation and metabolic/oxidative stress situations. It could be considered a biomarker of serum concentration of many acute phase protein, beyound, could be considered a systemic inflammatory biomarker since predict the risk for type 2 diabetes and cardiovascular diseases[7,16].

Non-diabetic normotensive obese individuals present the sialic acid concentration significantly increased as compared to non-obese controls. The sialic acid correlates positively with Homeostatic Model Assessment- Insulin Resistance formula (HOMA-IR), body mass index (BMI), waist and hip circumference and, negatively with Quantitative Insulin Check index (QUICKI) and insulinic sensitivity index (S_I)[17]. Yet, the siliac acid correlate to the following measurements of adiposity and /or metabolic syndrome components: glycemia, triacylglycerol, HDL-cholesterol, systolic and diastolic arterial pressure, besides it correlates with body weight, insulin, total cholesterol and LDL-cholesterol. In this same study, how much bigger the number of metabolic syndrome components, bigger was the sialic acid concentration. For each 0.34 mmol/L of increase in sialic acid concentration, a OR for metabolic syndrome was 2.5 (1.8 to 3.4), and persisted significant after the adjustment to BMI, with OR of 1.9 (1.3 ato 2. 6)[18,19]. In ARIC Study, individuals with the sialic acid and orosomucoid concentration bigger than average, showed *odds ratio* (OR) of 3.7 (1.4 to 9.8) e 7.9 (2.6 to 23.7), respectively, to develop type 2 diabetes. When adjusted for BMI and waist-hip index, the association of sialic acid and orosomucoid kept significants, with OR of 2.8 (1.01 to 8.1) and

7.1 (2.1 to 23.7), respectively[7]. In other study, individuals with sialic acid concentration on major quartile showed relative risk (RR) for cardiovascular mortality of 2.4 (2.0 to 2.8) and of 2.6 (1.9 to 3.6) in men and women, respectivelly[16].

Moreover, serum concentrations of sialic acid correlated to leptin concentrations, suggesting that high sialic acid concentration are related to biomarkers of obesity and adipose tissue metabolism, which could justify the fact of high concentrations of sialic acid precede the development of type 2 diabets and could also explain its function as cardiovascular risk indicator[20].

Then, the sialic acid correlate to adiposity measurement and/or metabolic syndrome components, besides predict the increase of the risk to develop metabolic syndrome and type2 diabetes, independently on BMI; as well as the increase of the risk to cardiovascular mortality. So, in individuals with elevation of its serum concentration, the sialic acid could add prognostic information that could be useful about obesity and chronic diseases.

Alpha$_1$-Antitripsin and the C-terminal fragment of alpha1-antitrypsin

The α_1-antitripsin is the main inhibitory endogenous human plasm proteases with serin which present a big variety of anti-inflammatory propriety, besides to execute an important function in reducing the proteolytic damage in the tissues. It is an acute phase protein and its concentration increases quickly in response to metabolic/oxidative stress due to infection and inflammation[21].

Even its production be mainly hepatic, some cells of immune system (neutrophils, monocytes, macrophages) also express it in response to one variety of inflammatory mediators. Some studies *in vitro* showed that alpha1- antitrypisin inhibit the TNF-β e da IL-1 syntesis and release, however it helps the inflammatory cytokines release, as IL-10 in human monocytes[21]. Addtionally, alpa1-antitrypsin is related to atherogenic process due to its union to LDL-cholesterol receptors and carriers receptors as the CD-36, which recognize the oxidate LDL-cholesterol and mediate the accumulation of lipid and foam cells formation. The result of this interaction is the pro-inflammatory molecules production by activated monocytes[22].

The C-36 carboxiterminal fragment, a product of alpha1-antitrypsin degradation, is found in atheroma and related to inflammatory transcription factors, as activation of NF-kβ, PPAR-α e PPAR-γ in primary cultivation of human monocytes[23]. Moreover, the C-36 modulates the human monocytes activation, actuating the TNF-α, IL-1β, IL-8 and the NF-kβ nuclear factor[24]. Then, alpha1-antitrypsin, as well as, the C-36 executes a very important function on expression of regulation and on modulation of pro-inflammatory and anti-inflammatory mediator[21].

In ARIC Study, individual with alpha1-antitrypsin concentration bigger than average, showed an OR of 1.8 (1.6 to 4.9) to develope type2 diabetes in the first 3years of following, after adjustments including smoking, glycemia in fasting, BMI and waist-hip index[7]. In other study, men with alpha1-antitrypsin concentration in major quartile when compared to smallers quartiles, the OR for type2 diabetes increased (OR= 3.9; 6.2; 19.2), in accordance to the increase of BMI (BMI = <25.0; 25 a 29.9; ≥30), respectively[6].

In relation to cardiovascular diseases, individual with alpha1-antitrypsin concentration in major quartile showed a RR of 2.3 (1.8 to 2.9), 1.3 (0.89 to 1.8) e 2.2 (1.7 to 3.0) to cardiacs events, acute myocardial infarction and cardiovascular mortality, respectively[9].

Nowadays, it is necessary more information about alpha1-antitrypsin and C-36 in relation to obesity. Nevertheless, it was published that elevated concentrations of sensitive proteins to inflammation, as alpha1-antitrypsin, could predict future gain of weight[5].

Then, alpha1-antitrypsin executes a relevant function on expression regulation, as well as, on modulation of pro-inflammatory and anti-inflammatory mediators[21]. Additionally, it seems to predict the increased chance to develop type 2 diabetes, irrespective of some conventional risk factors and dependently of BMI; as well as the increased risk to cardiovascular diseases. At last, individuals with elevation of alpha1-antitrypsin concentration could be come along with the inflammatory process, and high risk to develop chronic diseases, which could be aggravated by obesity.

Ceruloplasmin

The ceruloplasmin is a glucoprotein which is a family member of inflammatory sensitive protein, that include the alpha1-antitrypsin, haptoglobin, orosomucoid and fibrinogen[5,6]. It is considered the main copper transporter plasmatic protein (95%) for containing 6 copper atoms per molecule. It is syntetized, mainly, by liver, but other cells (monocytes, astrocytes, Sertoli cells) can also express it[25]. Its syntesis is increased in infection, inflammation and associated diseases situations[26] and its concentrations could be associated to cardiovascular risk factors, as hypertension, dyslipdemias, diabetes and increase in body weight[5].

Besides its transport function, ceruloplasmin exerts ferroxidase activity, modulation function of coagulation, angiogenesis, inactivity of biogenetic amines and defense to oxidative stress[27]. Due to its ferroxidase activity, the cerulolasmin relates to iron metabolism, since catalyzes the oxidation of ferrous to ferric iron. This activity is proposal as the mechanism in which the ceruloplasmin present antioxidant effect, reducing the oxidative stress through of inhibition of Fenton reaction, which uses the ferrous iron to generates reactive oxygen species (ROS)[3,27,28].

Otherwise, many researches have proposed a pro-oxidant effect[28]. The abdominal/visceral obesity, also relates to high ceruloplasmin concentration, postulating that the determination of this protein in patient with central obesity could be useful in order to identify patients with high risk to acute myocardial infarction (AMI)[29]. In other study, individuals with ceruloplasmin concentrations in bigger quartile showed RR of 2.1 (1.6 to 2.6), 2.0 (1.4 to 3.0) e 2.2 (1.6 to 3.1) for cardiac events, heart failure and cardiovascular mortality, respectively[9].

So that, high ceruloplasmin and copper concentrations associate with glucose tolerance and diabetes[30], as well as it is an important factor of cardiovascular risk when associated with homocistein concentrations[31]. In a study, men with ceruloplasmin concentrations in bigger quartile when compared with smallers quartiles, the OR for type 2 diabetes increases (OR= 4.2; 6.7; 18.4), in accordance to BMI increase (IMC= <25.0; 25 a 29.9; ≥30), respectively[6].

The suggested mechanism that ceruloplasmin could contribute to development of these diseases is related to situations which disfavour the oxidative stress promoting the copper release of the ceruloplasmine molecule and then, allowing the reation of free copper with pro-oxidants factors, which generate the free radical. Moreover, the enzymes activity in which the copper is a good cofactor (example: with the superoxide dismutase), will be prejudiced and the same way, the ferroxidase activity that depend on the molecule integrity, modifing the iron metabolism and favoring its accumulation[3,27].

So, high ceruloplasmin concentrations predict the increased of the risk to develop cardiovascular diseases, as well as, the increase of the chance to present type 2 diabetes, irrespective of BMI. But, it is important to stand out that high concentrations could not be pathologics, necessarily.

In clinical practice, the best way to know it, is understand the oxidative degree, and then, determine IF this elevation is pathologic[27]. Anyway, the ceruloplasmin could be pro-oxidant or antioxidant effect, depending on the integrity of its structure. The ceruloplasmin functions in stress oxidative situations and as biomarker of inflammatory state request new investigations[3].

Fibrinogen

The fibrionogen (Factor I) is a glucoprotein synthetized in the liver and is involved in final stage of coagulation, which consists on its conversion in fibrin under trombin action[15]. It is an acute phase protein, similar to C-reactive protein (CRP), its production is apparently controlled by IL-6[32]. The fibrinogen promotes the arterial and venous thrombosis through of increase in fibrin formation, plaquetary aggregation and plasma viscosity, promoting the atherosclerosis by proliferations of endothelial and smooth vascular muscle cells[15].

Since the obesity is associated with atherosclerosis, many researches have been conducted in order to know possible associations between the fibrinogen and obesity and its comorbities. In individual without diabetes, the fibrinogen concentration correlated to following adiposity measurement and/ or metabolic syndrome components: fasting glycemia, waist circumference, HDL-cholesterol, systolic and diastolic arterial pressure, besides BMI, insulin, pro-insulin values and S_I[33]. Additionally, overweight individuals have higher fibrinogen concentration when compared to normal[34]; and individuals with metabolic syndrome have fibrinogen concentrations significantly bigger than individual without metabolic syndrome[35]. Otherwise, fibrinogen concentrations could decrease in weight loss[36].

A study showed positive correlation between concentrations of insulin and fibrinogen, during many periods of glucose tolerance (normal tolerance, prejudiced tolerance to glucose and type 2 diabetes, respectively. The decrease of insulin sensitivity was an independent factor associated with high fibrinogen concentrations[37]. These results suggest that fibrinogen is a metabolic syndorme biomarker, in an insulin resistance state. In ARIC Study, individuals with fibrinogen concentrations in the bigger quartile, showed OR of 1.2 (1.0 to 5.0) to develop type 2 diabetes in a period of 7 years[7]. In other study, men with fibrinogen concentrations in the major quartile when compared with the smaller quartiles, the OR to type 2 diabetes increases (OR= 4.2; 7.8; 21.6), in accordance to the BMI increase (BMI = <25.0; 25 a 29.9; ≥30), respectively[6].

In relation to cardiovascular diseases, individuals with fibrinogen concentration in major quartile showed a RR of 2.3 (1.8 to 2.9), 1.9 (1.3 to 2.7) e 2.5 (1.8 to 3.4) to cardiac events, heart failure and cardiovascular mortality, respectively[9]. In a prospective cohort study, each increase of 100md/dL of fibrinogen level yielded a hazard ratio (HR) of 1.49 (1.11 to 2.22) for cardiovascular mortality, after adjusting for sex, age, hypertension, diabetes mellitus, obesity, total cholesterol, HDL-cholesterol/triacylglycerols ratio, smoking habit, and history of previous cardiovascular disease. Within the population of this study, fibrinogen is an independent predictor of cardiovascular mortality[38].

Fibrinogen concentrations also predict weight gain. It was demosntrated by a study in which individuals wich serum concentrations of fibrinogen in higher quartile, acquired approximately 0.23 kg/year when compared to them of smallers quartiles. The OR adjustmented for the biggest weigth gain (bigger than percentil 90) for that in the bigger quartile of fibrinogen concentration was de 1.65 (1.38 – 1.97) times when compared with them in the smaller quartile, for a period of 3 years. These OR values were also different for distinct degrees of obesity. Individuals with BMI < 25, 25 to 30 e > 30 kg/m^2 have their OR of 1.43, 1.59 e 2.02, respectively[39]. Then, high fibrinogen concentrations predispose the obese patients to higher risk to suffer thromboembolic complications[40], due to increase on oxidative and inflammatory state, favoured by increase in body adiposity.

The fibrinogen correlate to adiposity measument and/ or metabolic syndrome components and its concentrations increase proporcionally to insulin resistance state, increasing the chance to occur type 2 diabetes, which happen in dependent way of BMI. So, in clinical practice, the same could add usefull prognostic information to individuals with metabolic syndorme, an aggravated process by obesity, which predispose individuals to tromboembolic diseases. At last, there is an addicional advantage associated with the determination of fibrinoogen concentration, since it is considered an independent very important risk factor to cardiovascular diseases[2].

Haptoglobulin

The haptoglobulin (α_2-globulin) is a glucoprotein produced, mainly, in the hepatocytes, whose function principal is to fix the free hemoglobin and remove it of circulation by the reticuloendothelial system. As an acute phase protein, its synthesis is increased in inflammatory process[3]. Besides its hepatic synthesis, it has been demonstrated its presence in adipose tissue, as well as its release by primary cultivation. Its quantity is major in visceral than subcutaneous tissue, but in both cases are much inferior than circulant concentrations[41]. Amongst the cytokines, TNF-α, IL-6 and others, regulate its secretion in the liver and adipose tissue[42].

In healthy individuals, the haptoglobulin concentration correlate to following adipose measuriments and/or metabolic syndrome: insulin, total cholesterol, percentual of body fat, lipid oxidation[43], leptin, CRP[44] e BMI[43, 44]. The haptoglobulin, together the other plasmatic proteins sensitive to inflammation, relate to weight gain in long-term and, consequently, to the increase in cardiovascular risk in obese individuals[12].

In ARIC Study, individuals with haptoglobulin concentration bigger than average, showed an OR of 2.1 (0.7 to 6.0) to develop type 2 diabetes in 3 first years of following, after the adjustment including smoking, fasting glycemia, BMI and waist-hit index[7]. In other study, men with Hp concentration in the major quartile when compared to smallers quartiles, the OR to type 2 increase (OR= 3.2; 8.4; 21.6), accordant to BMI increase (BMI= <25.0; 25 a 29.9; ≥30), respectively[6].

In relation to cardiovascular diseases, individuals with haptoglobuin concentration in bigger quartile showed an RR of 2.0 (1.6 to 2.5), 1.9 (1.3 to 2.7) and 2.0 (1.5 to 2.7) for cardiac events, acute myocardial infarction and cardiovascular mortality, respectively[9].

Despite the found association between haptoglobulin concentration and adipose measurement and/or components of metabolic syndrome[42-44], as well as its capacity to predict the risk to type 2 diabetes[6,7], dependently of BMI, and to cardiovascular diseases[9], its use as marker of inflammatory state in clinical and epidemiologic researches should be done and elucidated with caution, due to different behaviour showed by its 3 main phenotypes (haptoglobulin1-1; haptoglobulin1-2 e haptoglobulin 2-2)[42].

Homocystein

The homocistein is an aminoacid which contain a thiol (sulphidril or SH-) that exerts an important function in folate and methionine metabolism. The homocistein is metabolized by two pathways: (a) when the metionin stock is sufficient, the homocistein enter on transulfuration pathway and is converted in cistein in one reaction catalyzed by β-sintase cistationin dependent on B6 vitamin; (b) when the metionin conservation is necessary, the homocistein receive a methyl group of N5 methyl-tetrahydrofolate (catalyzed by N5, N10 methylenetetrahydrofolate reductase enzime) and is converted to metionin by methionine synthase (demethylation), whose cofactor in this last reaction is the B12 vitamin[45,46].

The hyperhomocysteinemia was, recently, recognized as a factor of cardiovascular risk, independent on others risk factors as diabets, hypertension, hypercholesterolemia and smoking[47,48]. The estimated prevalence to hyperhomocysteinemia (Ho>14 μmol/L) vary between 5% and 30% in population[49,50], and occur in approximately 5 to 7% of population in generaly, and in 5 to 40% in patients with cardiovascular diseases[51]. In a populational study, HOOGEVEN et al., (2000) detected a prevalence of 25.8%[50].

The relation power between hyperhomocysteinemia and death seems to be stronger between individuals with diabetes than that one's without this disturb[50]. In fact, an interaction of hyperhocysteinemia with diabetes is biologically plausible. High homocystein concentrations could exert atherothrombotic effects by oxidative stress increase, which could induce endothelial disfunction[46]. The homocystein can also affect the propriety of extracellular matrix and increase the smooth muscle cells proliferation[46]. In diabetes, the oxidative stress is enhanced, and extracellular matrix alterations are proeminent features of diabetes. The both could become individuals with diabetes more susceptible to adverse effects of hyperhomocysteinemia[50]. Additionally, the oxidative stress caused by the increased concentration of tiacilglycerols and free fat acids is known in to cause the hyperinsulinemia and insulin resistance. This process could be aggravated in obesity in decurrency of the stock increase of body fat. The process is bidirectional and the insulin resistance could enhance the homocystein concentration[52].

However, the specific mechanism through the homocystein promote atherotrombose continuous unknowed, there are strong epidemiolgical evidences to association between hyperhomocysteinemia and atherotrombotic cardiovascular diseases[46,47]. Otherwise, as was showed by meta-analysis studies, the daily treatment with pholic acid could reduce the homocystein concentration in 15 up to 40% into 6 weeks[47]. Then, the hyperhomocysteinemia is an important risk factor but could be modified by diet habit.

In a prospective study, after the adjustment to the biggest cardiovascular risk factors, the serum albumin (marker of healthy general state) and glycated hemoglobin (HbA_{1c}), the OR to mortality into 5 years was 1.56 (1.07 to 2.30) to hyperhomocysteinemia and 1.26 (1.02 to 1.55) for each 5-μmol/L of homocystein increase. Morover, the OR pto mortality in 5 years to hyperhomocysteinemia was 1.34 (0.87 to 2.06) in individual without diabetes and 2.51 (1.07 to 5.91) in diabetics ($p<0,08$). For each 5-μmol/L of serum homocystein increment, the risk to general mortality in 5 years increase in 17% patients without diabetes and 60% in diabetics[50]. In other study, how much big was the quartile of homocystein concentration (Q1: <10.3; Q2: 10.3-12.49; Q3: 12.5-15.39; Q4: >15.4), bigger was the OR adjustment to heart failure, wich were 1.0, 1.2 (0.3 to 4.2), 2.6 (0.7 to 9.3) and 4.7 (1.1 to 20.0), respectively[45].

Then, the hyperhomocysteinemia is related to morbimortality of independent form of biggest cardiovascular risk factors[45,50] and seems to be a strong risk factor (about 2 times) to death in diabetics[50], which process could be aggravated in hyperinsulinemics[52]. So, in individuals with its high concentrations, the homocystein *per si* could be usefull in clinical practice to supply prognostic in relation to chronic diseases. Still, is important to stand out that the risk to chronic diseases, followed by the increase in homocystein concentration, could be modified by diet habit.

Plasminogen activator inhibitor-1

The PAI-1 is a protein that inhibits the tecidual plasminogen activator (tPA), which cleaves the plamine to plasminogen, thus is the first physiological inhibitor of fibrinolysis *in vivo*. It occurs while present the capacity to inhibit the plamine forerunner, whose function is the rupture of fibrine network, avoidding the thrombus formation[53].

The PAI-1 is produced in many tissues, including the liver and adipocytes. Many factores contribute to increase of the expression and release of PAI-1 in adipose tissue (specially, the visceral), amongst them there are insulin, TGF-β, PCR, IL-6, FNT-α and IL-1[15,54]. These factors associated with the stock increase of body fat can explain theirs enhanced concentration, generally, verified in obese individuals and/or insulin resistants[54].

These high concentration compromise the normal fibrine *clearance*, causing the fibrinolisys system deterioration, and consequently promote the thrombose, which is part of cardiovascular complication of obesity[15,53].

The PAI-1 concentration correlates to following adiposity measurements and/or metabolic syndrome components: waist circumference, insulin resistance and triacylglycerols concentrations. High concentration of PAI-1 predicts cardiac and coronary diseases and acute myocardial infarction. Then, high concentrations of PAI-1 found in obesity and metabolic syndrome could predispose to many micro and macrovasculars, arterials and venous, including the thrombose[15].

A study demonstrated positive correlation between insulin and PAI-1 concentration, during many periods of glucose tolerance (normal, prejudiced tolerance to glucose and type 2 diabetes), respectively. The decrease sensitivity to insulin was an independent factor associated with high PAI-1 concentrations[37].

The IRAS Study evaluated the link between PAI-1 and the incidence of type2 diabetes during 5 years and observed that PAI-1, which has a knowed relation to metabolic syndrome components, it seems to be a precocious inflammatory biomarker to type2 diabetes. The PAI-1 concentrations are enhanced in insulin resistant individuals, who become diabetics independently on insulin sensitivity and BMI[8]. These results suggest that PAI-1 is a metabolic syndrome marker, in insulin resistance state, due to reflect subclinical inflammation.

The PAI-1 activity coud be stimulated by others inflammatory mediators, as the PCR which stimulate the expression and PAI activity in endothelial cells. This effect is additional in hyperglicemia situation. So, the increase of PAI-1 concentration on diabetes and metabolic syndrome, is also due to a stimulation of monocytes and endothelial cells by PCR, in which situations are also increased[55,56].

A study with elders without diabetic, after to adjust the age, the PAI-1 concentrations correlated to following adiposity measurements and/or metabolic syndrome components: waist circumference, glycemia, HDL-cholesterol, body weight and insulin. In this study, the authors also related the PAI-1 concentration to body mass factors (body weight, waist circumference, insulin and glycemia). Then, individuals with high concentrations of PAI-1 together with body mass factor support the relation between obesity and prejudiced fibrinolysis[57].

Obeses with metablic syndrome have higher PAI-1 concentrations than individuls with normal weight without metabolic syndrome[35]. Additionally, in the same study, the PAI-1 concentrarion to whole group (obese with metabolic syndrome plus eutrofics) were associated with glycemia, insulin, HOMA-IR, triacilglycerol, HDL-cholesterol and CRP. Only in obese group with metabolic syndrome, the PAI-1 concentration was significantly associated with glycemia and HOMA-IR. So, the high serum concentrations of PAI-1 are found in obeses with metabolic syndrome, but these are dependents factors of others conditions for that inflammatory process occur. The association between PAI-1 and insulin resistance has been reported by many studies with eutrofic patients and obeses with insulin resistance[35].

Then, the determination of PAI-1 concentration has the advantage to be related to adiposity measurement and/or metabolic syndrome components and the fibrinolysis system. This parameter is also considered an important risk factor to type2 diabetes and to atherotrombotic cardiovascular diseases, aggravated by insulin resistance degree.

Inflammatory biomarkers set (fibrinogen, haptoglobuline, ceruloplasmine, orosomucoid, α_1-antitripsin)

In a study, the OR to type2 diabetes in men with high concetrations of biggest quartile for no biomarker (OR= 3.3; 3.8; 13.9), presence of one (OR= 4.0; 6.3; 12.8), for two (OR= 3.3; 6.7; 20.8), for three (OR= 5.4; 6.9; 16.9) e for four or five (OR= 2.5; 8.7; 28.0), when compared to smallest quartiles, increased with BMI (BMI= <25.0; 25 to 29.9; ≥30), respectively. The prevalence of diabetes was significantly associated with studied biomarkers concentrations among overweight and obese individuals, but not in individuals with BMI <25 kg/m^2. This association was similar to insulin resistance in accordance to HOMA-IR[6].

In this same study, individuals with and without diabetes, as well as the presence of two up to five biomarkers on fourth quartile, when conpared to lowest quartiles, had different OR to atherothrombotic cardiovascular diseases. After adjusted to age, smoking, cholesterol, triacilglycerols, sedentary life, systolic and diastolic arterial pressure and hypertension medicaments; men without diabetes, with two up to five biomarkers had OR of 1.6 (1.4 to 1.8); 1.7 (1.4 to 2.2); 1.6 (1.3 to 1.9); 1.4 (1.1 to 1.9) and 1.4 (1.0 to 1.9) for every cardiovascular mortality causes (cardiovascular diseases, cardiac events, infarct, ischemic infarct, respectively). And men with diabetes and presence of two up to five biomarkes had possuíam OR of 1.5 (0.93 to 2.4); 2.2 (1.2 to 4.2); 2.2 (1.2 to 3.8); 2.4 (0.98 to 5.8) and 2.0 (0.8 to 5.0) for all cardiovascular mortality causes (cardiovascular diseases, cardiac events, infarct, ischemic infarct, respectively). The authors concluded that on this study population (6.050 men), the diabetes was associated with the increase of inflammatory biomarkers concentrations (fibrinogen, haptoglobulin, ceruloplasmin, orosomucoid, α_1-antitripsin) between individuals with overweight and obesity, but not between individuals with normal weight. Additionally, high concentrations of inflammatory biomarkers increase the cardiovascular risk similary in diabetics when compared to individuals without diabetes[6].

Final Considerations

This review allows to verify the relation between some biomarkers (sialic acid, fibrinogen, haptoglobulin and PAI-1) with obesity and chronic diseases, since them presented correlation to adiposity measurements and/or metabolic syndorme components. The sialic acid predicts the chance increase to become type2 diabetic independently of BMI. Moreover, the α_1-antitripsin, ceruloplasmine, fibrinogen and haptoglobulin biomarkers, seem predict the chance increase to become type2 diabetic, dependently, of BMI. So, this process could be aggravated by obesity. The concentrations of fibrinogen, homocystein and PAI-1 increase proportionally to insulin resistance, showing its relation to metabolic syndrome (insulin resistance state) and with type2 diabetes.

In relation to cardiovascular diseases, every biomarkers reported in this review seem to increase the risk, becoming usefull in add important prognostic. It seems that fibrinogen and homocystein, in specially, have an additional advantage in its use in clinical practice, because they are considered independent risk factors to cardiovascular diseases.

The high concentration of ceruloplamin, should be interpretated with caution, because could be not necessarily pathologic. In the clinical practice, the best way to confirm this situation is to evaluating the oxidative state. In any way, the ceruloplasmin function of inflammatory biomarker in oxidative stress situation request new investigations.

It is important to stand out that the strong relation between inflamation biomarkers and chronic diseases could be equal as much in healthy as in sick individuals (after have been developed type 2 diabetes or had myocardial infarction), as well as in eutrofic and overweight individuals. In the same way, the cardiovascular risk varies widly between eutrofic and overweight individuals, with low or high concentrations of inflammatory biomarkers. Their relation to sensitive protein to inflammation contributes, but do not explain completly in increase of cardiovascular risk in obeses. It suggets that the contribution of insulin resistance on inflammatory process in not only a phenomenon restricted to individual with diseases or overweigth. All factors which modulate the liver and adipose tissue to product inflammatory biomarkers should be more explored; however, there is an inability in find differences on inflammatory biomarkers concentrations between healty and sick individuals, and between eutrofic and overweight individuals.

An context, specially on food patterns[58] and physical activity, should be considered into determinants of chronic diseases and not only in biochemistry and anthropometric values, and body composition.

The inflammatory process is a very complex reaction. All of these reported biomarkers, alone or together, seem execute many functions and following various biochemistry routs in human body metabolism[59]. At last, is necessary performing more studies to understand better the biological activity of these inflammatory biomarkers, and then, stablish their biological and clinical function.

References

1. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004; 109: 2-10.
2. Wu JT, Wu LL. Linking inflammation and atherogenesis: Soluble markers identified for the detection of risk factors and for early risk assessment. *Clin Chim Acta*. 2006; 366: 74-80.
3. Zulet Mª A., Puchau B, Navarro C, Martí A, Martínez JA. Biomarcadores del estado inflamatorio: nexo de unión con la obesidad y complicaciones asociadas. *Nutr Hosp*. 2007; 22(5): 511-27.
4. Volp ACP, Alfenas RCG, Costa NMB, Minim VPR, Stringueta PC, Bressan J. Capacidade dos biomarcadores inflamatórios em predizer a síndrome metabólica. *Arq Bras Endocrinol Metabol*. 2008; 52(3): 537-49.
5. Engstrom G, Stavenow L, Hedblad B, Lind P, Tydén P, Janzon L, et al. Inflammation sensitive plasma proteins and incidence of myocardial infarction in men with low cardiovascular risk. *Arterioscler Thromb Vasc Biol*. 2003; 23: 2247-51.
6. Engstrom G, Stavenow L, Hedblad B, Lind P, Eriksson K-F, Janzon L, et al. Inflammation sensitive plasma proteins, diabetes, and incidence of myocardial infarction and stroke. *Diabetes*. 2003; 52: 442-7.
7. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities Study): A cohort study. *Lancet*. 1999; 353: 1649-52.
8. Festa A, D'Agostino R Jr, Tracy RP, Haffner S. Insulin resistance atherosclerosis study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: The Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes*. 2002; 51: 1131-7.
9. Engstrom G, Lind P, Hedblad B, Stavenow L, Janzon L, Lindgarde F. Effects of cholesterol and inflammation-sensitive plasma proteins on incidence of myocardial infarction and stroke in men. *Circulation*. 2002; 105: 2632-7.
10. Kim F, Tysseling KA, Rice J, Pham M, Haji L, Gallis BM, et al. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. *Arterioscler Thromb Vasc Biol*. 2005; 25: 989-94.
11. Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Letters* 2008; 582: 97-105.
12. Engstrom G, Hedblad B, Stavenow L, Jonsson S, Lind P, Janzon L, et al. Incidence of obesity- associated cardiovascular disease is related to inflammation- sensitive plasma proteins: A population based cohort study. *Arterioscler Thromb Vasc Biol*. 2004; 24:1498-502.
13. Kannel WB, Wilson PW, Nam BH, D`Agostino RB. Risk stratification of obesity as a coronary risk factor. *Am J Cardiol*. 2002; 90: 697-701.
14. Jonsson S, Hedblad B, Engstrom G, Nilsson P, Berglund G, Janzon L. Influence of obesity on cardiovascular risk. Twenty-three-year follow-up of 22,025 men from an urban Swedish population. *Int J Obes Relat Metab Disord*. 2002; 26: 1046-53.
15. Darvall KAL, Sam RC, Silverman SH, Bradbury AW, Adam DJ. Obesity and thrombosis. *Eur J Vasc Endovasc Surg*. 2007; 33: 223-33.
16. Lindberg G, Rastam L, Gullberg B, Lundblad A, Nilsson-Ehle P, Hanson BS. Serum concentration of total sialic acid and sialoglycoproteins in relation to coronary heart disease risk markers. *Atherosclerosis*. 1993; 103: 123-9.
17. Rajappa M, Ikkruthi S, Nandeesha H, Satheesh S,Sundar I, Ananthanarayanan PH., et al. Relationship of raised serum total and protein bound sialic acid levels with hyperinsulinemis and indices of insulin sensitivity and insulin resistance in non-diabetic normotensive obese subjects. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2013;7:17-9.
18. Browning L, Krebs JD, Jebb SA. Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease. *Metabolism*. 2004; 53(7): 899-903.
19. Browning LM, Jebb SA, Mishra GD, Cooke JH, O`Connell MA, Crook MA, et al. Elevated sialic acid, but not CRP, predicts features of the metabolic syndrome independently of BMI in women. *Int J Obes*. 2004; 28: 1004-10.
20. Crook MA, Miell J, Ameerally P, Lumb, P, Singh N, Russell-Jones D, et al. Serum sialic acid, a reputed cardiovascular risk factor, is related to serum leptin concentrations in Fijians. *Clin Chim Acta*. 2003; 331:1-5.
21. Janciauskiene S, Larsson S, Larsson P, Virtala R, Jansson L, Stevens T. Inhibition of lipopolysaccharide-mediated human monocyte activation, in vitro, by alpha1-antitrypsin. *Biochem Biophys Res Commun*. 2004; 321: 592-600.
22. Janciauskiene S, Moraga F, Lindgren S. C-terminal fragment of alpha1-antitrypsin activates human monocytes to a proinflammatory state through interactions with the CD36 scavenger receptor and LDL receptor. *Atherosclerosis*. 2001; 158: 41-51.
23. Moraga F, Janciauskiene S. Activation of primary human monocytes by the oxidized form of alpha1-antitrypsin. *J Biol Chem*. 2000; 275:7693-700.
24. Subramaniyam D, Glader P, Von Wachenfeldt K, Burneckiene J, Stevens T, Janciauskiene S. C-36 peptide, a degradation product of alpha1-antitrypsin, modulates human monocyte activation through LPS signaling pathways. *Int J Biochem Cell Biol*. 2006; 38: 563-75.
25. Fox PL, Mazumder B, Ehrenwald E, Mukhopadhyay CK. Ceruloplasmin and cardiovascular disease. *Free Radic Biol Med*. 2000; 28: 1735-44.
26. Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. *Mol Aspects Med*. 2005; 26: 268-98.
27. Shukla N, Maher J, Masters J, Angelini GD, Jeremy JY. Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? *Atherosclerosis*. 2006;187: 238-50.
28. Giurgea N, Constantinescu MI, Stanciu R, Suciu S, Muresan A. Ceruloplasmin: Acute-phase reactant or endogenous antioxidant? The case of cardiovascular disease. *Med Sci Monit*. 2005; 11: 48-51.
29. Cignarelli M, Depergola G, Picca G, Sciaraffia M, Pannacciulli N, Tarallo M, et al. Relationship of obesity and body fat distribution with ceruloplasmin serum levels. *Int J Obes Relat Metab Disord*. 1996; 20:809-13.
30. Kim CH, Park JY, Kim JY, Choi CS, Kim YII, Chung YE, et al. Elevated serum ceruloplasmin levels in subjects with metabolic syndrome: A population based study. *Metabolism*. 2002; 51: 838-42.
31. Exner M, Hermann M, Hofbauer R, Hartmann B, Kapiotis S, Gmeiner B. Homocysteine promotes the LDL oxidase activity of ceruloplasmin. *FEBS Lett*. 2002; 531: 402-6.
32. Kerr R, Stirling D, Ludlam CA. Interleukin 6 and haemostasis. *Br J Haematol*. 2001; 115:3-12.
33. Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: The Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*. 2000; 102: 42-7.
34. Celik A, Saricicek E, Saricicek V, Sahin E, Ozdemir G, Kiline M., et al. Relation between the new anthropometric obesity parameters amd inflammatory markers in healthy adult men. *SCIRJ*. 2014; 11(3): 6-10.
35. Bahia L, Aguiar LG, Villela N, Bottino D, Godoy-Matos AF, Geloneze B, et al. Relationship between adipokines, inflammation, and vascular reactivity in lean controls and obese subjects with metabolic syndrome. *Clinical Sciences*. 2006; 61(5): 433-40.
36. Ditschuneit HH, Flechtner-Mors M, Adler G. Fibrinogen in obesity before and after weight reduction. *Obes Res*. 1995; 3:43-8.
37. Festa A, D`Agostino R, Mykkanen L, Tracy RP, Zaccaro DJ, Hales CN, et al. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance: The Insulin Resistance Atherosclerosis Study (IRAS). *Arterioscler Thromb Vasc Biol*. 1999; 19: 562-68.
38. Simarro M, Carbayo JA, Artigao LM, Massó J, Divisón JA, Ponce I., et al. Fibrinogen and mortality in a cohort population-based study. *Exp Clin Cardiol*. 2014; 20(5):3011-8.

39. Duncan BB, Schmidt MI, Chambless LE, Folsom AR, Carpenter M, Heiss G. Fibrinogen, other putative markers of inflammation, and weight gain in middle-aged adults: The ARIC Study. *Obesity Research*. 2000; 8(4): 279-86.
40. Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. *Obes Rev*. 2002; 3: 85-101.
41. Fain JN, Bahouth SW, Madan AK. Haptoglobin release by human adipose tissue in primary culture. *J Lipid Res*. 2004; 45: 536-42.
42. Taes YE, De Bacquer D, De Backer G, Delanghe JR. Haptoglobin and body mass index. *J Clin Endocrinol Metab*. 2005; 90: 594.
43. Heliovaara MK, Teppo AM, Karonen SL, Tuominen JA, Ebeling P. Plasma IL-6 concentration is inversely related to insulin sensitivity, and acute-phase proteins associate with glucose and lipid metabolism in healthy subjects. *Diabetes Obes Metab*. 2005; 7: 729-36.
44. Chiellini C, Santini F, Marsili A, Berti P, Bertacca A, Pelosini C, et al. Serum haptoglobin: A novel marker of adiposity in humans. *J Clin Endocrinol Metab*. 2004; 89:2678-83.
45. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet*. 1995; 346:1395-8.
46. Welch GN, Loscalzo J. Mechanisms of diseases: Homocysteine and atherothrombosis. *N Engl J Med*. 1998; 338: 1042-50.
47. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA*. 1995; 274: 1049-57.
48. Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol*. 1996; 27:517-27.
49. Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJC, Jakobs C, Bouter LM, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. *Arterioscler Thromb Vasc Biol*. 1998; 18: 133-8.
50. Hoogeveen EK, Kostense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ et al. Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes: 5-year follow-up of the Hoorn Study. *Circulation*. 2000; 101: 1506-11.
51. Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocysteine and cardiovascular disease: A critical review of the epidemiologic evidence. *Ann Intern Med*. 1999; 131: 363-75.
52. Sánchez-Margalet V, Valle M, Ruz FJ, Gascón F, Mateo J. Elevated plasma total homocysteine levels in hyperinsulinemic obese subjects. *J Nutr Biochem*. 2002; 13(2): 75-9.
53. Juhan-Vague I, Alessi MC. PAI-1, obesity, insulin resistance and risk of cardiovascular events. *Thromb Haemost*. 1997; 78(1): 656-60.
54. Samad F, Loskutoff DJ. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. *Mol Med*. 1996; 2(5): 568-82.
55. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*. 1999; 19: 972-8.
56. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: Implications for the metabolic syndrome and atherothrombosis. *Circulation*. 2003; 107:398-404.
57. Sakkinen PA, Wahl P, Cushman M, Lewis MR, Tracy RP. Clustering of procoagulation, inflammation, and fibrinolisis variables with metabolic factors in insulin resistance syndrome. *Am J Epidemiol*. 2000; 152: 897-907.
58. Volp ACP, Hermsdorff HHM, Bressan J. Glycemia and insulinemia evaluation after high-sucrose and high-fat diets in lean and overweight/obese women. *J Physiol Biochem*. 2008; 64(2):103-114.
59. Jung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci*. 2014;15:6184-223.