Impact of the Glucolipotoxicity *In vivo* and Insulinotoxicity Inducing in Fibroblast Aortic of Sand Rat (*Psammomys obesus*)

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Abstract

It was established that the sand rat *Psammomys obesus* is an experimental model of type diabetes 2 and dyslipidemia, in this *in vivo* study the sand rats were submitted to natural diet associated with fat (10%) and sucrose (20%) enriched diet /rat per day for 6 months.

The initial time and once of the months, plasma parameters were evaluated and at 6th month aortic morphology were examined. To simulate hyperinsulinemic stress, the effects of high dose of insulin 10 UI/ml during 6 h were investigated on subcultured aortic control fibroblasts which were implicated in extracellular matrix remodeling during the atherosclerosis process. In this work report, the proliferation rates, morphological aspects of apoptosis, few markers of oxidative stress (malonaldehyde and nitric oxido) and type I and III collagen contained in extracellular compartment were evaluated. Others animals received a high fat high sucrose diet showed hyperglycemia associated with hyperinsulinemia, dyslipidemia, hyperproteinemia, increase of cardiac markers, low and very low density lipoproteins and decrease high density lipoprotein versus control group. The aortas of diabetic animals were exhibited an important disorganization and remodeling of vascular extracellular matrix. In *in vitro* results indicated a decrease in cellular proliferation rate, the apparition of some morphological characters of apoptosis (hypertrophy fibroblasts, hypercondensation chromatin, oncosis) and increase of malonaldehyde, total nitrite production in intracellular compartment and increase of total proteins, type I and III collagen especially type III were observed in extracellular compartment. The high fat high sucrose diet induced diabetes associated with hyperinsulinemia and development of extracellular matrix remodeling in sand rats similar to human advanced atherosclerotic lesions. The high insulin dose induced an increase of both some markers of oxidative stress caused apoptosis and extracellular type III collagen especially.

Keywords: *Psammomys obesus*; Insulin; DT2; High fat and high sucrose; Oxidative stress; Apoptosis

Introduction

Atherosclerosis is a multifactorial disease that is associated with many risk factors including dyslipidemia, diabetes, obesity and hypertension. Chronic hyperglycemia characteristic of type 2 diabetes is responsible for the accelerated atherosclerosis process and increased cardiovascular risk [1], it is established that cardiovascular complication are the major causes of diabetic patients morbidity and mortality worldwide [2,3].

In case of diabetics, atherosclerosis development occurs earlier and in more severe form [4,5], it is established that diabetes induced various defects in extracellular matrix (10 aouli 227). Robert and Robert [6] have described quantitative and qualitative alterations of the level of total collagen. Theses variations would imply a decrease of collagen solubility [7] and the premature ageing of diabetic connective tissue [8].

Type 2 Diabetes and insulin resistance are risk factors for atherosclerosis characterized by high concentration of insulin and/or glucose [9]. Insulin resistance contributes to the pathogenesis of atherosclerosis, the role of hyperinsulinemia needs further investigation in this regard. It has recently been shown that hyperinsulinemia inhibited the release of nitric oxide (NO) by decrease the expression of endothelial nitric oxide synthase (e-NOS) [10].

Oxidative stress, a potentially harmful imbalance between the level of pro-oxidants and anti-oxidants in favor of the former, is thought to be an important source of vascular injury [11]. All oxidative reactions are a continuous source of potentially cytotoxic reactive oxygen species (ROS), which play an important role in living systems both through their beneficial and detrimental effects [12]. Under physiological conditions, ROS are fully inactivated by an elaborate cellular and extracellular antioxidant defense system [13]. However, under certain conditions increased generation of ROS and/or reduction of the antioxidant capacity leads to enhanced ROS activity and oxidative stress. Oxidative stress can cause cellular injury and tissue damage by promoting several cellular reactions, e.g. lipid peroxidation [13,14]. Diabetes mellitus is characterized by increased production of ROS, sharp reduction in antioxidant defense and altered cellular redox status.

We found it interesting to analyze *in vivo* effect of insulin-resistance and diabetes of *Psammomys obesus* when receiving a high fat and high

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sucrose diet. Earlier studies have shown that the sand rat (*Psammomys obesus*), naturally feeding on low-caloric plants, was a good animal model for developing spontaneous obesity, diabetes and dyslipidemia, when submitted to dietary stress [15].

Secondly, we investigated the effect of high insulin concentration during 6 h on subcultured fibroblast aortic control to simulate the hyperinsulinemia that characterizes the obesity and diabetic state. Some markers of oxidative stress and extracellular total collagen have evaluated and type I and III collagen were separated.

**Materials & Methods**

**Biological material**

*Psammomys obesus* gerbils, also known as the "fat sand rat," were captured in the Algerian Sahara, and housed in individual cages in an animal facility (university of Algiers) under controlled temperature and lighting conditions, with free access to food and water. During a 2 week acclimation period, the animals were fed the halopholic plants, rich in water and mineral salts, (Dalay and Dalay, 1973) that they normally eat in the desert [16-18]. Thereafter, they were separated into three groups. The first group (n = 8) and the second group (n = 5, for in vitro study) were fed natural plants from the same halophilic family during 6 months , but growing along the edge of sea (salicornia ; composition of egg per 100 g: water 80.8 g ; mineral salts 6.9 g ; lipids 0.4 g ; proteins 3 g ; carbohydrates 8.4 g ; and 45-50 kcal/100 g). The third group (n = 8) received a high-fat (10%) and high sucrose (20%) diet, comprising the saccharose diet. Earlier studies have shown that the sand rat (*Psammomys obesus*), naturally feeding on low-caloric plants, was a good animal model for developing spontaneous obesity, diabetes and dyslipidemia, when submitted to dietary stress [15].

**Culture of adventitial fibroblasts:** This technique was used according to Bourdillon et al. and Aouichat et al. [18,21,22]. Cultured adventitial fibroblasts, explants were obtained from control *P. obesus* (non diabetic). They were prepared after removing media collagenase action at 0.1% (type IA Sigma Aldrich) and incubated in cultured flask containing Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, USA), supplemented with 10% fetal calf serum (FCS) (Sigma,USA), 1% antibiotics (streptomycin 50 µg/ml, penicillin 50 IU/ml), 1.2% L-glutamine at 200 mM (Gibco, USA). The explants are maintained into the incubator at 37°C under air: CO₂ (95%: 5%) atmosphere until they reached confluence. Then, they were trypsinized (trypsin at 0.1%and subcultured. For the experiment, the hyperinsulinic stress was induced in adventitial fibroblasts at the 7th passage. After incubation during 48 h, the medium was eliminated and the cells were submitted to DMEM 10% FCS supplemented with 10 UI / ml insulin (Novo nordisk) during 6 h and 24 h in DMEM prepared with 1.2% L glutamine, 1% antibiotic without FCS. Flasks of control cells were subjected to the same conditions without insulin.

**Electrophoresis of plasma lipoproteins:** The detection and quantification were performed by kit REP. LIPID Lp (a) of the KPC (cassette COBAS INTEGRA) by horizontal electrophoresis on agarose gel (1%) using the method of Kalwakami [19].

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**Histology of the aorta:** Animals were killed at the end of the experiment (n = 8) for control group and (n = 8) for submitted at high fat and high sucrose diet group. Specimens of thoracic aorta were fixed in Bouin’s solution, dehydrated, embedded in paraffin and cut at 5 µm. The sections were stained with Masson’s trichrom [20].

**Determination of Malondialdehyde (MDA):** The MDA was measured in the intracellular compartments of control fibroblasts during 48 h of incubation and submitted at 10 UI/ml of insulin, after reaction with thiobarbituric acid (TBA) (Sigma) [9]. Intracellular compartments of control fibroblasts and those submitted to insulin were centrifuged at 10,000 g for 20 min at 4°C in buffered (Na2HPO4/NaH2PO4) 0.2 M, pH 6.5. The MDA contained in the supernatant in presence of 10 % TCA reacts with TBA and causes the formation of a complex read at 532 nm.

**Determination of Nitric Oxide (NO):** NO formation is typically and indirectly by determining the concentrations of nitrates and nitrates that are products of oxidative degradation of NO. The intracellular compartments of control fibroblasts and those submitted to insulin were deproteinized by centrifugation at 10,000 g for 10 min at -20°C. The determination of nitrates and nitrites is produced directly from the obtained supernatant.

The Griess reaction only allows the measurement of nitrates. Nitrates should be reduced to nitrite prior to be quantified. The concentration thus measured represents the sum of nitrates and nitrites. The conversion of nitrates to nitrites is based on reduction reaction of by cadmium (Fluka) and regenerated using a solution of CuSO4 in 5 mM glycine-NaOH buffer, in contact for 5 min. The nitrates contained in the intracellular compartments of control fibroblasts and those submitted to insulin were deproteinized by centrifugation at 10,000 g for 10 min at -20°C. The determination of nitrates and nitrites is produced directly from the obtained supernatant.

**Total proteins and collagen:** The subcultured fibroblasts control and submitted at insulin, for 24 h in medium cultured without fetal calf serum defined of total proteins and collagen ; extracellular fractions were submitted to 2 successive dialyses, at 4°C for 24 h, respectively against running water and 0.5 M of acetic acid. A first aliquot was taken to assess that rat of total protein.
To determine the total collagen, samples extracellular (ECC) compartments were treated at the end of the incubation by a dialysis pepsinization (200 μg/ml of pepsin; biochemical) against 0.5 mM acetic acid at 4°C for 24 h [24-26]. The total proteins and pepsino-resistant proteins (collagen) contained in the ECC reacted with the Bradford reagent, resulting in the formation of a blue colored complex legible at wavelength of 595 nm [27].

**Densitometric analysis of α1(I+III) and α2 (I) collagen chain and collagen type:** After pepsination, the ECC was lyophilized and resuspended in buffer solution (Tris- HCL 0.05 M, sodium dodecyl sulfate (SDS) 14%, bromophenol blue 0.05 %, and EDTA 2 mM). The collagen samples were separated by vertical electrophoresis on 10% polyacrylamide gel (SDS-PAGE) in reducing conditions with 0.25% β-mercaptoethanol according to the procedure of Laemmli [28]. Gel were stained in colored solution (Cooamassie blue 0.025 %, propanol 25 %, acetic acid 10 %) for 24 h and discolored in 10 % acetic acid; α1 and α2 collagen chain levels were evaluated by densitometry.

**Statistical analysis**

Data are expressed as mean values ± SEM and were compared with analysis of variance (factor: time) and student t-test. A significant difference was considered as P < 0.05.

**Results**

**In vivo study**

In animals subjected to natural diet supplemented with rich high fat high sucrose diet (NDFS) for 6 months of experiment, we recorded a significant increase in body weight in the first months of experiment followed by a very highly significant increase at the end of the experiment compared to control groups submitted to the natural diet (ND) (236.06 ± 1.26 vs 92.38 ± 0.52; p <0.0001) (Figure 1).

In our experiments, we noted an increase rate of glyceremia in *Pobesus* submitted to NDFS until the end of the experiment (2.18 ± 1.29 g / l vs 0.58 ± 0.07 g / l (p ≤ 0.01) (Figure 2A). At 3th month and until 6th month, we recorded a significant increase of triglycerides (441.67 ± 124.05 mg / dl vs 100.84 ± 5.89 mg / dl in control group (p < 0,001)) (Figure 2B). We noted an increase of cholesterolemia greater at 6th months of experiment from 2.01 ± 0.49 mmol / l in control group to 10.84 ± 0.07 (p <0.0001) (Figure 2C) indicating insulin resistance. Insulin returned to near-baseline values at month 6. This state revealing the evolution towards insulin deficiency.

Analysis of plasma lipoproteins in *P. obesus* submitted to NDFS revealed an important disturbance of all plasma lipoproteins report in hypertriglyceridemia and hypercholesterolemia. Our results indicated increase levels of atherogenic lipoproteins (LDL, VLDL) until the end of the experiment (89.60 ± 4.62 % vs 36.90 ± 0.17 % at the initial time (P < 0.001)). The cardioprotective lipoprotein (HDL) showed a very highly significant decrease at the end of experiment (10.39 ± 4.62 % vs 63.13 ± 4.22 % at the initial time (P <0.0001)), (Figure 4A,B).

The observation of histological sections of thoracic aortas of animals submitted to NDFS revealed an important lesions which showed hypertrophy of endothelial cells, lipid and collagen deposition (Figure 5B-5D). The disorganization of the media was marked by the accumulation of extracellular compartment (ECM) especially collagen sign of remodeling, breaking of elastic fibers responsible of the occurrence of aneurysms, increased density, migrated and disoriented smooth muscle cells, (Figure 5B,5C), thickening of the tunica adventitia which showed a significant infiltration of fibroblasts in the media and a very significant secretion of collagen marking an ECM remodeling (Figure 5E and F) compared with the control group (Figure 5A).

Our in vitro results showed a highly significant decrease of the rate proliferation in cells exposed at 10 UI/ml of insulin, compared to their controls 0, 35 ± 0.44 vs X10<sup>1.07</sup> ± 0.11 (p <0.0001) (Figure 6A). We noted also an increase in the number of nucleoli in the fibroblasts submitted at insulin (Figure 6B).

Observation under the inverted microscope enabled us to note that the cells exposed at 10 UI/ml of insulin during 6 h are characterized by a vacuolization of the cytoplasm, sign of oncosis (Figure 7C), an hypertrophy of cell and a hypercondensation of chromatin sign of apoptosis (Figure 7B), compared to the control. A morphological analysis of the apoptotic cells is carried out by coloring with orange acridine. Moreover, compared to the control, the fibroblasts subjected at insulin showed an intense fluorescence in the perinuclear area, marking the hypercondensation of chromatin (Figure 7E,7F) and nuclei fragmentation (Figure 7G).

The rate of total nitrite measured in the intracellular compartment of adventitial fibroblasts control submitted to 10 UI/ ml insulin during 6 h indicated an increase of 700 % (p <0, 0001) (Figure 8).

The evaluation of products of the MDA levels contained in the intracellular compartments of aortic fibroblasts exposed at insulin, showed an increase of 266 % (p <0.0001) (Figure 9).

Our results showed a very significant growth of the quantity of total proteins contained in the intracellular compartment of adventitial...
fibroblasts submitted at insulin compared to corresponding controls (Figure 10).

The high insulin concentration during 6 h induced a pronounced increase of the level of total collagen contained in the extracellular compartment (269%; p <0.0001) (Figure 11).

Electrophoresis profiles showed the presence of 4 bands in the extracellular compartment, just as in treated control fibroblasts but at a different absorbance. The collagen III fibers were mainly represented by three chains α1, the collagen I fibers were represented by 2 chains α2 and 1 chain α1. In our experimental conditions, we noted an increase in the bands absorbance corresponding to α1 chains of collagens I and III relative to the α2 chain of collagen I in the extracellular compartment (ECC) of aortic fibroblasts exposed to insulin compared to corresponding controls. The evaluation of the absorbance in the ECC fibroblasts subjected to insulin an increase in the absorbance of the bands as well as an increase in the absorbance of α1 chains of collagen I and III compared to α2 chain of collagen I. Collagen III is thus more represented than collagen I (Figure 12).

Figure 2: Evaluation of, glycemia (A), triglyceridemia (B), cholesterolemia(C), total protein (D) in Psammomys obesus for 6 months initial time (0), a subjected a natural diet (ND) or natural diet comprising at high fat and high sucrose (NDGL) diet.

Figure 3: Evaluation of, CPK (A), CKMB (B) and insulinemia (C) in Psammomys obesus for 6 months initial time (1), a subjected a natural diet (ND) or natural diet comprising at high fat and high sucrose (NDGL) diet.
Discussion

In vivo study which involved the impact of high fat high sucrose diet during 6th month in sand rat Psammomys obesus and on the other hand, in vitro study that examined the influence of a high dose of insulin (10 UI/ml) on the physiology of aortic fibroblasts of Psobesus.

In vivo results

The body weight study, we noted an elevation from 6th months of experiment confirm the results obtained by Aouichat and al. (2001) in Psammomys subjected to a high carbohydrates diet [29], to a high fat diet [30-32], to a rich in sucrose diet [33] and high fat high sucrose diet [34].

In our study, we founded an hyperglycemia associated with hypertriglyceridemia, hypercholesterolemia and hyperinsulinemia characteristic of type 2 diabetes [29,34]. According to Reaven et al. and Bourgoin [34,35] in rats subjected high fat and high sucrose diet. According to Park et al. a high fat and high sucrose diet administrated to female rats creates an imbalance oxidant / antioxidant [36-38]. Confirm the result obtained by Bourgoin, who noted that an hypercholesterolemia and an hyperglycemia may induce uncoupling of eNOS and also lead to excessive production of superoxide anions O2- in endothelial cells in vitro induces the installation of an oxidative stress. A high fat diet induced the accumulation of TG associated with increased membrane localization of receptor free fatty acid, CD36 that allow myocardial uptake of lipids [39,40]. The work of Bedard et al. and Perreault et al. have shown both that the iNOS is associated with a decrease in glucose transport stimulated by insulin in skeletal muscle isolated from rats fed a high fat diet and also is involved in the development of metabolic disorders during chronic inflammation such as obesity and atherosclerosis [41,42]. In 1999, Jiang et al. have shown that hyperinsulinemia has the potential atherogenic because its ability to stimulate the MAPK cascade [43]. Indeed, compensatory hyperinsulinemia and hyperglycemia that accompany insulin resistance may have an important direct impact on the body and cardiovascular complications. In addition, several biochemical pathways including glucose autoxidation, protein glycation and activation of the polyol pathways stimulated by hyperglycemia can cause the production of free radicals derived from oxygen and affect the bioavailability NO [44]. Hyperglycemia can play an important role in the deterioration of endothelial function commonly reported in individuals with diabetes or high blood pressure and obesity in animal models of insulin resistant [34].

Our results show an increase in cardiac marker CPK. The elevated CPK is an indicator of cardiac complications characterized by smooth muscle cells lysis [45]. Indeed, histological sections of the heart of P. obesus showed cardiac myocytes and fibroblasts to apoptotic or necrotic. In a state of necrosis, the cells release enzymes and proteins in particular phospho-creatine kinase in the blood. Muscle weakness corresponds to high levels of CPK, excessive levels indicate a rapid degeneration of the muscle by apoptosis or necrosis, this increase is related to the increase of NO due to hyperglycemia and hyperlipidemia [46].

Similarly, our results showed an increase in the other cardiac marker, the CKMB. Our results confirm the result obtained by

Figure 4: Evaluation in plasma lipoproteins VLDL-LDL (A), HDL (B) in in Psammomys obesus for 6 months initial time (1), a subjected a natural diet (ND) or natural diet comprising at high fat and high sucrose (NDGL) diet.

Figure 5: Morphological structure in aorta of control group (A) and submitted at high fat and high sucrose (B,C,D,E,F) of Psammomys obesus during 6 months (A,B,C,E : GX400); (D and F: GX1000).
Chinnapu et al. (2004) in patients with acute coronary syndromes, in hypertensive patients and in patients who underwent coronary bypass surgery [47]. The enzymes related to the function of the heart such as CKMB are considered an indicator of the death of cardiac myocytes.
Our results showed the occurrence of dyslipidemia. Indeed, electrophoresis profile analysis of *P. obesus* plasma lipoproteins subjected to a high fat and high sucrose diet showed a very significant growth in LDL-VLDL and very significant reduction of the HDL at the end of the experiment, according of Hamlat et al., (2006) in *Psammomys* and Rat submitted a high fat diet [50]. The increased VLDL level in type 2 diabetes, is created by the increased of novo lipogenesis in the hepatocyte and the increase expression of the transcription factor, SREBP-1c which activates enzymes of lipogenesis including ACC1 [51-53]. Moreover, the increase in TG-rich VLDL in type 2 diabetes induced activation of the CETP which promotes the exchange of TG against cholesterol giving rise to these small dense LDL cholesterol enriched. LDL particularly atherogenic small increased risk of coronary events [54]. The TG-rich lipoproteins promote the overexpression of pro-inflammatory phenotype of macrophages and induce apoptosis of endothelial cells [55]. In addition, small dense LDL accumulates preferentially in macrophages to the advancement of foam cells, they have an increase oxidizability and greater affinity for intimal proteoglycans facilitating their retention in the arterial wall [56]. In addition, another marker of the lipoprotein type 2 diabetes is the

and slightly increased after heart surgery following an associated risk of mortality via an increase in long-term [48]. The CK-MB is in the cytosol and facilitates the passage of high energy phosphates out of the mitochondria via ATP K+ channels that prevent ischemia, it is distributed in many tissues, even in skeletal muscle. The complex Phosphatidylinositol-3-kinase/Akt is currently estimated to be one of the main factors responsible for the intracellular antiapoptotic signaling and cell survival [49].

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decreased concentration of HDL [57,58]. These have a key process with their biological atheroprotective properties and they induce cellular cholesterol efflux, and promote the anti-oxidative, anti-inflammatory and anti-apoptotic. The efflux of cholesterol is carried by HDL so in the membranes of peripheral cells especially muscle cells via interaction with ABCA1 and ABCG1 transporters and hepatic cells via SR-B1 transporter [59,60]. The major goal of the physiological antioxidative action of HDL is to inhibit LDL oxidation [61,62]. The antioxidative activity of HDL is related to the presence of apolipoproteins that have an antioxidative activity including the API, the ApoE, ApoJ of the ApoA- II and ApoAIV and enzymes particulary PON1, PAF-AH, LCAT and GPx. The anti-inflammatory activity of HDL is illustrated by the ability of HDL to reduce the expression of adhesion molecules in endothelial cells and inhibit their adhesion induced by cytokines [62].

Regarding the histological sections of the aorta, we observed at the intima the thickening due to hypertrophy of endothelial cells and thickening of the endothelial space under due to deposition of collagen and fat indicator of remodeling important to the intima. According to Hamlat and Han et al. [30,31] by a high fat diet and sucrose diet which have shown in rats and pigs diabetic loss of elastin content and increased intimal fragmentation due to the action of metalloproteinases [63].

According with [63], we observed several alterations in relation with an important pathophysiological state of oxidative stress marked by platelet activation, vascular hypertrophy, proliferation and migration of vascular SMCs. These changes contribute to the development and progression of atherosclerosis and cardiovascular disease [34].

The media has a marked thickening with deposits of collagen, a major realignment of leaf springs up to the total lysis as well as infiltration of adventitial fibroblasts in the medium caused by the rupture of elastic lamina including the external elastic lamina. Large lipid deposits are observed between the leaf springs. In addition, the intense accumulation of collagen and fibroblasts in the media is a sign of matrix remodeling and these results are in accord with Anita M et al. [64]. All these changes indicate the birth of atheroma and the development of aneurysms that indicate a major remodeling of the media [65]. The progression of atherosclerotic lesions, migration and proliferation of SMCs of the media to the intima produce growth factors and participates in the formation of the fibrous cap of atheroma [65]. In addition, hyperglycemia contributes to diabetes-related interstitial fibrosis by increasing collagen produced by adventitial fibroblasts [64,65].

Our in vitro study enabled us to analyze some physiopathological aspects of the adventitial fibroblasts of P. obesus in culture under the influence of high dose of insulin. The fibroblasts exposed at 10 U/ml of insulin showed a decrease in the rate of proliferation compared to the control. Our results are in agreement with that of Jeschke et al. who observed a decreasing of the proliferation rate in hepatocytes in culture from 20% at 10 U/ml insulin compared to corresponding controls [66], in β cell proliferation in response to insulin resistance [67-69], and in β cell proliferation subjected to insulin [65]. In contrast, Muller and Coll observed that increasing the concentration of insulin or supraphysiological doses of insulin stimulate the proliferation of β cells [70], a mitogenic effect of insulin and anti-apoptotic β cells at doses between 2-20 nM. However, beyond these doses, stimulate opposite effects [66,71,72].

In our morphological analysis, we noted in the cytoplasm vacuolization of fibroblasts exposed at insulin after staining with MGG. Our results confirm the result obtained by Boumaza et al. in fibroblasts aortic P. obesus in culture exposed at hydrogen peroxide to 1.2 mM [73,74], in the aortic fibroblasts in culture submitted a high dose of glucose (0.6%) during 7days [33], Weidong and coll observed the same images in vascular endothelial cells subjected to the insulin dose-dependent 300 µU/ml and 3000 µU/ml after staining with alizarin red. We also observed cytoplasmic vacuolization of cells subjected to insulin and it is linked to the increased production of reactive oxygen species induced by high doses of insulin that increase oxidative stress [75]. The monocyte precedes apoptosis [66]. Apoptosis is associated with intracellular oxidative stress [74,76,77], an imbalance in the concentration of Ca++ [74,78,79] and increased expression of genes involved in apoptosis such that: cufs and c-myc [79,74]. A high dose of insulin in endothelial cells induces oxidative stress and inflammatory response [80,81].

In our study, we noted an increase in production of nitric oxide ICC of fibroblasts submitted at insulin, this increase can be explained by impaired membrane permeability due to lipid peroxidation, the insulin stimulates the production of NO in human umbilical endothelial cells and increased expression of eNOS in human aortic endothelial cells (HAEC), its long-term expression induces a vasodilator effect [82,83], by the same authors noted an increased expression of eNOS dose-dependent in human aortic endothelial cells in culture subjected to 100, 1000 µU/ml insulin, and in endothelial cells of human umbilical vein subjected to 100 nM insulin for 20 h [82-85]. The stimulation of NO production under the effect of insulin has been shown in preparations of endothelial cells in culture and in microvessels of normal rats [82]. Insulin stimulates NO production by endothelial cells by stimulating eNOS, activation of eNOS by activating PI3K/AKT leading to vasorelaxation [86]. The insulin stimulates the production of NO by means of PI3K [82], the NO may have an antithromrogenic effect and inhibit L. NG monomethyl arginine (L. NMNA) that a proatherogenic effect in animals [87]. Furthermore, pathophysiological stimuli such as hypercholesterolemia, hyperglycemia, oxidized LDL and peroxynitrite, have been identified in vitro can induce uncoupling of eNOS and thus lead to excessive production of O2-in endothelial cells [34].

In our study, we have analyzed the effect a high dose of insulin on the production a lipid peroxidation marker (MDA) in the intracellular compartment (ICC) of fibroblasts; we noted a very significant growth production of MDA compared to the control. Our results confirm those of Mark et al. (2005) who observed that hyperinsulinemia induces an increase the rate of lipid peroxidation [88]. The oxidative stress can cause cell and tissue damage by lipid peroxidation and DNA damage [87,89]. The increase of oxidative stress and insulin resistance are observed in patients with type 2 diabetes [90,91]. The MDA levels increase in Wistar rats injected with 0.1 U/ml insulin, after a period of 180 min, from the time of injection [92].

In our study, we found an increase of total protein and total collagen rate in the ECM of fibroblasts submitted at insulin. Our results confirm those of Hesketh et al. showed that administration of 1 mU/ml insulin in 3T3 cells in culture, increases protein synthesis dependent time after 1 pm and 3 pm to incubation [93]. Insulin inhibits protein degradation by metalloproteinases [39,87] and induced overexpression of VCAM1 via the pathway of P38 [76,90] and ICAM, PECAM and the selectins via the MAPK pathway [81,86,94,95].

The Typing of collagen showed an in-crease and intensification of bands corresponding to the γ fragments representing the trimer chain α1 (coll I and III), the β fragments representing the dimeric chain α1 (coll I and III), α1 chains (coll I and III) and α2 chain (coll I). Aouichet et al. observed a large increase in type I and type III collagen in cultured...
aortic smooth muscle cells diabetic of *Psammomys obesus*, Berdjia et al. observed an increase in type I and type III collagen in cultured aortic fibroblast control of *P. obesus* submitted at high concentration of glucose (0.6%), Chen et al. (2000) noted an increase in α1 chain after diabetes onset [96], insulin growth factor increase the expression of a collagen in a cardiac fibroblast [97].

**Conclusion**

The glucolipotoxicity administered to animals by high fat and high sucrose diet revealed very metabolic disorders including hyperglycemia and hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia and hyperproteinemia reflecting the severity of disorders recorded, marking the installation of type 2 diabetes. The increase of CPK and CKMB levels has shown a dramatic indicated the occurrence of cardiomyopathies. Analysis of plasma electrophoretic profiles revealed an increase of LDL-VLDL and decreased of HDL.

The study of hyperinsulinemia on fibroblasts aortic *in vitro* exposed at 10 U/ml insulin during 6 h after 48 h of incubation showed a decrease in proliferation. Morphometric analysis revealed an increased number of nuclei and increased cellular hypertrophy adaptation to a stressful situation, hypervacuolisation characteristic of oncose, an increase in type I and type III collagen in cultured aortic smooth muscle cells. Influence of insulin. Int J Exp Diabetes Res 2: 37-46.

It would be interesting to characterize some key molecules implicated in insulin resistance (Glut4, IRS1), in inflammation (VCAM, ICAM, MCP1) and apoptosis (caspases family).

**References**

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