CORRELATIONS BETWEEN ADIPOCYTOKINES AND ANTROPOMETRIC MARKERS IN NEWLY DIAGNOSED OBESE TYPE 2 DIABETES MELLITUS

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Both obesity prevalence and cardiovascular risk profile increases with age and it has been proven that this increase in visceral adipose tissue mass represents one of the most important factors associated with the development of an atherogenic metabolic profile for type 2 diabetes patients. Our study aimed to evaluate several clinical, biochemical and antropometric parameters together with their correlation with insulin secreting function of β-pancreatic cells and adipocytes function in obese newly diagnosed type 2 diabetes (ND-T2D). The study included 120 ND-T2D (74 men and 46 women) and 34 healthy subjects. The ND-T2D group were classified according to their body mass index (BMI) into two subgroups: overweight ($25 \leq BMI < 29.9$ kg/m^2) and obese ($BMI \geq 30$ kg/m^2). All participants underwent antropometrical evaluation and routine blood parameters. Serum insulin, proinsulin, C peptide, leptin and adiponectin levels were also measured by ELISA method. We have found highly statistically significant differences between the control group and the other two subgroups of diabetic patients (overweight and obese), who have recorded values of double or more compared to the control group regarding clinical and biochemical measurements. Moreover, differences of 10-20% have also been observed between the overweight diabetic group and the obese diabetic group for proinsulin, C peptid and adiponectin ($p<0.05$). Serum insulin level was positive associated with weight, creatinine and waist circumferance (WC) while serum proinsulin was positively correlated only with WC ($p<0.005$). Leptin was positively associated with BMI ($p<0.05$) while adiponectin was negatively correlated with BMI ($p<0.001$). High proinsulin levels, confirm an overload of the β-pancreatic cells in overweight/obese subjects while decreased adiponectin levels in obese ND-T2D make excess weight an essential risk factor for diabetes even before the decompensation of blood glucose regulation.

Key words: adipocytokines, type 2 diabetes, obesity.

INTRODUCTION

Obesity is the main risk factor for type 2 diabetes (T2D). Epidemiological studies have repeatedly presented an independent risk factor for the onset of diabetes associated with an overweight or obese status, with a well-defined dose-dependent relationship along progressive categories of body mass increase. Although the importance of increased body mass in the etiology of diabetes is unequivocal, it is a known fact that adipose mass distribution offers additional explanations to the risk of developing diabetes[1].

Since as early as 1947, Vague observed that *android type obesity* (masculine, central, troncular) is associated with a poor metabolic profile in comparison with *ginoid type obesity*[2]. These clinical observations regarding the detrimental effects of central obesity were strengthened by the results of several large-scale prospective studies concerning the incidence of diabetes, in which the pattern of body fat dispositionm was estimated using body surface measurements, especially waist circumferance (WC) and waist/hip ratio (WHR) as well as skin thickness. Whereas these studies reported an increased incidence of diabetes

correlated with waist circumference or WHR in comparison with BMI, this is far from becoming a general rule. In recent metaanalyses, BMI, waist circumference and WHR have presented similar associations with diabetes incidence[1, 3-5].

It was estimated that the physiological process of free fatty acids (FFA) recycling which is independent from the very low density lipoprotein/ adipose tissue lipoprotein lipase could play an important part in the discret less partitioning of fat to the upper body part and the one toward the lower body regions in women, but not men. An increased accumulation of the FFA tracing agent per adipocyte corresponding subcutaneously to the lower region of the body, proves that in women, the adipocytes of this specific compartment accumulates larger quantities of FFA in obese women, compared to the subcutaneous upper body and omental regions. The partitioning of FFA plays a key role in favorising fat storage in the lower body in ginoid type women[6]. These findings suggest that omental adipocyte hypertrophy, and not a subcutaneous one, is associated with an altered lipis profile independent from body composition and body fat distribution in women[7].

Reaven has shown that 25% of healthy subjects without overweight or altered glucose tolerance manifest a lower degree of sensitivity to insulin similar to those characteristic to early stages of type 2 diabetes mellitus. Numerous studies have proven that diabetic patients with a lower sensitivity to insulin who only present minor alterations in blood glucose regulations also manifest increased blood pressure values as well as specific lipid metabolism alterations[8].

Often, both a familial character as well as several elements from one's childhood make excess weight the main detectable pathologic state, possessing however the advantage that it is usually easily visible. Several confusion elements can occur due to the proportion between adipose mass/muscular mass. By using height, weight and abdominal circumference, a child, a teenager, a young and especially an elderly person can be easily characterised as belonging to one of the 5 main anthropometric categories: underweight (BMI < 18), normal-weight (BMI > 18 but < 25), overweight (BMI > 25 but < 30), obese (BMI > 30 but < 40) or morbidly obese (BMI > 40)[9].

The aim of the study was to evaluate several clinical and biochemical parametres (plasma glucose, glycated haemoglobin, total cholesterol, HDL-cholesterol, triglycerides, urea, creatinine, uric acid) and antropomentric markers (height, weight, body mass index, waist and hip circumference, visceral fat) together with their correlation with insulin secreting function (by determining the serum levels of insulin, proinsulin and C peptide) and adipocytes function (by determining serum levels of leptin and adiponectin) in newly diagnosed obese type 2 diabetes (ND-T2D). The ND-T2D group was compared to a control group of healty subjects.

MATERIALS AND METHODS

Regarding the achievement of the proposed objectives, two groups were selected: (1) a group of patients with newly-diagnosed diabetes mellitus, included in the "N. Paulescu "National Institute for Diabetes, Nutrition and Metabolic Diseases (NIDNMB) (group ND-T2D; *n=120*) and (2) a control group, recruited from the institute's medical personnel (group CTRL; *n = 34*). The carrying out of the study was approved by the ethics committee of "N. Paulescu" NIDNMB and informed consent was obtained from all participants. ND-T2D group was divided into two subgroups according to their body mass index (BMI) as follows: group 1 – overweight patients (BMI between 25 – 29.9 kg/m^2; n=46) and group 2 – obese patients (BMI $\geq$ 30 kg/m^2, n=74)

Study *inclusion criteria* were: ages ranging between 40 and 80 years old and a recent diagnosis of type 2 diabetes mellitus (less then 6 months). Diabetes was diagnosed using standard diagnosis criteria according to the WHO recommandations: glycemia levels of $\geq$ 200 mg/dL accompanied by classical symptoms such as polyuria, polyfagia, polydipsia as well as a weight loss of over 10 kg or *à jeun* plasma glucose levels $\geq$ 126 mg/dL or glycemia levels $\geq$ 200 mg/dL obtained after performing the oral glucose tolerance test if the values of *à jeun* glycemia were comprised between $\geq$110 mg/dL and $\leq$126 mg/dL[10]. *Exclusion criteria* were: excessive alcohol consumption, acute myocardial infarctions or ischemic or haemmorhagic strokes in the prior 6 months, epilepsy or other severe neurological diseases, pancreatic head carcinoma as well as other malignancies, renal diseases with a serum creatinin concentration of >1,36 mg/dL, proteinuria or microalbuminuria.

For the control group (34 aparently healthy subjects), only those subjects with normal glycemia as well as glycated hemoglobin levels <5.9% were included in this group, without any suspicion of diabetes or glucose intolerance. Their exclusion was carried out on the basis of similar exclusion criteria specified for the diabetic group.

For all subjects were carried out the following clinical and biochemical characteristics: age, gender, smoking, weight, height, waist and hip circumference, visceral fat by using a resistometric evaluation (body composition analyser MC-980 from TANITA). The concentration of fasting glucose (mg/dL), glycated hemoglobin (%), total cholesterol (mg/dL), HDL-cholesterol ("high density lipoprotein"; mg/dL), total triglycerides (mg/dL), urea (mg/dL), creatinine (mg/dL), uric acid (mg/dL) were measured using current biochemical methods (Hospitex Diagnostincs Eos Bravo Forte Analyser). Serum concentrations of insulin, proinsulin, C peptide, leptin and adiponectin were determined by ELISA method on Multiskan Ex-Thermo Electro Corporation using commercially available kits (EIA-2935, EIA-1560, EIA-1293, EIA-2395 and EIA-4177; DRG Instruments, Germany) following the manufacturer's guidelines.

RESULTS AND DISCUSSION

Clinical and biochemical characteristics

The clinical characteristics and routine biochemistry tests of the diabetic patients and of the healthy subjects are shown in Tables 1 and 2.

Diabetic patients compared with controls had higher serum triacylglycerol and uric acid levels (p<0.05) and also high TG/HDL-cholesterol ratio (p<0.001) (Figure 1). As shown in Figure 2 the insulin and proinsulin were positive correlated with WC (2A and 2B) but only insulin was also positive correlated with weight (2C) and creatinine (2D). Moreover obese ND-T2D compared with overweight ND-T2D had significantly higher mean levels of triglyceride, uric acid and fasting glucose (p<0.05).

The results of ELISA measurements of insulin, proinsulin, C peptide, leptin and adiponectin are presented in Table 3. A similar distribution model for serum leptin levels as well as insulin levels can be observed in Figure 3A and 3C. Leptin value in control group subjects was 6.95 ± 0.49 ng/mL, more than two times lower compared to the ND-T2D group, thus proving the leptin resistance phenomenon which progressively settles in and gradually evolves in diabetic patients, especially if obesity is also a present factor. We did not recorded a significant diferance between subgroups studies regarding serum leptin levels. As shown in Figure 3B and 3D serum proinsulin and adiponectin levels had the same tendency and the difference between the overwheight and obese groups was statistically significant (p<0.05). Serum proinsulin level,

proinsulin/insulin and proinsulin/adiponectin ratio were also significantelly increased in group 2 *versus* group 1 (p< 0.05). Moreover, leptin was positively associated with BMI (p< 0.05) while adiponectin was negatively correlated with BMI (p<0.001) (Figure 4A and 4B).

Our study aimed to evaluate several clinical, biochemical (plasma glucose, glycated haemoglobin, total cholesterol, HDL-cholesterol, triglycerides, urea, creatinine, uric acid) and antropometric parameters (height, weight, body mass index, waist and hip circumference, visceral fat) together with their correlation with insulin secreting function of beta-pancreatic cells (by determining the serum levels of insulin, proinsulin and C peptide) and adipocytes function (by determining serum levels of leptin and adiponectin) in obese newly diagnosed type 2 diabetes (ND-T2D). The ND-T2D group was compared to a control group of healty subjects.

The hypothesis that has represented a starting point for our study was the pathogenic influence of adipose tissue excess, supposed to be related to an increase in free radicals production and a decrease in antioxidant capacity[11], thus leading to an increase in proflammatory adipokines secretion while oversoliciting the insulin secreting beta-cells. At the same time, we have set out to confront literature data regarding the role of excess weight measured through anthropometric indexes on the pathogenesis and accelerated evolution towards diabetes mellitus, together with multiple cardiovascular risk factors.

Table 1

The clinical characteristics of the studied groups

Parameters	Healthy subjects (lot CTRL) (n=34)	Diabetic patients (Lot ND-T2DM) (n=120)	Group 1 (n=46)	Group 2 (n=74)	p-value diabetic patients *verso* lot CTRL	p-value group 1 *verso* group 2
Sex (Male/Female)	14/20	74/46	30/16	44/30	NS	NS
Age (Years)	56 ± 4	60 ± 1	61 ± 1.77	59 ± 1.33	NS	NS
Smokers/Nonsmokers	10/24	49/71	18/28	31/43	NS	NS
Weight (kg)	68 ± 3.87	89 ± 1.52	78 ± 1.53	96 ± 1.79	< 0.001	<0.05
Waist Circumferance (cm)	93 ± 3.92	108 ± 1.09	99 ± 1.17	113 ± 1.21	< 0.05	<0.05
Body Mass Index (BMI)	21 ± 1.12	31 ± 0.39	27 ± 0.36	35 ± 0.53	<0.001	<0.001
Visceral Fat (%)	22.8 ± 0.2	35.43 ± 1.8	31.02 ± 1.2	39.85 ± 0.04	<0.001	<0.001
Systolic blood pressure (mmHg)	114 ±7.05	141 ± 15. 06	138 ± 32.05	144 ± 20.11	< 0.05	NS
Diastolic blood pressure (mmHg)	70 ± 4.85	79 ± 8.36	75 ± 7.38	85 ± 8.78	< 0.05	<0.05

Data are expressed as mean ± SD (standard deviation). NS = not significant

Table 2

The routine biochemistry tests of the healthy subjects and diabetic patients

Parameters	Healthy subjects (lot CTRL) (n=34)	Diabetic patients (Lot ND-T2DM) (n=120)	Group 1 (n=46)	Group 2 (n=74)	p-value diabetic patients *versus* lot CTRL	p-value group 1 *versus* group 2
Glycemia (mg/dL)	97±2.48	172±5.86	182±9.9	166±7.14	<0.001	<0.05
HbA$_{1c}$ (%)	5.57±0.05	7.49±0.18	7.73±0.3	7.56±0.24	<0.001	NS
Serum total cholesterol (mg/dL)	199±17.51	208±4.8	204±7.35	212±5.02	NS	NS
Serum HDL-cholesterol (mg/dL)	52±2.33	40±0.98	43±1.55	38±1.25	NS	NS
Serum triacylglycerol (TG) (mg/dL)	102±8.56	198±14.2	207±28.28	190±14.95	<0.05	<0.05
TG/HDL-cholesterol	2.09±0.25	5.23±0.41	5.42±0.87	5.08±0.39	<0.001	NS
Urea (mg/dL)	31±1.74	36±1.15	36±1.6	37±1.61	NS	NS
Creatinine (mg/dL)	0.81±0.06	0.88±0.02	0.87±0.04	0.89±0.03	NS	NS
Uric Acid (mg/dL)	5.35±0.21	5.77±0.19	5.2±0.34	6.12±0.23	NS	<0.05

Data are expressed as mean ± SD (standard deviation). NS = not significant.

Overwheight/obesity present in 99% of diabetic patients, induces multiple metabolic alterations including: an atherogenic lipoprotein profile (hypertriglyceridemia, decrease in plasma HDL-cholesterol, increased levels of LDL-cholesterol, postprandial hyperlipidemia), increased glycemic levels as well as arterial hypertension [9,12].

In the past few years it has been observed that for the regulation of energetic metabolism, both insulin secreted by the β-pancreatic cell as well as leptin secreted by adipocytes cooperate at hypothalamic level in order to maintain weight homeostasis[13].

Considering that the β-pancreatic cells is the main center of control which integrates the regulation of energy metabolism, and the adipocyte is the main energy storage site with a significant role in maintaining metabolic homeostasis, it is logic to think that a desequilibrum between the β-pancreatic cells and the adipocyte pool, could be found at the early phase of diabetes. Thus, an increase in proinsulin/insulin ratio and decrease in proinsulin/adiponectin ratio could stimulate adipogenesis as well as excess weight[14]. The statement that obesity precedes diabetes is correct if it refers to hyperglycemic diabetes, which constitutes a late stage in adipogenesis associated with a drastic decrease in ß cellular mass[15].

An important role of adipose tissue in regulating energetic metabolism is attributed to leptin, an adipokine which is secreted proportional to the volume of adipose tissue [16]. Its role is that of signaling the level of fuels present in storage sites to the central nervous system (hypothalamic region). When adiposity decreases, leptin levels decrease; when adiposity increases, leptin levels also increase. This secretory behaviour is identical to that of insulin. Older experimental data[17] as well as more recent data[14] prove that plasma levels of insulin increase with weight gain and decrease with weight loss. A study regarding the effects of bariatric surgery on insulin secretion is eloquent[18] proving that insulinemia decreases rapidly after the surgical procedure, together with the respective weight loss. This decrease in plasma insulin levels, simultaneously associated with a decrease in plasma glucose levels is interpreted by us as a result of decreasing the biochemical pressure inside the energy system of the human body[19].

It is a unanimously accepted fact that obesity is a heterogenous condition from a metabolic point of view. It is also a known fact that ginoid type obesity, more frequently present in women, was not associated with the expected complications of obesity. Prospective studies which used the WHR or WC as indicators of obesity confirmed that android type obesity (also called "abdominal obesity") is much more frequently associated with a proatherogenic metabolic profile, as well as with a higher risk of developing diabetes and cardiovascular diseases[6].

Numerous studies have proven that overwheight diabetic patients who only present alterations "signals" of glycemic regulation, also manifest by the increased arterial blood pressure values and characteristic lipid metabolism alterations (hypertriglyceridemia, decreased concentrations of

HDL-cholesterol)[19-21]. It has also been proven that leptin to adiponectin ratio is associated with carotid artery intima-media thickness being an independent predictor in obese subjects[19]. Measurements performed in this study have illustrated similar results to literature data, with a proportion of two times higher in the diabetic group compared to the control group, associating at least two illnesses which present great cardiovascular risk, including arterial hypertension and dyslipidemia. Data from our study have showed a positive relationship between BMI and leptin while adiponectin was negativelly correlated with BMI (Figure 4A and 4B).
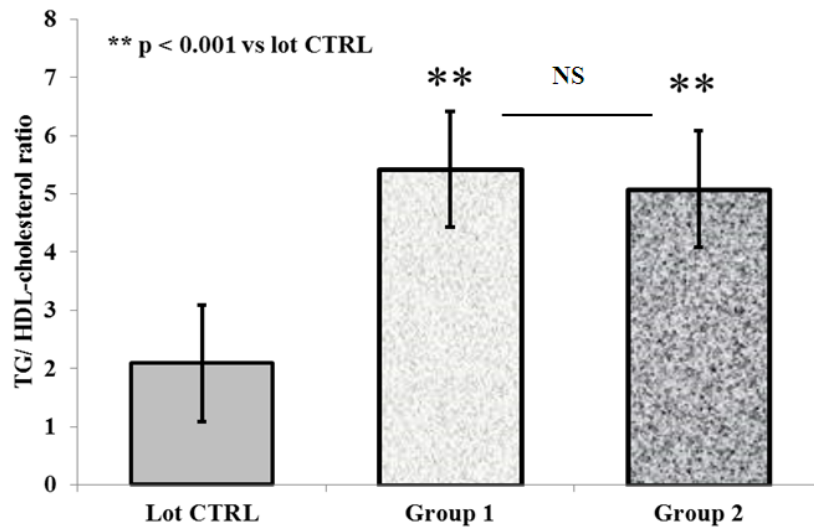

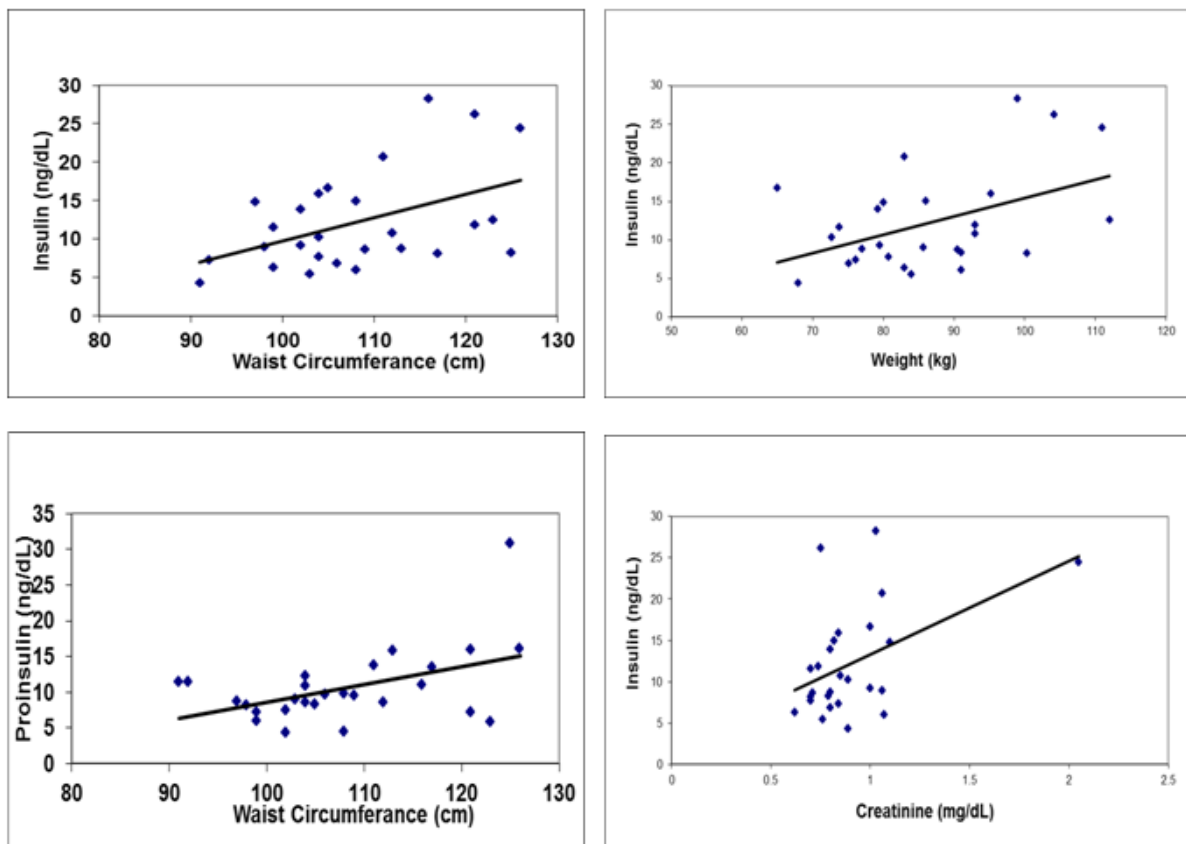Figure 1. TG/HDL-cholesterol ratio of the healthy subjects (lot CTRL) and diabetic patients (group 1 and group 2).

Figure 2. Correlation between insulin and waist circumferance **(2A),** proinsulin and waist circumferance **(2B),** insulin and weight **(2C)** and between insulin and creatinine **(2D)** in ND-T2D patients.

Table 3

The results of ELISA measurements for the ND-T2D and control groups

Parameters	Healthy subjects (lot CTRL) (n=34)	Diabetic patients (Lot ND-T2DM) (n=120)	Group 1 (n=46)	Group 2 (n=74)	p-value diabetic patients *versus* lot CTRL	p-value group 1 *versus* group 2
Insulin (ng/dL)	6.81±0.62	12.16±1.22	10.95±1.23	13.13±1.98	<0.001	NS
Proinsulin (ng/dL)	7.05±0.64	10.61±1	8.44±0.73	12.34±1.58	<0.001	<0.05
C peptide (ng/mL)	1.10±0.02	1.22±0.01	1.17±0.01	1.27±0.02	<0.05	<0.05
Leptin (ng/mL)	6.95±0.49	14.84±2.88	14.20±3.95	15.42±4.35	<0.001	NS
Adiponectin (ng/mL)	12.46±0.81	5.60±0.68	7.38±1.18	4.97±0.84	<0.001	<0.05
Proinsulin/Insulin ratio	0.85±0.25	1.49±0.15	0.99±0.20	1.28±0.21	<0.05	<0.05
Proinsulin/Adiponectin ratio	0.55±0.09	2.15±0.32	1.58±0.31	2.88±0.49	<0.001	<0.05

Data are expressed as mean ± SEM (standard error of the mean). NS = not significant

By using biochemical measurements, we have found interesting results regarding the relationship between total cholesterol, HDL-cholesterol and triglycerides which followed an pathogenic evolution pattern. Whereas in the control group mean values did not reach a dyslipidemic threshold, maintaing total cholesterol levels under 200 mg/dL, triglyceride levels under 150 mg/dL and HDL-cholesterol over 45 mg/dL, the diabetic group exceeded threshold values judging by calculated mean values, being progressively increased for total cholesterol and triglycerides at levels of over 150 mg/dL and decreasing progressively for HDL-cholesterol at values under 40 mg/dL. For a more suggestive illustration of the certain differences between lipid fractions in the control group, the overweight diabetic group and the obese diabetic subgroup, we have designed a proportion between triglycerides and HDL-cholesterol (Table 2 and Figure 1), used in numerous studies due to its relevant correlations with the development of cardiovascular diseases[21,22]. We have obtained a value of 2.09±0.25 in the control group, a subsequent double value for the overweight/obese diabetic group. To conclude, not only do the majority of diabetic patients suffer from dyslipidemia, but it can be also stated that the degree of dyslipidemia (correlated with a decrease in HDL-cholesterol and by means of the TG/HDL-cholesterol ratio) advances progressively in raport to BMI.

Previous studies[22] on obese and non-obese diabetic patients have concluded that increased levels of leptin, C reactive protein, FFA and IL-6 are more likely associated with obesity than the diabetic disease. Even though higher values were obtained in the case of diabetic patients as compared to control group subjects, statistically significant differences have been recorded between subgroups of overweight and obese diabetic patients, which is why a key role was attributed to excess weight as a main factor responsible for increased values of leptin[22].

In a first step, an increase in insulin secretion according to the increase in the number of insulin dependent cells will return glycemia levels to normal values[12]. However, a chronic stimulation, through its unphysiological character, will force the β pancreatic cell to not only secrete insulin, but also a higher concentration of proinsulin, which possesses modest hypoglycemic actions[12]. In numerous studies from the past decade, along with an increase in insulin levels, an increase in plasma levels of proinsulin has also been observed in the case of diabetic patients, along with its consequences related to the weak hypoglycemic actions that it possesses[23,24]. Research carried out consequent to this study has indeed illustrated an average insulinemia value of 12.16±1.22 ng/mL, almost double compared to the mean value present in the control group (6.81 ± 0.62) and an average proinsulinemia value of 10.61 ±1 *versus* 7.05 ± 0.64 in control group. By correlating values with the BMI of all subjects, who were organised according to this specific parameter, an ascension curve proportional to the BMI value of the entire group was obtained. It was also interesting to observe a first peak of insulin level ascension in the only 3 overweight subjects in the control group, which suggests a high insulin respons in subjects with excess weight in a first phase, even in the absence of diabetes. Other insulinemic peaks have also been recorded especially in the case of obese diabetic patients, proportional to an increase in BMI. The same characteristics were noted for proinsulin level, with a more increased statistically significant value in the diabetic group as compared to the control group, also positively correlated with an increase in excess weight.
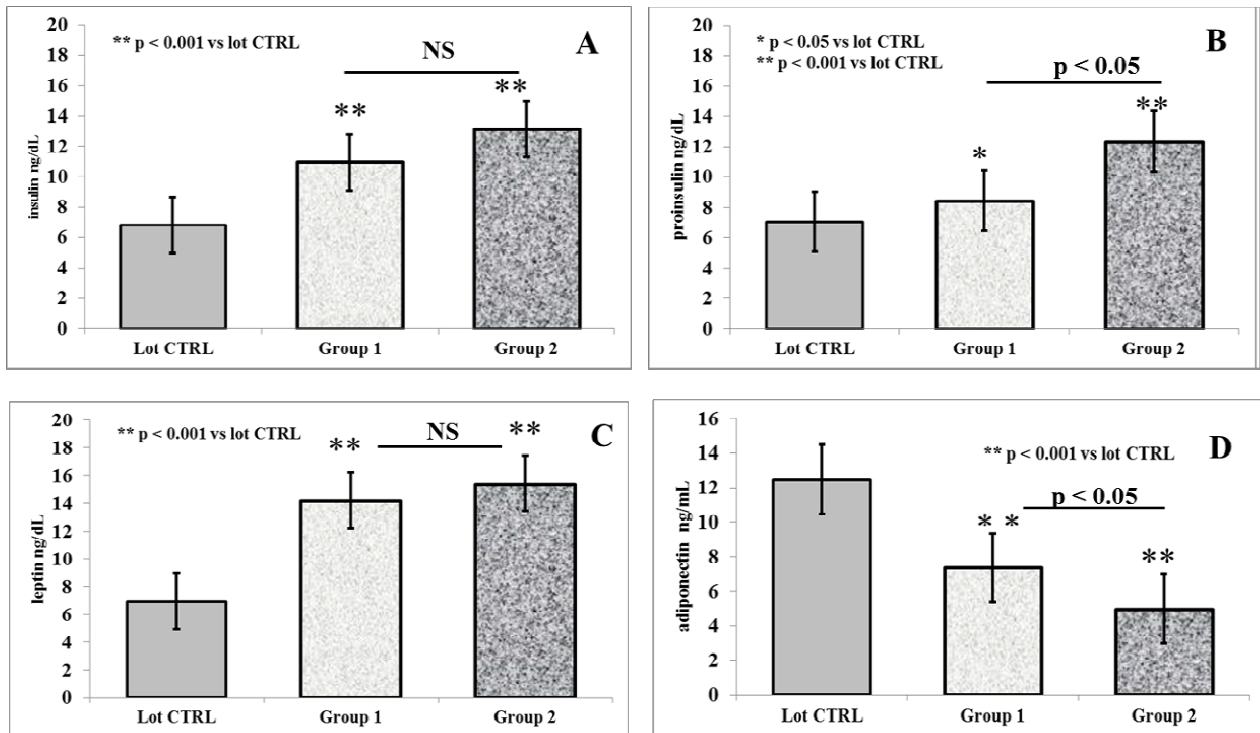
Figure 3. The measured serum insulin **(A)**, proinsulin **(B)**, leptin **(C)** and adiponectin **(D)** levels in the ND-T2D (overweight-group1 and obese-group2) patients and in the healthy subjects (lot CTRL).
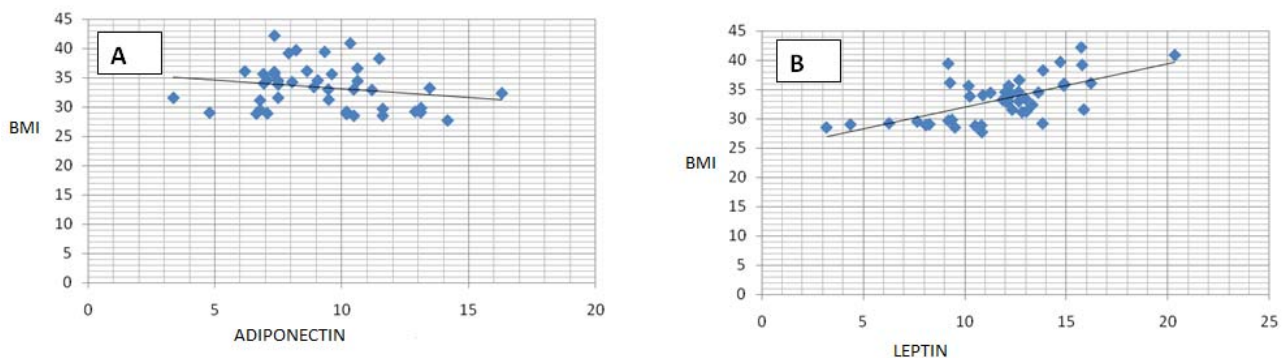

Figure 4. Correlation between adiponectin (A) and leptin (B) with BMI in ND-T2D patients.

CONCLUSION

Clinical research that we have carried out after analysing and permanently comparing the groups and subgroups included in the study have concluded the following findings:

1. plasma insulin levels increased statistically significant in the diabetic group versus the control group;

2. plasma insulin levels positively correlated with the degree of adiposity, recording several differences between overweight patients and obese ones, thus making excess weight an essential risk factor for diabetes even before the decompensation of blood glucose regulation;

3. plasma insulin values have correlated positively with creatinin levels, raising the possibility of opening a new direction in the study of renal complications associated with type 2 diabetes;

4. high plasma proinsulin levels, confirm an overload of the pancreatic β-cells in overweight/ obese subjects;

5. Plasma proinsulin levels correlated positively with waist circumference, thus establishing a key role of adipose tissue in alterations of the β-cells function.

6. Leptin hyperproduction and decreased plasma adiponectin levels present in diabetic patients, which gradually increases or decreasing with the degree of adiposity, emphasis the leptin resistance mainly due to excess weight.

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