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Research Article

Experimental Model of Oxidative Stress Markers in Subclinical Atherogenesis Associated with Metabolic Syndrome

Tarán Mariana D[1], Baez María C[1-3], María de La Paz Scribamo Parada[1], Ariel Balceda[1], Blencio Sergio[1], Binci Miriam[2] and Moya Mónica[1-3]*

[1]*Faculty of Medical Sciences, Universiad Nacional de Córdoba, Argentina*
[2]*Faculty of Clinical analysis, Laboratorio LABAC, Argetina*
[3]*Faculty of Medical Sciences, Universiad Nacional de La Rioja, Argentina*

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***Corresponding author**

Moya Mónica, MD, PhD Professor of Biomedical Physics, Cardiovascular Biomarkers Laboratory, Los Médanos 3155, Alto Verde, 5009, Córdoba, Argentina, Tel/Fax: 54-351-4332020; Email: monicamoya@hotmail.com

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Abstract

The study of oxidative markers in an experimental model of atherogenesis induced by Hyperfibrinogenemia (HF) and Metabolic Syndrome (MS) proposes to analyze the relationship between inflammatory biomarkers and oxidative stress associated with insulin resistance to determine their involvement in ischemic vascular lesions and explain the potential pathophysiological mechanisms.

Methods: Seventy two male Wistar strain rats were divided in six groups: control (A), HF for 30 days (B) and HF for 60 days (C), rats with MS (D), HF for 30 days+MS (E) and MS+HF for 30 days (F). Induction of HF to trigger the proinflammatory process is carried out by medium and paramedian laparotomies every seven days. For induction of the metabolic syndrome fructose 10% was administered diluted in the drinking water for 6 weeks. We determined plasma levels of insulin, glucose, triglycerides, HDL, fibrinogen, L-citruline and superoxide dimustase. Statistically analysed by an ANOVA, $p<0.05$ level of significance.

Results: The MS group (D) showed increased insulinemia, glucose and triglycerides with respect to group (A), (B) and (C) ($p<0.001$). Similiar modifications showed groups (E) and (F), with insulinemia, glucose and triglycerides increased with respect to (A) ($p<0.01$, $p<0.001$ respectively). HDL significantly decreased in the groups (D), (E) and (F) compared to control ($p<0.001$) and groups (B) and (C) ($p<0.001$).

Conclusion: The potential importance of vascular wall inflammation in diseases, subclinical atherosclerosis and MS, as a therapeutic target remains an area not yet fully explored, where new knowledge on the involvement of inflammatory mediators may be relevant as the score validated risk assessment does not currently include these components, and their inclusion could assess the actual risk patients.

Introduction

The constant study and review of the risk factors plays an important role in strategies for the prevention of ischemic cardiovascular disease because is the leading cause of death [1]. Subclinical atherosclerosis affects all vascular walls, many years before a cardiovascular event clinically apparent [2]. The evaluation of multiple vascular walls through an individual marker for atherosclerosis provides additional information on future risk of cardiovascular events [3]. The concept of association of hypertension, obesity, dyslipidemia and Diabetes Mellitus (DM) is not new, it is an association of health problems that may occur simultaneously or sequentially in the same individual, caused by a combination of environmental factors genetic and related to a lifestyle in which insulin resistance is considered the fundamental pathogenic cause. This syndrome called Metabolic Syndrome (MS) is characterized by the convergence of several risk factors where in each individual contributes to cardiovascular risk and its association increases it exponentially, and not merely additive manner [4]. It is also a predictor of the development of DM; therefore the diagnosis could be useful in identifying individuals with a number of risk factors that could benefit from relevant changes your lifestyle to reduce the risk of developing DM and coronary artery disease [5]. Because individuals with MS and evidence of subclinical atherosclerosis have higher rates of overt cardiovascular disease comparable to those with diabetes, coronary disease considered cardiovascular risk equivalent [6], it is essential to diagnose MS and to evaluate the presence of markers of atherosclerosis subclinical, not just for specific treatment, but to identify those whose adverse metabolic state justifies it's early detection and control.

It has been established in previous studies that have different risk factors prooxidative with common pathway that negatively affect mitochondrial function.

In addition, increasing evidence supports the hypothesis that mitochondrial dysfunction may be the most important mechanism since the intracellular biochemical site where the agent acts adversely affecting aerobic respiration.

Would, on which depends the mitochondrial oxidative phosphorylation and training Adenosine Triphosphate (ATP) to explain the unification of the atherogenic action of major cardiovascular risk factors [7].

The study of oxidative markers in an experimental model of atherogenesis induced by hyperfibrinogenemia and MS proposes to analyze the relationship between inflammatory biomarkers and oxidative stress associated with insulin resistance to determine their involvement in ischemic vascular lesions and explain the potential pathophysiological mechanisms.

Material and Methods

Male Wistar rats employed in this study were bred and housed under controlled conditions; they were maintained at room temperature (20ºC ± 2ºC); after weaning, rats were randomly assigned to single sex groups of twelve with food (Cargill) and water ad libitum. The investigation was carried out according to the guide for care and use of Laboratory Animals published by the US National Institute of Health, NIH publication (Nº58-23, revised 1996). Resides, the Ethic Committee from the Medicine School (National University of Cordoba), has also approved the experimental animal procedures. Seventy two rats weighing between 250 and 300 g were divided as follows:

A) Control (n = 12)

B) Hyperfibrinogenemia (HF) induced by 30 days. (HF x 30 days) (n = 12)

C) HF induced by 60 days. (HF x 60 days) (n = 12)

D) With Metabolic Syndrome (MS) induced by additional water with fructose to 10% in drinking water for 6 weeks (n: 12)

E) HF induced by 30 days (HF x 30 days) + MS. (n: 12)

F) MS + HF induced by 30 days (HF x 30 days) (n:12)

Hyperfibrinogenemia was induced by medium and paramediam laparotomies for periods of 30 days, even seven days.

For induction of the metabolic syndrome were used fructose by 10%; it was administered diluted in the drinking water for 6 weeks [9].

Blood was obtained after sacrifice of animals previously anesthetized by ketamine (Ketal®) (10mg/kg/rat), after fasting for 24 hours, completed the experimental period studied at 72 hours after the last LAPAROTOMIE induction in coincidence with the 30 and 60 days and immediately centrifuged at 3000 rpm for 15 minutes to obtain plasma. Plasma glucose levels (mg/dL) were determined by enzymatic colorimetric method using commercial kits (Wiener, Buenos Aires, Argentina). Fibrinogen (mg/dL) was determined by spectrophotometry using Ratnoff and Menzie's method [10] and insulin (uU/mL) by radioimmunoassay (National Diabetes Data Group, 1979). Analysis of lipid profile (mg/dL) was determined by enzymatic methods (Report of The National Cholesterol Education Program, 2000) and L-Citrulline by spectrophotometry [11]. The quantification of superoxide dismutase activity in red blood cell lysates was made by spectrophotometry, using Randox Kit [12].

Results are expressed as mean ± SE and after were analyzed with an ANOVA lineal model (Levene and Shapiro-Wilks test) for the continuous variables. A $p < 0.05$ level of significance was established in every case.

Results

The levels of plasma insulin are illustrated in Figure 1.

At the end of the experimental period, the group (D) (2.5 ± 4.52) showed a significant increase in plasma of the insulin levels ($p < 0.001$) compared with the control group (A) (4 ± 0.82) and with the injured groups for 30 (B) (4.52 ± 1.72) and 60 days (C) (6.22 ± 1.3) ($p < 0.01$). MS + HF group x 30 days (F) (13.7 ± 3.4) and group (E) (9.68 ± 1.61) showed a significant increase in plasma insulin levels ($p < 0.05$) compared with control (A) (4 ± 0.82) Insulin was significantly higher in the group (F) (13.7 ± 3.4) compared with group (E) (9.68 ± 1.61) as shown in the Figure 1.

The levels of plasma glucose are illustrated in Figure 2.

Chronic administration of fructose to 10% occurred in the experimental group (D) (176 ±17.3) produced a statistically significant increase in glucose levels ($p < 0.001$) compared with the control group (A) (115 ± 1.1) and with the injured groups for 30 (B) (120 ± 3) and 60 days (C) (114 ± 2.6) as noted in the attached Figure 2.

The levels of triglycerides are illustrated in Figure 3.

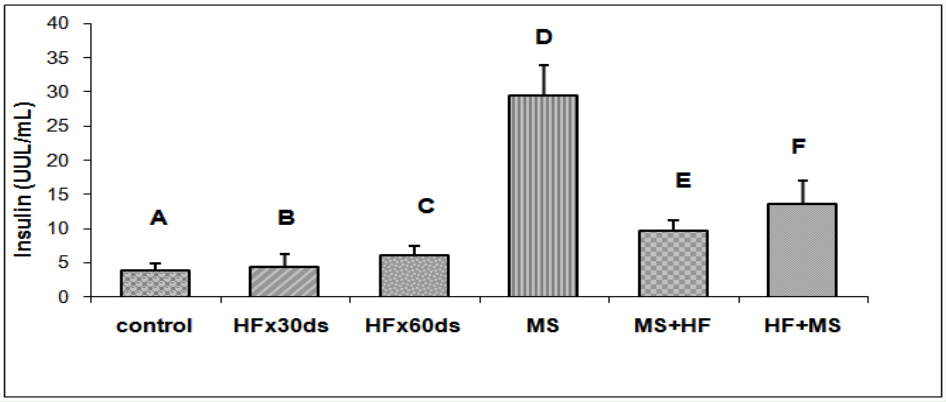

Figure 1: Insulin levels in rats con HF y MS.
ME ± SE: (A) vs (B) y (C) NS, (A) vs (D): ($p<0.001$); (C) vs (B): NS, (E) vs (F): NS; (A) vs (E) y (F): $p<0.01$.

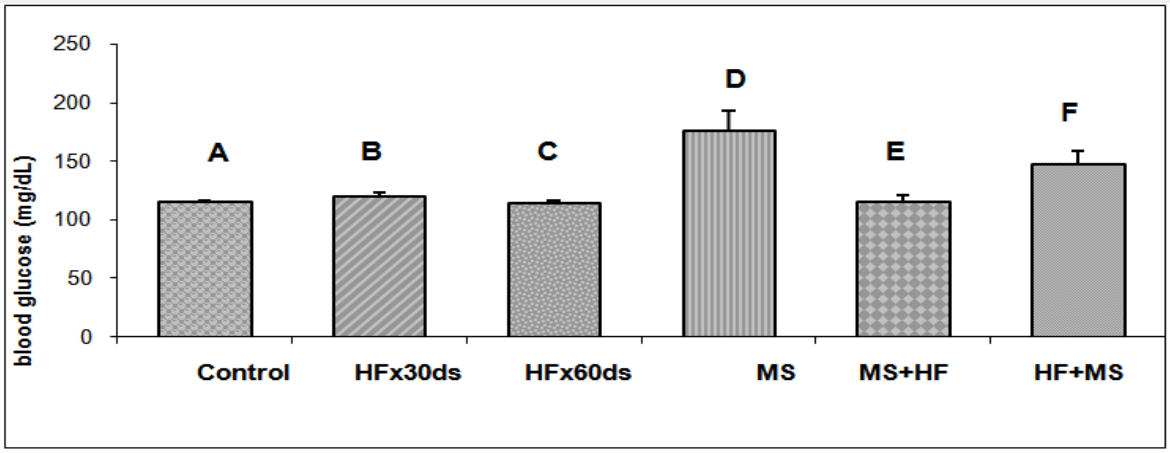

Figure 2: Blood glucose concentrations in rats with HF y MS.
ME ± SE: (A) vs (B) vs (C) NS; (A) vs (F) (p< 0,05); (A) vs (D) (p<0.001).

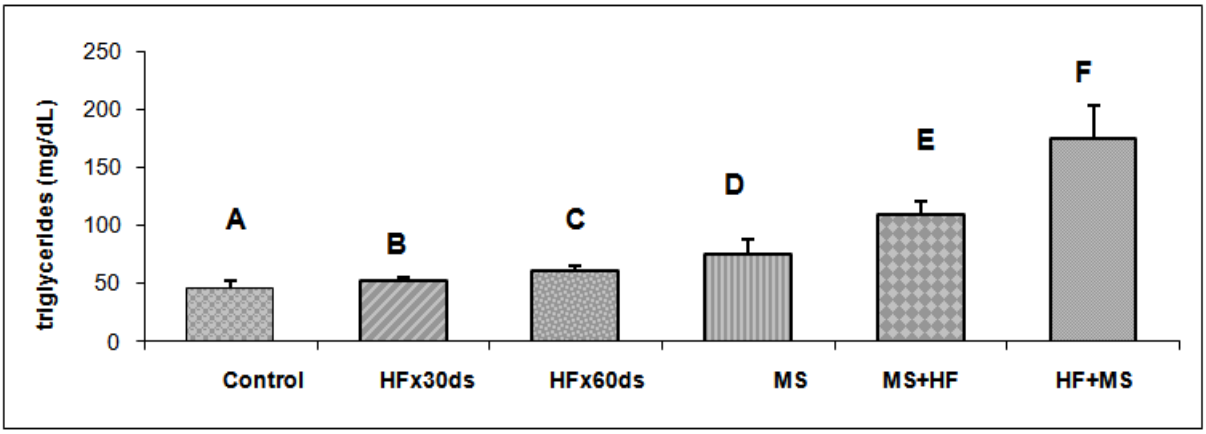

Figure 3: Values of triglycerides in rats with multiple tissue injuries by 30 to 60 days and in rats with MS.
ME ± SE: (A) vs (D), (E) and (F) p <0.001. A vs (B) NS. (B) vs (E) and (F) : p<0.001.

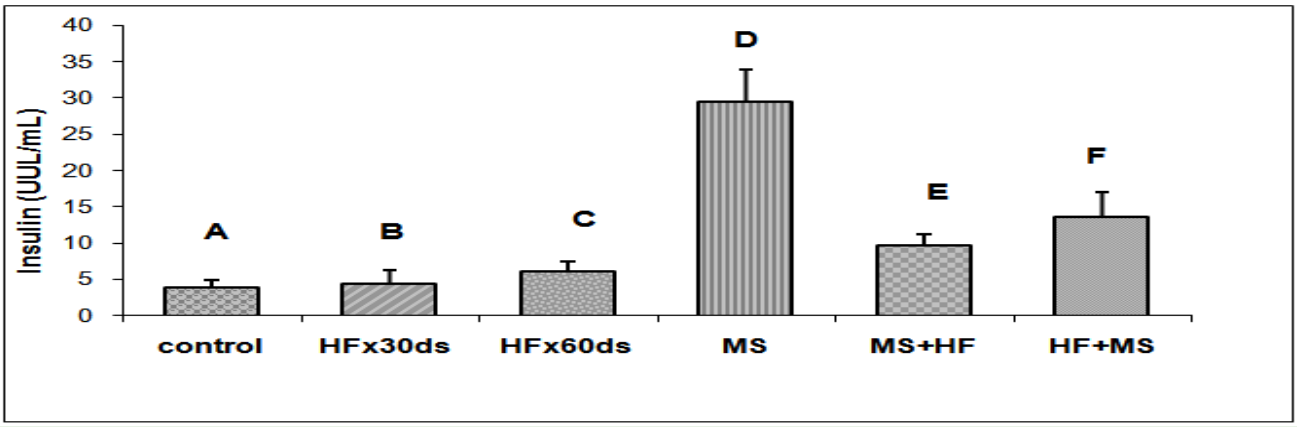

Figure 4: Levels of HDL in rats with HF and MS.
ME ± SE: (A) vs (D), (E) and (F): p<0.001; (A) vs (B): NS, (B) vs (C), (D), (E) and (F): p<0.001, (C) vs (D), (E) and (F): p<0.001.

In our results found a statistically significant difference when comparing the levels of triglycerides in the group with experimentally induced MS (D) (75 ± 12.9), group (E) (108.75 ± 12) and (F) (175 ± 28.3) respect control group (A) (46.2 ± 6).

There was no difference when compared group (B) (HF x 30 days) (52 ± 3) respect (A) (46.2 ± 6). A statistically difference was obtained when compared group (B) (52 ± 3) respect (E) (108.75 ± 12) and (F) (175 ± 28.3) ($p<0.001$).

The levels of HDL are illustrated in Figure 4.

Plasma HDL decreased in groups (D) (28.3 ± 1.14), (E) (17.3 ± 0.08) and (F) (35 ± 8.3) compared with control group (A) (61 ± 0.01). There was no difference when compared group (B) (HF x 30 days) (61 ± 0.01) respect (A) (61 ± 0.01). A statistically difference was obtained when compared group (C) (57 ± 0.02), (D) (28.3 ± 1.14), (E) (17.3 ± 0.08) and (F) (35 ± 8.3) respect (B) (61 ± 0.01) ($p<0.001$) as shown in the Figure 4.

The levels of fibrinogen are illustrated in Figure 5.

A statistically hiperfibrinogenemia was observed in group with HF x 30 days (B) (266 ± 9) and (E) (286 ± 23) compared with the control group (A) (203 ± 9) ($p<0.001$). On the other side, in groups HF x 60 days (C) (359 ± 11.7) group (D) (292 ± 11) and MS+ HF x 30 days (F) (295 ± 19) plasma fibrinogen levels increased when compared with the control group (203 ± 9) (A) ($p<0.001$).

The levels of L-citrulline are illustrated in Figure 6.

L-citrulline levels increased in groups (B) (4.24 ± 0.14), (C) (4.56 ± 0.09), (D) (4.01 ± 0.1), (E) (3.9 ± 0.12) and (F) (4.1 ± 0.2) compared with the control group (A) (3.03 ± 0.9) ($p<0.001$).

The levels of SOD are illustrated in Figure 7.

Increase plasma fibrinogen triggers the alteration of oxidative stress modifying the enzymatic activity of SOD. The persistent fibrinogen induces a significant increase in SOD enzyme activity in groups (B) (264 ± 7.6), (C) (225.42 ± 25), (D) (180.15 ± 6.3), (E) (248, 3 ± 6, 26) and (F) (215 ± 7, 3) respect the control group (A) (138, 5 ± 3, 6) ($p <0.001$). The persistence of vascular inflammatory stimulus

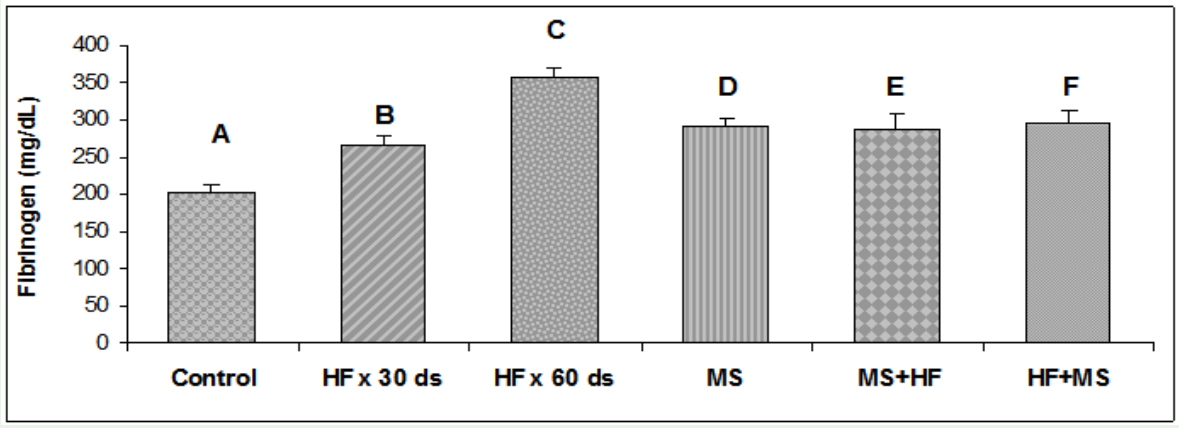

Figure 5: Behavior of fibrinogen in multiple tissue injured rats and rats with MS.
ME ± SE: (A) vs (B) and (E) $p <0.01$; (A) vs (C) (D) and (F) $p <0.001$.

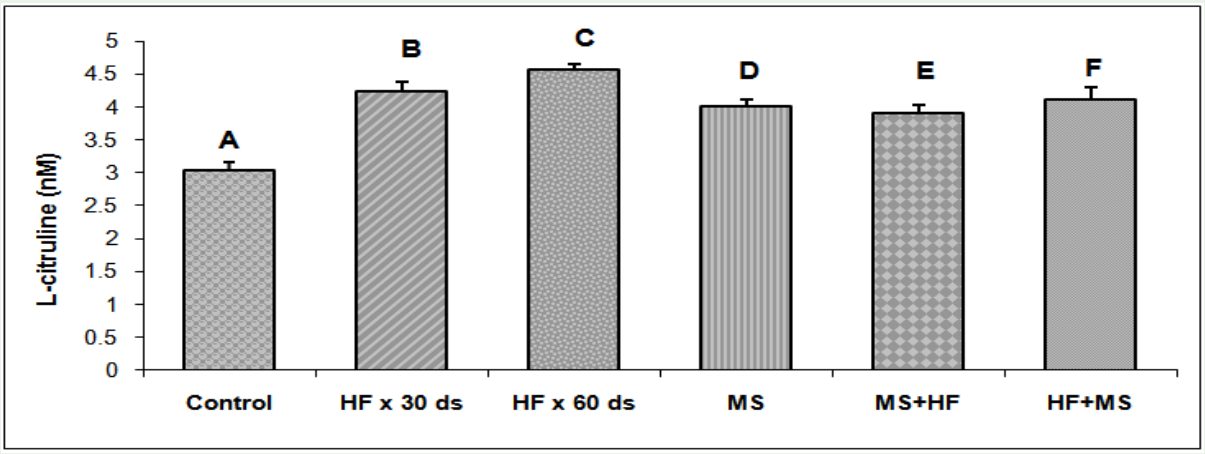

Figure 6: Levels of L-citrulline in rats with HF and rats with MS.
ME± SE: (A) vs (B) (C) (D) (E) y (F): $p < 0,001$.

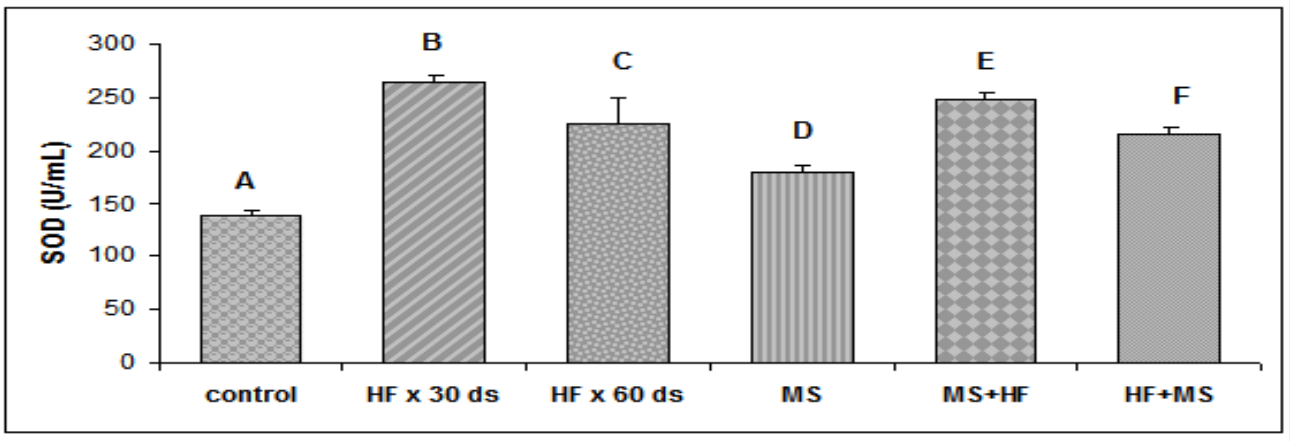

Figure 7: Activity of superoxide dismutase in rats with HF for 30 and 60 days and in rats with MS.

ME ± SE: (A) vs (B) (C) (D) (E) and (F) p <0.001; (C) vs (D), (E) and (F): NS.

level produced a significant increase of enzyme activity of SOD in all groups respect the control group. There was no difference when compared group (C) (HF x 60 days) (225. 42 ± 25) respect (D) (180.15 ± 6, 3), (E) (248.3 ± 6.26) and (F) (215 ± 7.3).

Discussion

Many in vivo studies have shown that hyperglycemia induces endothelial dysfunction in both normal and diabetic subjects [13]. Hyperglycemia produces oxidative stress by auto-oxidation of glucose with production of end products of oxigen free radicals.

On the other hand, is known the relationship between dyslipidemia and the potential risk of cardiovascular disease. It demonstrated the association between dyslipidemia, DM 2 and MS as a factor in the high cardiovascular risk with a characteristic changes of lipid pattern with low HDL and qualitative changes in particles of Low Density (LDL) as saw in our results. Moreover, in previous work has shown that increased fibrinogen synthesis via neuroendocrine and nervous to occur via stimulation of free nerve endings induced by tissue injury, was demonstrated in the experimental model that the increase in fibrinogen synthesis occurs extraadrenal mainly via inducing an increase in hepatic expression of it reflecting the increase in plasma concentrations reviled groups. Fibrinogens participate in early changes at the vascular wall, confirming their presence from the early stages of atherogenesis. This research correlates that fibrinogen is a atherogenic risk factor, and is also a good biomarker for the disease with a high predictive value of the same, as modified in early stages of the disease.

Another variable studied, L-citrulline showed a significant increase of its concentration in this experimental model, having no correlation with NO dosed under the same experimental condition.While the L- citrulline is considered a biological marker of atherosclerosis and its output is “X” molar respect to the NO production probably, the increase in plasma is due to L- citruline follow a different route to NO. Possibly the increase in L-citrulline, is a physiological mechanism by which tries to compensate for the low bioavailability of NO in the atherogenic process carried out in cells of the aortic vessel wall during the initiation of atherogenesis.

Endothelial production of reactive oxygen species, especially superoxide anions, is an important mechanism in endothelial dysfunction present in atherosclerosis. Increased oxygen species causes an increase in SOD enzyme activity with the consequent rise in the plasma. Increased production of free radicals and reactive oxygen species and / or decreased antioxidant defenses favors the oxidative process and accelerates the progression of atherosclerosis.

Studies made in experimental models have shown that the main pathogenic feature of the metabolic syndrome is insulin resistance. Rats receiving chronic fructose provide an interesting experimental model of induced insulin resistance through diet; it is postulated that vascular inflammation and oxidative stress are key components of pathophysiologic mechanisms involved in vascular changes associated with metabolic syndrome, such as demonstrated by our results.

On the other hand, MS is associated with a 5-fold increase in the prevalence of type 2 diabetes mellitus and 3 times for Cardiovascular Disease (CVD), being the main cause of death and disability in industrialized countries and its incidence is increasing [14]. Morbidity and mortality associated with these two diseases could unbalance the health budgets of many countries [15]. The potential importance of vascular wall inflammation in diseases, subclinical atherosclerosis and MS, as a therapeutic target remains an area not yet fully explored, where new knowledge on the involvement of inflammatory mediators may be relevant as the score validated risk assessment does not currently include these components, and their inclusion could assess the actual risk patients.

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