

## ATHEROGENIC INDEX OF PLASMA (AIP): CAN BE A BIOMARKER FOR EARLY ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE IN EXPERIMENTAL MODELS OF OBESITY RATS?

Andreea Clim<sup>1</sup>, Minela Aida Maranducă<sup>1,4\*</sup>, A. C. Pînzariu<sup>1</sup>, Ludmila Lozneau<sup>2</sup>,  
A. Alexandru<sup>5</sup>, I. F. Faur<sup>5,6</sup>, R. A. Băisan<sup>7</sup>, A. Szilagyi<sup>3</sup>, B. I. Tamba<sup>3</sup>,  
D. N. Șerban<sup>1</sup>, Ionela Lăcrămioara Șerban<sup>1</sup>

“Grigore T. Popa” University of Medicine and Pharmacy Iasi, Romania  
Faculty of Medicine

1. Department of Morpho-Functional Sciences (II) - Division of Physiology

2. Department of Morpho-Functional Sciences (I) - Division of Histology

3. Advanced Research and Development Center in Experimental Medicine (CEMEX),

“Sf. Spiridon” County Clinical Emergency Hospital Iasi, Romania

4. Internal Medicine Clinic

“Victor Babes” University of Medicine and Pharmacy Timisoara, Romania

5. Faculty of Medicine / Department of General Surgery

6. Emergency County Hospital, Timisoara, Romania / II<sup>nd</sup> Surgery Clinic

“Ion Ionescu de la Brad” University of Agricultural Sciences and Veterinary Medicine Iași,

7. Faculty of Veterinary Medicine / Department of Clinical Sciences

\*Corresponding author. E-mail: minela.maranduca@umfiasi.ro

ATHEROGENIC INDEX OF PLASMA (AIP): CAN BE A BIOMARKER FOR EARLY ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE IN EXPERIMENTAL MODELS OF OBESITY RATS? (Abstract) **Objective:** Our experimental study evaluated the influence and correlation among the Atherogenic index of plasma and early histological endothelial dysfunction in an experimental model of obesity rats. **Materials and methods:** Twenty-one Wistar rats, male, aged 4–6 months (450-550g), were allocated into III groups. The control group consumed exclusively a standard food, while the II group was administered HFD (High-Fat-Diet) with 2% cholesterol and the last group received HFD and treatment with Atorvastatin 20mg/kg/day by gavages. Before and after 4 weeks of oral administration of diet and treatment, serum lipid profile and glycemia were measured. The Atherogenic Index of Plasma, Castelli risk indices (I and II) and lipoprotein combined indices (LCI) are evaluated with a formula. Also, for histological study, aortas were extracted for the histological investigation and the principal extracellular matrix components, including collagen and elastin, were examined with a microscope for indications of aortic degeneration. Endothelial dysfunction was evaluated with biochemical levels of lipid profile, abdominal ultrasonography and histological study. **Results:** Four-week diet supplementation with HFD for obesity rats caused an increase in serum lipids levels; AIP levels indicate a high risk for cardiovascular events, histological aspect we observed endothelial damage, with degenerative modification in the aortic media, indicating by the dissociation of elastic fibers and accumulation of collagen. **Conclusions:** In our study, AIP, lipoprotein combined index and Castelli’s risk index I and II are correlated with endothelial injury. Obesity disturbs the lipid profile, increasing AIP in experimental models of obesity rats and can be a risk factor for endothelial dysfunction. Treatment with statin, healthy food and change in the

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modifiable risk factors can improve endothelial function and early stage of atherosclerosis.

**Keywords:** CARDIOVASCULAR RISK FACTOR, ATHEROGENIC INDEX OF PLASMA, EXPERIMENTAL MODELS OF OBESITY, ENDOTHELIAL DYSFUNCTION.

### **INTRODUCTION**

Cardiovascular diseases (CVD) are considered the primary cause of death in the population, affecting women and men. The World Health Organization (WHO) predicted that in 2023, 23.3 million people will die caused by cardiovascular disease (1). Also, diabetes mellitus, obesity and metabolic syndrome continue to be the main factors that limit life (2) and are a major cause of reduced quality of life and disability.

The normal equilibrium of the vascular endothelium is considered the fundamental part of vascular health. The vascular endothelium has been considered, for a long time, only a simple cellular barrier, new is defined as an active monolayer that covers the cells of the vascular lumen, thus separating the vascular wall from the circulating blood. Over the years, authors have offered multiple definitions of endothelial dysfunction – ED (3). Endothelial dysfunction is definitely like the imbalance between vasodilation and vasoconstriction with excessive reactive oxygen species (ROS) and proinflammatory agents and decreased nitric oxide (NO) bioavailability (4).

Atherosclerosis, dyslipidemia and oxidative stress (OS), at present, are considered risk factors associated with CV and are responsible for millions of deaths annually (5). A major cause of premature mortality among CVD is atherosclerosis. It is considered a chronic, systemic, complex disease, who is affecting the vascular endo-

thelium, characterized by lipid deposition in the arterial wall. Recent scientific research supports that atherosclerosis can be slowed or stopped, by initiating specific therapeutic measures and lifestyle modification and developing new therapeutic strategies capable of improving endothelial function and cardiovascular prognosis. The pathophysiology of atherosclerosis involves inflammation, abnormalities in lipoprotein metabolism and oxidative stress, which result in the accumulation of cholesterol esters in the macrophages of the arterial wall (6).

The primary contributing cause of coronary artery disease and endothelial dysfunction is dyslipidemia. Also, dyslipidemia plays a significant role in the carcinogenesis of various tumor types (renal, prostate and ovarian cancer (7)). Atherogenic dyslipidemia is characterized by an abnormal lipid profile, a low level of high-density lipoprotein cholesterol (HDL-C) and increased levels of total cholesterol (TC), also, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B and triglyceride (TG). Atherogenic dyslipidemia is the same, implicated in the pathogenesis of endothelial dysfunction, also, microvascular coronary pathology and atherosclerosis - contribute to the progression of atherosclerotic plaques (8, 9). Oxidized LDL (ox-LDL) contributes to atherosclerotic plaque development because it inhibits endothelium-dependent vasodilation by decreasing the activity of nitric oxide synthesis (10). The principal role of HDL is to protect

endothelial cells from the damaging consequences of LDL and increase their function (11). Recent studies have shown that hypertriglyceridemia inhibits endothelial function in a variety of ways (12). TG also promotes atherosclerosis by producing proinflammatory cytokines, fibrinogen and coagulation factors (13).

Furthermore, early diagnosis of endothelial dysfunction has an essential role in the prevention and detection of CVD; this could be a reversible first step in atherosclerosis. Endothelium dysfunction causes very small TG-high lipoproteins and residue molecules, recognized like ApoB which contain lipoproteins, to traverse the endothelium cell barrier, accumulating fat and atherosclerosis.

In 2001, Dobiášová and Frohlich discussed for the first time the Atherogenic Index Of Plasma (AIP) which represents the interaction between protective lipoprotein and atherogenic biochemical markers(14). A relatively recent marker of atherogenicity is the atherogenic index of plasma (AIP) and is evaluated with the classical formula *as log<sub>10</sub> (TG/HDL-C)*. It is considered a good biochemical predictor for cardiovascular pathology, which includes coronary artery physiopathology and a significant biomarker to estimate the risk of atherosclerosis.

According to previous studies, AIP was stratified into three groups: low (<0.11), intermediate (0.11–0.21) and high-risk (>0.21) (15). The AIP is considered a new complex lipid index that measures the equilibrium between the atherogenic and anti-atherogenic components and has also been the subject of research in recent years.

Previous research has proved that the atherogenic coefficient, calculated as Non-

HDL-C/HDL-C, Castelli risk indices I represent the report between [TC]/HDL-C and Castelli risk II - LDL-C/HDL-C had better predictability for CVD events and significant correlations with cardiovascular disease than simple lipid parameters.

The comprehensive lipid indexes, compared with single lipid parameters, such as non-HDL-C (TC minus HDL-C), TC/HDL-C, LDL-C/HDL-C, non-HLD-C/HDL-C (atherogenic index, AI), and TC\*TG\*LDL/HDL-C (lipoprotein combine index, LCI), are considered to be better predictors for cardiovascular diseases (16).

## MATERIALS AND METHODS

Twenty-one Wistar male rats, 4-6 months (450-550g), come from the “Cantacuzino” Institute, Bucharest, Romania and then accommodated within the CEMEX animal research “Grigore T. Popa University of Medicine and Pharmacy”, Iasi, Romania was included in an experimental study. The animals were placed in individually ventilated cages, with a specially designed room that had a temperature of  $20 \pm 4$  °C, standard humidity ( $50 \pm 5\%$ ) with 12 hours of cycle light/dark. They also had unlimited access to food and drink.

The experimental investigation was conducted according to the European Directive 2010/63/EU, with approval from the “Grigore T. Popa” University of Medicine and Pharmacy Iasi Ethics Committee (no. 277) and authorization from the Romanian National Sanitary Veterinary and Food Safety Authority (no. 61).

The present study was performed on three groups of 7 Wistar male rats, aged 4-6 months, weighing 150 - 200 g for group control and weighing 450 - 550 g for a group with obesity. After 2 weeks of ac-

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climatization, all the rats received food and treatment according to the experimental protocols.

The animals from the I group (control) have standard food and drink water ad libitum. The second and the III groups were High-Fat-Diet (HFD) with a 2% cholesterol-containing diet for 20 days and experimental groups (group III) were separately treated with Atorvastatin 20 mg/kg/day, every day, for the next 20 days, with gavages and water ad libitum. A recent study reported that using a dose of 20 mg/kg/day for the next Twenty days has not described any toxicity in rats and this can be a safe dose (17). Before being administered orally, Atorvastatin (Sortis, Pfizer, no NM4027) was taken in distilled water and administered once a day by gavages, using a sterile disposable for every animal feeding needle (20G × 1.1/2, Popper and Sons). Water was given to the control group via the same method as the treated groups. Every day, the quantity of water and food received was measured. Also, and on the same day, every week, body weight was measured.

Before and after 4 weeks of oral administration of Atorvastatin levels of lipid parametric (Triglyceride, Total Cholesterol, HDI-cholesterol, LDI-cholesterol) and Glycemia were analyzed. The AIP, atherogenic coefficient (AI), Lipoprotein combined index (LCI), and Castelli risk indices I and II are evaluated with the formula (results are notated in tables I and II).

Also, all the subjects were anesthetized and investigated with EKG performed in 6 leads, and abdominal ultrasonography. After 4 weeks, for histological study, the aortas were removed. The major extracellular matrix components, including collagen

and elastin, have been evaluated microscopically as indicators of aortic modification.

Endothelial dysfunction has been evaluated with biochemical markers, abdominal ultrasonography and histological study.

**Experimental procedures. Physiological and Biochemical markers.** On the 20th day after oral administration of treatment, euthanasia was conducted on all subjects, followed by an intracardiac puncture to obtain 3-4 mL of terminal blood samples, which were then collected in 3 mL special vacutainer tubes with clot activator for biochemical assays. Following a half-hour harvesting period, the tubes were centrifuged for 15minutes at 1500x g, a temperature of 4°C. We separated serum samples for the subjects for biochemistry analysis. Following the manufacturer's explicit instructions, all reagents for the determination of biochemistry were obtained from *BioSystems S.A. (Barcelona, Spain)* and utilized with the automatic analyzer system *ACCENT-200 (PZ Cormay, Poland)*.

**Paraclinical Evaluation: Abdominal ultrasonography and Histopathology.** The ultrasound was performed with the Vevo 2100, Visual Sonics, with a linear probe (MS 250S, 13-24 MHz). Subjects were abdominal trimmed and maintained in a supine position under inhalation anesthesia. The abdominal aorta was assessed in the longitudinal section. In B-mode the aortic wall, the aortic caliber at the mid-abdominal level and in pulsed Doppler mode the aortic flow velocity was measured.

**Histopathology.** Each animal had a thorough postmortem examination, which

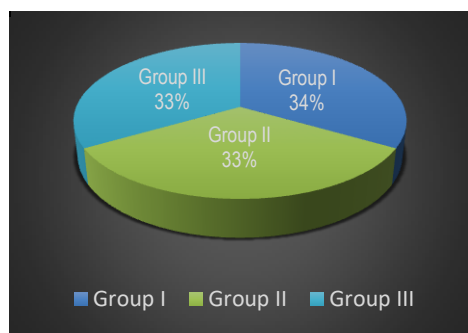
included a rigorous examination of the bodies. During this examination, the abdominal aorta was removed and preserved in 10% formalin and let for up to 48 h for fixation, in full accordance with the updated guides for rats and mouse organ sampling and trimming for a more extensive histological study. After that, two sections of the abdominal aorta were placed on a microscope slide and stained according to the usual staining methodology for hematoxylin and eosin (H&E).

**Statistical analysis.** Through a comparative study between every group, all measurable parameters were evaluated. The data analysis process was carried out using *SPSS version 23.0* for Windows (SPSS,

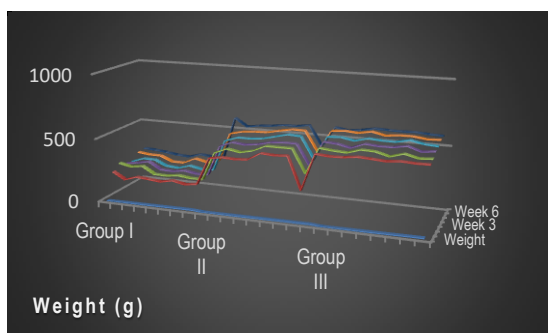
USA). Two-sided testing was used to evaluate with a statistically significant value of  $p < 0.05$ .

**RESULTS**

At the finally of the experiment study, all the animals were examined carefully. During surgery, we observed abdominal fat accumulation in group II and group III, even retroperitoneal fat was not evaluated for the classification of obesity. However, at the macroscopic evaluation, we don't observe aortic atherosclerosis in the control or obesity groups. In figure 2 shows the changes in body weights in all groups. In the tables I, II and III will see the biochemical changes in lipid profile, before and after treatment/experiment.



**Fig. 1.** Distribution of groups



**Fig. 2.** Weight of the groups for six weeks

TABLE I.

**Comparison of each study group before (initial) and after treatment(final) for lipidic profile and glycemia. Values as Mean ± Standard error.**

Parameter \ Study group	Glycemia		LDL Cholesterol		HDL Cholesterol		Total Cholesterol		Triglyceride	
	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)
Group I	84.14± 4.55	77.43± 4.51	38.43± 1.99	38.43± 0.74	52.14± 2.62	52.29± 0.68	85.71± 3.34	85.71± 3.34	96.71± 2.62	96.71± 2.62
Group II	91.29± 5.19	101.0± 2.57	51.71± 3.96	53.71± 2.42	53.29± 2.97	49.86± 2.89	178.43± 12.23	230.57± 18.19**	168.29± 9.2	182.86± 3.31
Group III	119.0± 19.34	131.29± 19.95	49.71± 1.64	39.14± 2.82**	53.29± 2.15	56.14± 3.67	270.0± 23.71	169.86± 3.36**	209.57± 10.02	182.14± 12.61**

\*OB- obesity, AT- Atorvastatin; I- Initial, Finally, \*\*Statistically significant with  $p < 0.05$ ;

\*\*\*Statistically significant with  $p < 0.001$  (paired t-test between before and after treatment values).

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In the first group control, we see that there are no changes in biochemical values.

In group II- with obesity and HFD (cholesterol 2%), Total cholesterol levels increased, from an average of 178.43±12.23 mg/dL to 230.57±18.19 mg/dL, significantly (p <0.05) and correlated with food received. LDL-cholesterol levels increased from 51.71±3.96 mg/dL to 53.71±2.42 mg/dL, but not statistically significant. Also, triglyceride levels increased from 168.29±9.2 to 182.86±3.31 but not statistically significant. Blood glucose levels increased, but not statistically significant during monitoring, from an average of 91.29±5.19 mg/dL to 101.0±2.57 mg/dL. In group III- with obesity and HFD (cholesterol 2%) and received Atorvastatin 20mg/kg/day, for the 20 days, it can be noted that the level of total cholesterol decreased from 270.0±23.71mg/dL to 169.86±3.36 and LDLc the same, from 49.71±1.64 mg/dL to 39.14±2.82 mg/dL (statistically significant with p <0.05). The level of Glycemia and HDL<sub>C</sub> increased but was not statistically significant. The experiment revealed that in

group III, initial blood glucose values were above 119.0±19.34 mg/dL (the value considered physiological). We believe that these increased values were primarily due to stress caused by restraint, medication administration and explorations. The mean blood glucose value at the study's end was 131.29±19.95 mg/dL.

In all groups, we calculated the Atherogenic Index of Plasma- AIP with standard formula as TG/HDL<sub>C</sub> and Atherogenic index – AI (non-HLD<sub>C</sub>/HDL<sub>C</sub>) (tab. II). In control groups, AIP increased but was not statistically modified. For group II, the obesity group with HFD (with 2% cholesterol) AI and AIP index levels increased, from an average of 2.60±0.23mg/dL to 3.42±0.46 mg/dL, also, 3.41 ± 0.18 to 3.49 ± 0.18, but not significantly and correlated with food received, age and weight and no treatment administrated. In group III, which received treatment with statin, we observed significantly lower levels of TC, LDLc, and TG (tab. I). Also, AIP and AI decreased statistically significantly with p <0.05 in the final experiment, from levels 3.83 ± 0.29 to 2.48 ± 0.29\*\*\* and 4.04±0.73 to 2.21±0.12\*\* for the AI index (tab. II).

TABLE II.

**Comparison of each study group before(initial) and after treatment for AIP and AI index. Values as Mean ± Standard error.**

Parameter Study group	(TG/HDL <sub>C</sub> ) ATHEROGENIC INDEX OF PLASMA (AIP)		Non-HDL <sub>C</sub> /HDL <sub>C</sub> ATHEROGENIC INDEX (AI)	
	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)
Group I	1.85 ± 0.04	1.85 ± 0.04	0.62±0.06	0.64±0.06
Group II	3.41 ± 0.18	3.49 ± 0.18	2.620±0.23	3.42±0.46
Group III	3.83 ± 0.29	<b>2.48 ± 0.29***</b>	4.04±0.73	<b>2.21±0.12**</b>

\*OB- obesity, AT- Atorvastatin; I- Initial, Finally, \*\*Statistically significant with p <0.05;

\*\*\*Statistically significant with p < 0.001 (paired t-test between before and after treatment values).

Table III was completed for all groups with lipid profiles and calculated Castelli risk index I and II and also, LCI. The values level of the lipid profile is evaluated with the standard logarithm of the molar ratio of the comprehensive lipid indexes, compared with single lipid parameters, such as non-HDL<sub>C</sub> (TC minus HDL<sub>C</sub>), TC/HDL<sub>C</sub>-Castelli risk index I, LDL<sub>C</sub>/HDL<sub>C</sub>- Castelli risk index II and TC\*TG\*LDL/HDL<sub>C</sub> (Lipoprotein combine index, LCI).

In control groups, all the lipid index in-

creased but was not statistically modified.

For group II, the obesity group with HFD (with 2% cholesterol) Castelli risk index I and II levels increased, not significantly and is correlated with food received, age, weight and without treatment administered. Therefore, Non-HDL<sub>C</sub> novels were modified statistically significantly from 128.57±10.72 to 177.29±18.71. Also, the Lipoprotein Combine Index has the same, increased final levels of lipid profile from 32738.11±4776.05 to 43561.26±4996.11.

TABLE III.

**Comparison for each study group before(initial) and after treatment(final) for CASTELLI RISK INDEX I and II, Lipoprotein combine index and Non-HDL<sub>C</sub>. Values as Mean ± Standard error.**

Parameter Study group	TC/HDL <sub>C</sub> CASTELLI RISK INDEX I		LDL <sub>C</sub> /HDL <sub>C</sub> CASTELLI RISK INDEX II		Non-HDL <sub>C</sub> (TC minus HDL <sub>C</sub> )		TC * TG * LDL/HDL <sub>C</sub> LIPOPROTEIN COMBINE INDEX	
	I	F	I	F	I	F	I	F
Group I	1.64± 0.06	1.64± 0.06	0.73± 0.03	0.74± 0.03	33.43± 3.23	33.57± 3.17	6097.69± 429.61	6111.18± 422.56
Group II	3.60± 0.23	4.42± 0.46	1.05± 0.09	1.04± 0.10	128.57± 10.72	<b>177.29± 18.71**</b>	32738.11± 4776.05	<b>43561.26± 4996.11 **</b>
Group III	5.04± 0.73	<b>3.21± 0.12**</b>	0.91± 0.07	<b>0.75± 0.08**</b>	213.86± 25.80	<b>116.57± 2.69**</b>	52008.64± 6960.58	<b>16262.94± 1445.30 **</b>

\*OB- obesity, AT- Atorvastatin; \*\*Statistically significant with p <0.05;

\*\*\*Statistically significant with p < 0.001 (paired t-test between before and after treatment values).

In group III, which received treatment with statin, we observed significantly lower levels of TC, LDL<sub>C</sub>, and TG (tab. I). Also, AIP and lipoprotein combined index, TC/HDL<sub>C</sub> and TC \* TG \* LDL/HDL<sub>C</sub> decreased statistically significantly with p <0.05 in the final experiment.

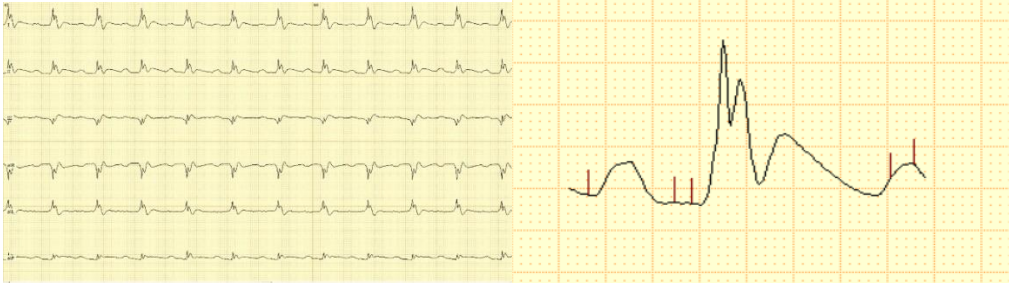
As expected, the values of lipid profile - Castelli risk index I and II decreased statistically significantly from 5.04±0.73 to 3.21±0.12, 0.91±0.07 to 0.75±0.08 with

statin treatment.

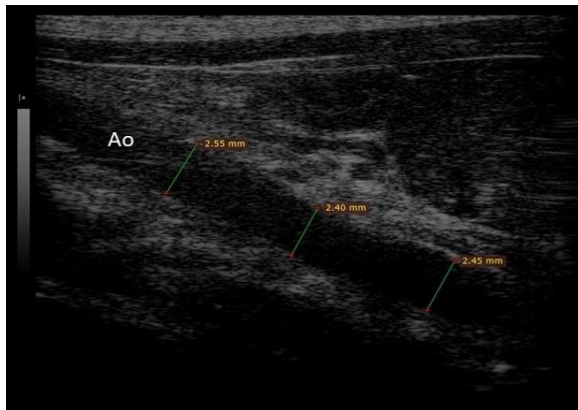
Finally, in the study, the recorded electrocardiograms did not reveal any pathological changes. EKG was performed on anesthetized subjects, induction with 5% isoflurane and maintenance with 2% isoflurane and 20 L/min oxygen (figs. 3, 4).

Abdominal ultrasonography performed during the present study revealed no structural changes, aortic wall thickness, atheroma plaque, or calcifications (fig. 5).

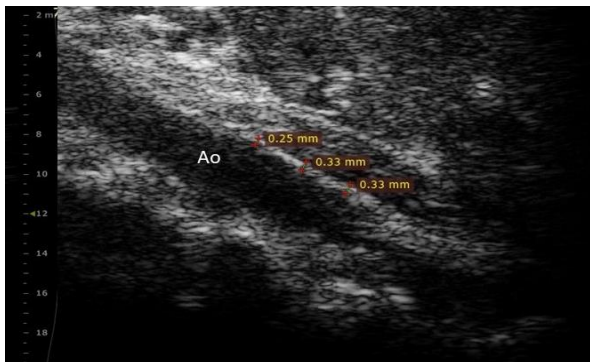
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**Figs. 3 and 4.** Electrocardiogram performed in 6 leads, hex axial system; sinus rhythm, heart rate 400 bpm. P wave - 32 msec, 0.05 mV, PQ - 57 msec, R wave - 17 msec, 0.23 mV; QT - 88 msec, T - 0.19 mV. Calibration 200 mm/sec, 40 mm/mV.



**Fig. 5.** Longitudinal abdominal aorta: example of aortic diameter measurement in systolic



**Fig. 6.** Longitudinal abdominal aorta: example of aortic wall thickness measurement

***Histological and structural characteristics***

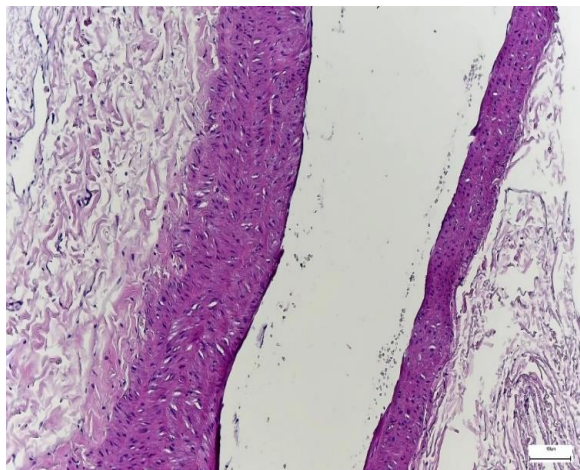
Slight aortic histomorphological modifications have been identified in the group with obesity *versus* the control group. All

three groups have three distinct tunics: intima, media and adventitia. In all groups with obesity, the integrity of the endothelial layer was preserved but plaque for-

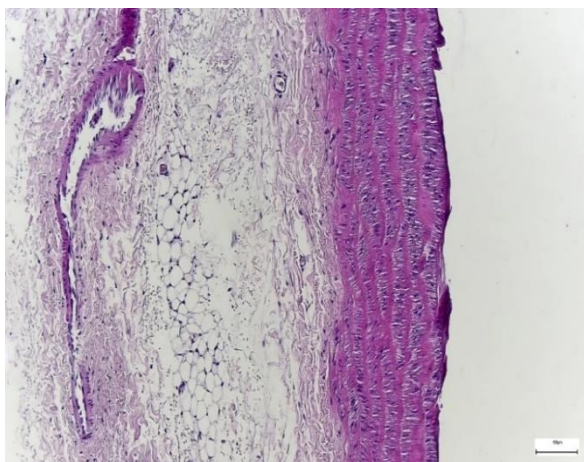


mation within the arteries was not seen in any group. In groups II and III, the histopathological aspect was modified for early endothelial injury (figs. 7, 8). In groups with obesity, the aorta wall with an asymmetrical thickness of tunica media with focal hypertrophy of tunica media, irregularly arranged elastic lamellae, slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis

slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis. Also, media thickening of the aortic wall, with an increase of collagen and a moderate amount of variably basophilic matrix extracellular matrix and many adipocytes in subjacent tunica adventitia in all groups (fig. 7).



**Fig. 7.** Aorta wall with asymmetrical thickness of tunica media with focal hypertrophy of tunica media (on one side of the aorta in section), irregularly arranged elastic lamellae, slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis (group 2 x10 HE)



**Fig. 8.** Media thickening of the aortic wall, with an increase of collagen and a moderate amount of variably basophilic matrix extracellular matrix and many adipocytes in subjacent tunica adventitia (group 2 x10 HE)

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### **DISCUSSION**

Recently, AIP has been a strong marker for predicting the risk of cardiovascular diseases (18). According to previous studies, dyslipidemia is considered the most important factor for CVD. It is commonly known that standard lipid measurements, such as TC, LDL<sub>C</sub> and TG, are associated with a significant risk for CVD events. Lipid ratios such as TC/HDL<sub>C</sub>, LDL<sub>C</sub>/HDL<sub>C</sub>, LCI, AI and Castelli risk I and II are also considered to be a very important indicator of CVD. As a consequence, small-density LDL (sdLDL) is more easily oxidized to oxLDL and invades arterial deposits more easily than LDL because of the measure of particle size, it is very small. Macrophages become foam cells when oxLDL is phagocytized by macrophages, which influences arteriosclerosis and cardiovascular events.

Recent research has indicated that sdLDL is a significant predictor of arteriosclerosis and clinical application has been proposed. However, because it is a complex determination and has a high cost, the evaluation of sdLDL has limitations in clinical applications, AIP may be a good biochemical, economical and reliable indicator of coronary diseases and early stage of endothelial dysfunction.

**Atherogenic Index of Plasma (AIP):** In the present experiment, we observed that dyslipidemia in obesity groups, without treatment, increased the lipid profile, high AIP index, and lipoprotein combined index, then healthy controls but the summary clinical investigation is normal. In the present results, we discovered that in the obesity groups, the AIP value was more substantial than the values of controls ( $4.04 \pm 0.73$  vs.  $0.64 \pm 0.06$ ). Also, the lipo-

protein combine index decreased statistically significantly ( $p < 0.05$ ) in group III ( $52008.64 \pm 6960.58$  vs  $16262.94 \pm 1445.30$ ) and increased statistically significantly with ( $p < 0.05$ ) in groups II- groups without treatment ( $32738.11 \pm 4776.05$  vs.  $43561.26 \pm 4996.11$ ). To conform literature data and classification, we included Group II (with obesity) and Group III (obesity with statin treatment) in high risk for AIP. Furthermore, HDL<sub>C</sub> contains an antioxidant enzyme and carries cholesterol from secondary tissues to the liver and is a component of the AIP. Decreased HDL<sub>C</sub> levels are associated with increased levels of pro inflammatory cytokines, TNF-alpha and interleukin-6 (19, 20) and can play an important role in the early stage of atherosclerosis. In our study level of HDL cholesterol was lower and can be improved with early treatment.

Date for literature reported the important effect of smoking, blood pressure, physical exercise and glycemia on AIP. Therefore, the authors reported a remarkable correlation between BMI, TC, and AIP in patients with cardiovascular diseases (21). In our experimental study, obesity and dyslipidemia can be the reason for high values of all parameters in the group with obesity.

In the present experimental study, will discover that all the lipid profiles were high values of the different atherogenic indices, Castelli risk and LCI. The ESC recommended, in primary prevention, to use of these lipid indexes and these parameters to evaluate and estimate the CVD risk (22).

Date from the literature confirmed several times the efficiency and benefits of treatment with a statin, in the experimental and clinical studies (23, 24, 25, 26,

27, 28, 29).

In an experimental study, animals with AIP height values and natural treatment have low-risk factors for atherosclerosis (30). Noor *et al.* show that in an experimental model of obesity rats, confirm that total cholesterol and LDL<sub>C</sub> levels were positively correlated with the atherogenic index and treatment with methanolic extract in hyperlipidemic rats reduced the improved lipid profile and AIP (31). Lianto *et al* described that treatment with statin and high-dose Vitamin D improves levels of HDLc (32).

Previous epidemiologic studies on human subjects have shown contradictory results (5). For example, in a cross-sectional study, authors showed that the value of AIP was positively associated with waist circumference and body mass index and was inversely associated with physical activity (33). In the Turkish population-prospective study, AIP predicted CAD independently. Another study on human patients revealed that AIP was not an independent factor determining the impact of the risk of cardiovascular disease in post-menopausal women (34). In another prospective cohort study, authors evaluated the relationship between the AIP value and major adverse cardiovascular events during intensive hospitalization in patients with acute myocardial infarction (35).

**Endothelial Dysfunction.** Vascular endothelium has an important role in hemostasis and cardiovascular diseases. Obesity and dyslipidemia are the same, important factors for CVD because is accelerate atherosclerosis and increase cardiovascular risk. The current study used an experimental study to investigate the correlation that exists between AIP and endothelial

dysfunction and compared the biochemical level of serum with histopathological aspects. In our research, we have found significantly high values of biochemical lipidic profile and AIP index, lipoprotein combines index compared with control groups. However, increased LDLc is another important marker of endothelial dysfunction since it can oxidize and generate oxidized Lipoprotein lipase cholesterol (LPL-cholesterol). This can increase the synthesis of caveolin-1, which inactivates eNOS and reduces NO production (36).

In our experimental study, non-efficient dyslipidemic profile control and high levels of LDLc can be considered the most important factors for endothelial dysfunction in obesity rats.

**Lipid Profile.** In practical terms, we know that dyslipidemia constitutes an important risk for CVD. Our results observed that group III who received treatment with Atorvastatin 20mg/day by gavage, profile lipid, AIP, atherogenic index, Castelli index and also lipoprotein combined index decreased significative statistically.

**Abdominal and carotid ultrasonography** in our results confirm that calcification and atherogenic plaque are too early to see. In daily practice, it's an investigation basically, but not relevant to endothelial dysfunction in the incipient stage.

**The histopathological** aspect of the abdominal aorta confirmed that dyslipidemia induced and determined incipient modified for endothelial injury, vascular endothelium is affected with asymmetrical thickness of tunica media, focal hypertrophy of tunica media, also, irregularly arranged elastic lamellae, slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis. Our study confirmed the associa-

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tion between biochemical and histopathological aspects can be an early stage of atherosclerosis.

High AIP was the most common abnormality among the atherogenic indices (37) in practice. According to our results, AIP can be more easily applied in practice than other lipid ratios and traditional lipid indicators. It was also found that AIP can be a substantial predictor of the probability of cardiovascular disease. Prevention and early treatment for dyslipidemia can improve the lipid profile, Atherogenic Index of Plasma, lipoprotein combined index, Castelli index and endothelial function.

Our research concluded that the AIP, atherogenic coefficient (AI), Lipoprotein combined index (LCI) or Castelli's risk indices I and II can be powerful biochemical predictors of early endothelial dysfunction and cardiovascular diseases in the experimental group with obesity. Therefore, the AIP can be usually calculated in medical practice, using only a standard lipid profile, making it an easily, economical and accessible biomarker (38).

### **CONCLUSIONS**

The prevention of cardiovascular disease remains the main objective of modern

medicine and a controversial issue. Our study confirms that the AIP can be a better biochemical predictor for endothelial dysfunction and atherosclerosis, than individual cholesterol risk factors and also a good predictor of cardiovascular risk. As a result, we believe that the AIP can significantly improve the cardiovascular disease risk algorithm. Furthermore, a reduction in AIP values, which includes lowering LDLc levels, might be a crucial part of the therapy for dyslipidemia.

### **CONFLICTS OF INTEREST AND FUNDING**

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