Exploration of Insulin Sensitivity, Insulin Resistance, Early Insulin Secretion and β-cell Function, and Their Relationship with Glycated Hemoglobin Level in Normal Weight Patients with Newly Diagnosed Type 2 Diabetes Mellitus

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When discussing insulin resistance and insulin sensitivity, data from literature focuses on obese and overweight patients. In our study on, 110 patients with normal body-mass index with newly diagnosed type 2 diabetes mellitus, with the help of glucose tolerance test, we explored insulin resistance, sensitivity, early insulin secretion and β-cell function assessed by using the following indexes: HOMA-IR, ISI, IGI and HOMA-β. We compared the results from our reference group with a control group of 109 overweight patients with newly diagnosed type 2 diabetes mellitus. Normal weight patients had a statistically significant lower HOMA-IR index than overweight patients (2.65 vs. 3.55, p<0.01), however in both groups HOMA-IR was above the cut-off value of 2.5. HOMA-β was statistically significant lower in normal weight patients than in overweight patients (55.08 vs 65.36, p<0.01). ISI index was in an inverse proportional relationship with HOMA-IR, statistically significant higher in normal weight individuals (5.97 vs.3.48, p<0.01). IGI index was not statistically significant lower in normal weight patients (3.63 vs.3.95, p=0.07). It is important to observe that although they have a normal BMI these patients are insulin-resistant confirming the hypothesis of metabolic obese normal weight patients that develop type 2 diabetes mellitus. The indexes that correlate with HbA1c in normal weight patients, predicting glucose status, are HOMA-β (negative correlation), ISI (positive correlation) and IGI index (negative correlation).

Keywords: insulin, β-cell, glycated hemoglobin, type 2 diabetes mellitus

Although the pathophysiological modifications of insulin action have been intensely studied in obese and overweight patients that develop type 2 diabetes mellitus (T2DM), these modifications have been less explored in normal weight individuals. The explanation is the increased prevalence of T2DM in obese or overweight individuals; more than 80% of newly diagnosed cases appear in persons with a body-mass index (BMI) > 25kg/m^2. It is not a surprise that the scientific data regarding T2DM pathophysiology are less abundant regarding normal body weight individuals. T2DM appears when β cells are not capable to produce enough insulin to compensate insulin resistance [1]. In obese and overweight individual, fat tissue is responsible for the production of certain substances named adipokines, such as leptin, TNF-α, resistin, MCP-1 that block the insulin signaling pathway [2]. As a result, the β cells are forced to produce more insulin, it has been demonstrated that plasmatic levels of insulin are 4 times greater in obese T2DM patients than in individuals with normal weight [3]. An inverse relationship exists between insulin secretion and insulin sensitivity in these individuals [4-9].

In normal weight individuals the reasons why T2DM appears are more complex and there are many theories regarding this subject [10-13]. The early theories state that in these individuals the β cell dysfunction is the main factor that leads to hyperglycemia as opposed to overweight patients where the main factor is insulin resistance [14]. This theory is supported by the fact that in normal weight T2DM patients, insulin therapy is initiated early and insulin predominates in their treatment [15]. The risk factors that predispose to this early β cell failure, were identified in some studies: low birth weight, malnutrition in childhood, smoking, alcoholism and pancreatitis [16]. These predisposing factors could alter the structure and capacity of insulin secretion of the β-cell. Another theory is that normal weight patients with T2DM develop the disease because every individual has a personal fat threshold and if this limit is exceeded T2DM appears even if the BMI is normal [17].

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In the context of such complexity regarding the mechanism that leads to T2DM development in normal weight individuals, we wanted to explore the insulin secretion and insulin resistance behavior in a cohort of new-onset T2DM patients where these processes have not been altered by any administered drug that influences insulin resistance or β-cell secretion capacity.

Experimental part

Material and method

This study included patients with newly diagnosed T2DM, from the Clinical County Emergency Hospital of Oradea, each patient signing an informed-consent form before inclusion; Our research was approved by the Ethics Commission of the Council of the Faculty of Medicine and Pharmacy, University of Oradea, and conducted in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki, 1967).

The patients were distributed in two groups: a reference group and a control group, according to their BMI. The reference group included normal weight newly diagnosed T2DM patients and the control group included overweight newly diagnosed T2DM patients. The following inclusion criteria were used for selection: patients' ages between 18-65 years; BMI of the patients between 18.5-24.9 kg/m^2 for the reference group and BMI between 25-29.9 kg/m^2 for the control group; patients diagnosed with T2DM based on the diabetes diagnosis criteria; patients with negative glutamic acid decarboxylase (GAD) antibodies specific for type 1 diabetes mellitus patients; interval between diabetes diagnosis and laboratory work-up (specific for our study) shorter than 1 week; patients that did not receive any antidiabetic medication before the laboratory work-up, in order not to influence the results of the study; patients that gave their written consent for the participation in the study. Exclusion criteria were: patients that from the moment of diagnosis to the moment of selection received antidiabetic medication; patients with BMI<18.5 kg/m^2 or with BMI>29.9 kg/m^2, patients with any disease that modifies the metabolic state and can influence insulin resistance or insulin-sensitivity (autoimmune diseases, hyperthyroidism, hypothyroidism, neoplastic diseases, etc.)

Patients that were diagnosed with T2DM in the Ambulatory of the Clinical County Emergency Hospital of Oradea in the period 01 April - 01 July 2018 were considered for the inclusion in the study. The inclusion method was the following: we included every 4th patient diagnosed with T2DM that was overweight and every 2nd patient diagnosed with T2DM that had normal weight. In the beginning were selected 118 normal weight patients and 120 overweight patients. All patients were explained the content of the study and signed a written consent. The research was conducted according to the principles of the Helsinki Declaration and with the approbation of the Ethics Commission of the Faculty of Medicine and Pharmacy of Oradea.

After the patients were selected at Visit 1, they were evaluated clinically. Information was collected regarding: age, rural or urban area, education, medical history, treatment for the comorbidities. The BMI was calculated after determining the weight and the height of the patient, the waist circumference was also measured. Patients were instructed to present two days later at the Diabetes Clinic from the Clinical County Emergency Hospital of Oradea for the laboratory work-up. After the end of visit 1, 4 patients from the reference group and 10 patients from the control group were excluded from the study.

At visits 2, laboratory tests were effectuated. We performed the oral glucose tolerance test (OGTT). The test was performed in the morning after 8 hours of fasting. The patient drank 75g of oral glucose solution. We determined the plasma glucose and insulin at 0, 30, 60, 120 and 180 minutes. These results were noted for every patient. The symbols for the glucose results at every moment were: GLU0, GLU30, GLU60, GLU120 and GLU180 and for insulin were: INS0, INS30, INS60, INS120 and INS180. The level of glycaemia GLU0 was considered as fasting plasma glucose (FPG) and the level of glycaemia GLU120 was considered as 2-h plasma glucose (2-h PG).

Also, the following analyses were effectuated: plasma GAD antibodies, HbA1c, total cholesterol, LDL-cholesterol, triglycerides, HDL-cholesterol. The indexes specific for the evaluation of insulin action were determined based on the G0-180 and INS0-180 results [18]:

- HOMA-IR – the index for evaluation insulin resistance:
$$HOMA - IR = fasting\ insulin\ (INS0)\ (\mu U/L)\ x\ fasting\ glucose\ (GLU0)\ (mmol/L)/22.5.$$
- HOMA-β – the index for evaluation of β cell function:
$$HOMA - \beta = 20\ x\ fasting\ insulin\ (INS0)\ (\mu U/L)\ /\ (fasting\ glucose\ (GLU0)\ (mmol/L) - 3.5).$$
- ISI - insulin sensitivity index:
$$ISI = 10000/\sqrt{(INS0)(\mu UI/L) * (G0)(mmol/L) * mean\ (G0 - G120) * mean(INS0 - INS12)}$$
- IGI – early insulin secretion:
$$IGI = (INS30(\mu UI/L) - INS0(\mu UI/L))/(GLU30)(mmol/L) - GLU0(mmol/L))$$

Analyzing the results of the laboratory tests conducted, the patients with positive plasma GAD antibodies were excluded from the study. At the end, 110 normal weight patients and 109 overweight patients remained in the study. Data extracted from these patients were further analyzed in order to explore the relationship between the above indexes and glycated hemoglobin.

The statistical analysis was performed using Biostat software. A value of $p<0.05$ was considered statistically significant. For comparison of variables with normal distribution t-test was used and for comparison of variables that were not normal distributed Mann-Whitney u-test was used. Variables with normal distribution were expressed as mean values and standard deviations while variables without normal distribution were expressed as mean values and quartiles.

Results and discussions

In the normal weight patients and in overweight patients with newly diagnosed diabetes mellitus we observe that the level of HbA1c is similar (7.35% vs. 7.36%, p=0.29) meaning that the glycemic misbalance in the past three months and the mean glycaemia are very close. After the administration of 75g of glucose in the two groups and the measurement of glycaemia at 0, 30, 60, 120 and 180 minutes we observe that the level of glucose in plasma is similar at every interval; there is not any statistically significant difference between the two groups at the moments when we determined the glycaemia (Table 1, Figure 1). However, when we assessed the insulin level at 0, 30, 60, 120 and 180 from the moment of administration of 75 g glucose in the two groups, we observed that the level of plasmatic insulin is statistically significant higher in overweight patients that in normal weight patients (Table 2, Figure 2). Other differences between the two groups are: the level of triglycerides is statistically significant higher in overweight patients (2.00 mmol/L vs 1.56 mmol/L, $p<0.01$), the level of HDL cholesterol is statistically significant higher in normal weight patients (1.41 mmol/L vs. 1.27 mmol/L, p=0.01), systolic blood pressure is statistically significant higher in overweight patients (134.60 mmHg vs. 129.40 mmHg, p=0.01) and diastolic blood pressure is statistically significant higher in overweight patients (83.88 mmHg vs. 79.28 mmHg, $p<0.01$).

Table 1
CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE INCLUDED PATIENTS

Parameter	Normal Weight (n=110) Mean Values and Standard deviation/(Quartile1/Quartile 3)	Overweight (n=109) Mean Values Standard deviation/(Quartile1/Quartile 3)	Statistical Significance (p-value)
Sex (% Men)	52.72%	52.29%	0.94
Age (years)	56.08 ±11.43	56.25±10.65	0.90
BMI (kg/m^2)	21.50±2.02	26.23±2.52	<0.01
WC (cm)	79.53±6.91	89.97±5.86	<0.01
GLU0 (mmol/L)	7.16 (6.1, 7.8)	7.38 (6.3, 8.3)	0.83
GLU30 (mmol/L)	11.32 (9.85, 13.05)	11.39 (9.79, 12.80)	0.72
GLU60 (mmol/L)	14.64 (12.60, 16.25)	14.70(12.55, 16.65)	0.91
GLU120 (mmol/L)	14.56 (11.80, 17.05)	14.30 (11.90,1 6.57)	0.71
GLU180 (mmol/L)	10.96 (7.45, 14.70)	10.20 (7.25, 13.05)	0.26
INS0 (μIU/L)	8.29 (5.56, 9.49)	10.85 (6.99, 13.22)	<0.01
INS30 (μIU/L)	21.46 (10.17, 23.84)	28.25 (12.46, 37.35)	<0.01
INS60 (μIU/L)	34.78 (16.25, 42.8)	46.52 (21.60, 64.48)	<0.01
INS120 (μIU/L)	46.45 (21.52, 64.50)	57.51 (28.36, 78.58)	0.01
INS180 (μIU/L)	26.93 (14.18, 32.96)	34.74 (17.52, 46.81)	0.01
HbA1c (%)	7.35 (6.4,7 .9)	7.36 (6.2, 8.12)	0.29
TC (mmol/L)	5.04 (4.28, 5.66)	5.02 (4.38, 5.53)	0.72
TG (mmol/L)	1.56 (0.89, 1.96)	2.00 (1.20, 2.40)	<0.01
HDL (mmol/L)	1.41 (1.14, 1.56)	1.27 (1.04, .41)	0.01
LDL (mmol/L)	3.12 (2.52, 3.55)	3.15 (2.63, 3.53)	0.36
SBP (mmHg)	129.40±16.97	134.60±14.73	0.01
DBP (mmHg)	79.28±10.74	83.88±8.83	<0.01

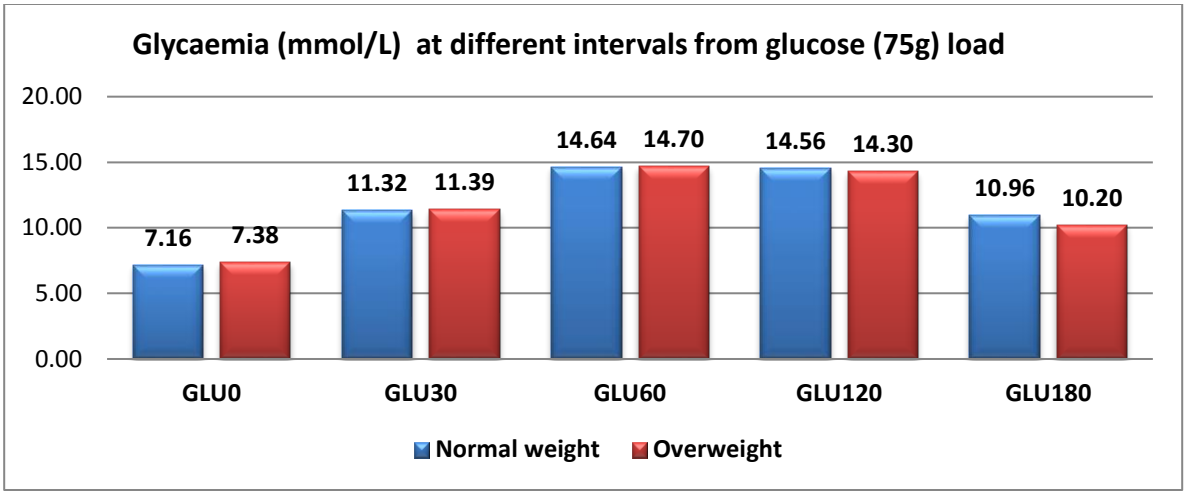


Fig. 1. Glycaemia of the normal weight and overweight patients, at different intervals after 75 g glucose administration

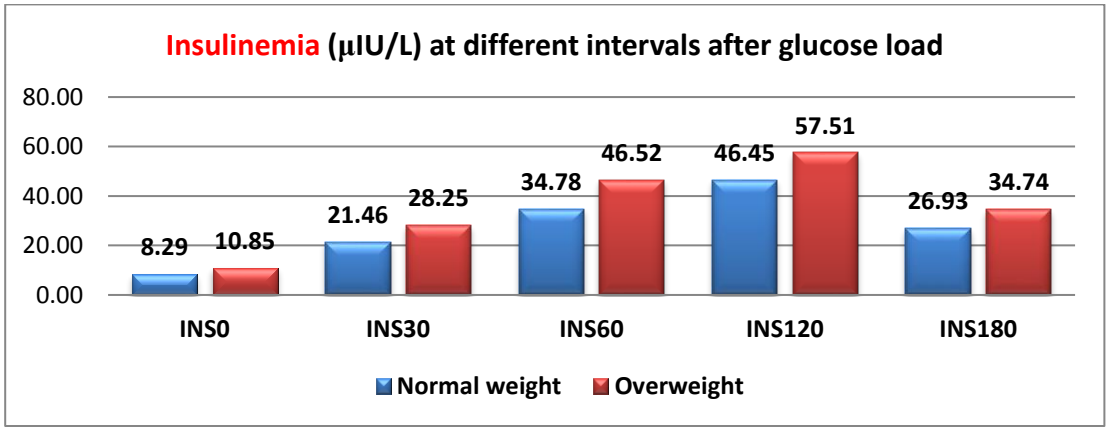


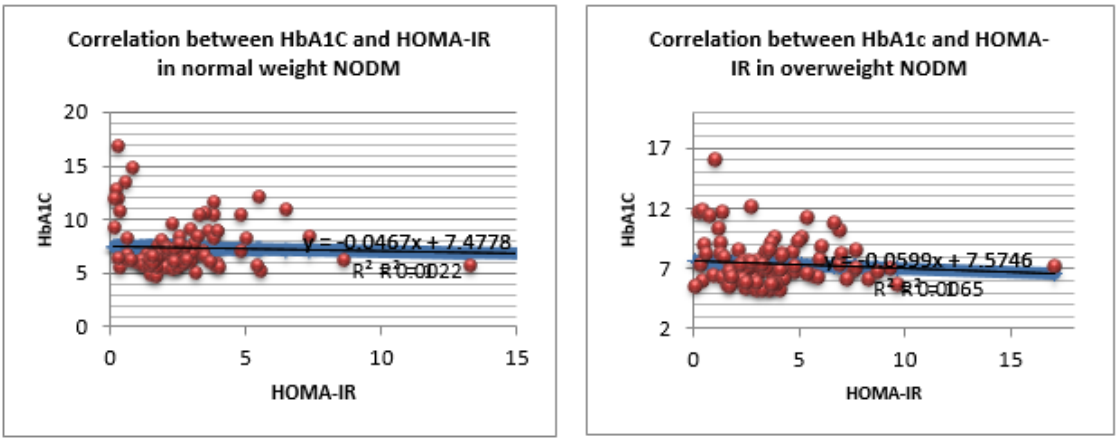
Fig. 2. Insulinemia of normal weight and overweight patients at different intervals after 75g glucose administration

Table 2
INDEXES FOR INSULIN SECRETION, INSULIN RESISTANCE, INSULIN SENSITIVITY AND β CELL FUNCTION

Parameter	Normal Weight (n=110) Mean Values and Standard deviation/(Quartile1/Quartile 3)	Overweight (n=109) Mean Values and Standard deviation/(Quartile1/Quartile 3)	Statistical Significance (p-value)
HOMA-IR	2.65 (1.53, 3.14)	3.55 (2.27, 4.17)	<0.01
HOMA-β	55.08 (27.88, 64.16)	65.36 (31.29, 77.31)	0.02
ISI	5.97 (0.40, 1.06)	3.48 (0.21, 0.77)	<0.01
IGI	3.63 (0.76, 3.74)	3.95 (0.91, 5.65)	0.07

In overweight patients diagnosed with T2DM, insulin resistance assessed by HOMA-IR index was statistically significant more increased than in patients with normal weight (2.65 vs. 3.55, $p<0.01$). Individuals with increased BMI had a mean value of insulin secretion or a β cell function assessed by HOMA-β index, statistically significantly higher than individuals with normal BMI (65.36 vs. 55.08, $p=0.02$). Normal weight patients diagnosed with T2DM had a mean value of insulin sensitivity as assessed by ISI index, statistically significantly higher than in the case of overweight patients (5.97 vs. 3.48, $p<0.01$). In overweight individuals, early insulin secretion as assessed by IGI index was not statistically significantly higher than in normal weight individuals (3.95 vs. 3.63, $p=0.07$).

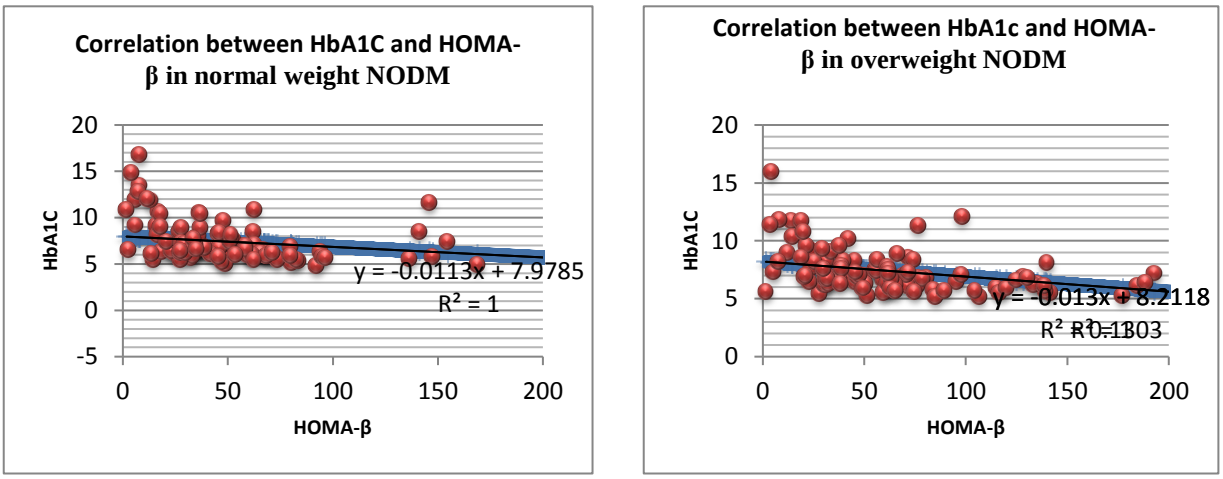
Both in normal weight patients and overweight patients with newly diagnosed diabetes mellitus (NODM) there is not any correlation between HbA1c and HOMA-IR ($p=0.62$ for normal weight individuals and $p=0.40$ for overweight individuals) (Figure 3a and b).



a b

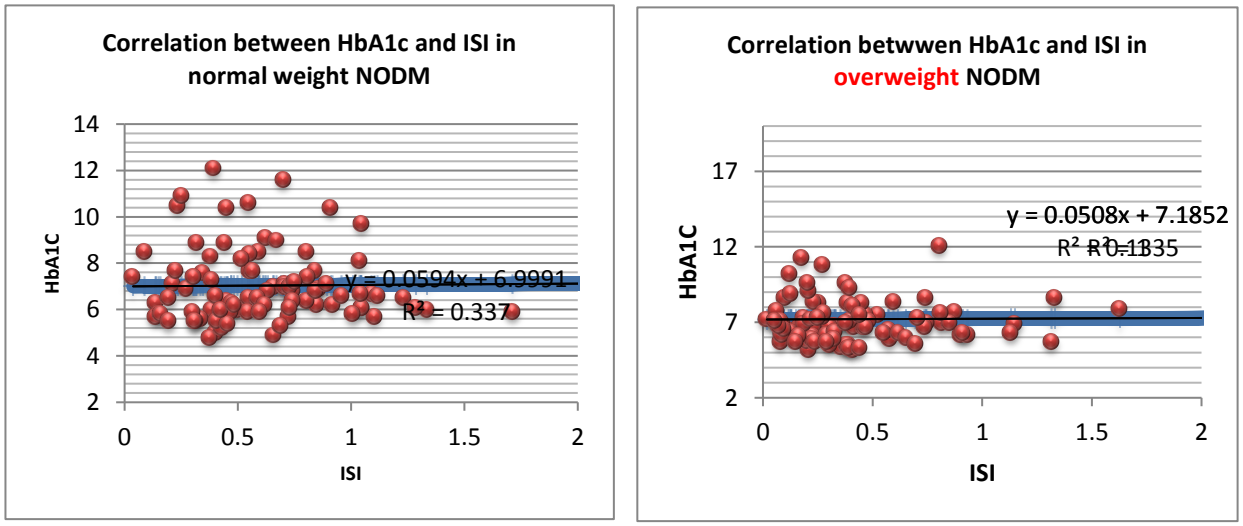
Fig. 3. Correlation between HbA1c and HOMA-IR in normal weight (a) and overweight patients (b)

There is a statistically significant correlation between HOMA-β representing the insulin secretion of β cells and HbA1c in both normal weight (p=0.001) and overweight (p=0.0001) individuals with NODM (Figure 4a and b), as well as between insulin sensitivity assessed by ISI index and HbA1c both in normal weight NODM patients (p<0.01) and overweight NODM patients (p<0.01) (Figure 5a and b). In normal weight NODM patients there is a statistically significant correlation between early insulin secretion assessed by IGI index and HbA1c (p=0.01), but in overweight NODM patients there is no statistically significant correlation between IGI index and HbA1c (p=0.05) (Figure 6 a and b).



a b

Fig. 4. Correlation between HbA1c and HOMA-β in normal weight and overweight patients



a b

Fig. 5. Correlation between HbA1c and ISI index in normal weight (a) and overweight patients (b).

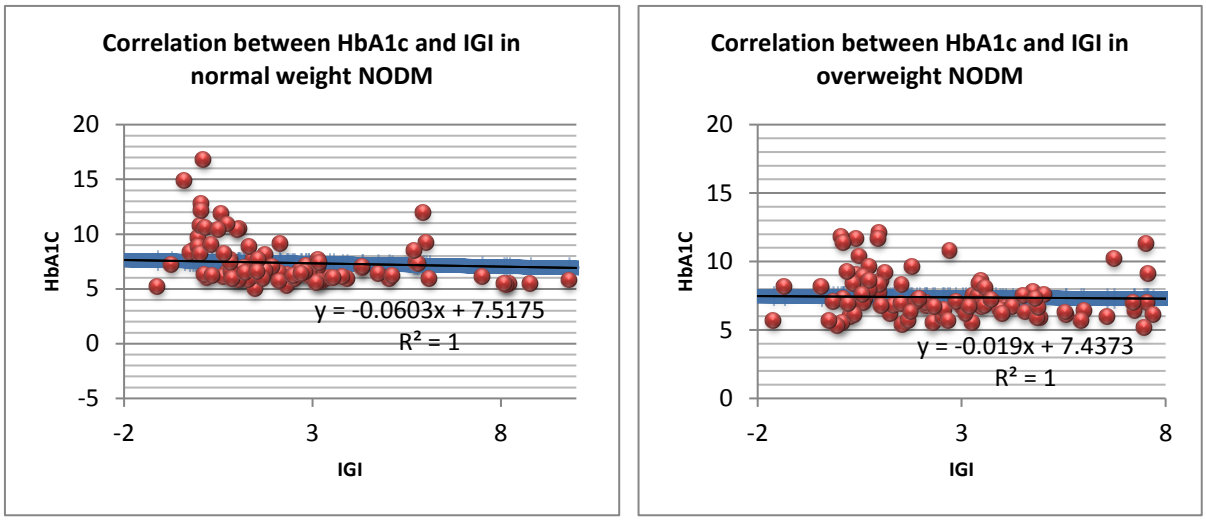


Fig. 6. Correlation between HbA1c and IGI index in normal weight (a) and overweight patients (b).

a. b.

We observed that the level of HbA1c and the values of glycaemia in the two groups were comparable but in overweight patients in order to achieve almost the same HbA1c and GLU0, GLU30, GLU60, GLU120 and GLU180 the levels of insulin values INS0, INS30, INS60, INS120 and INS 180 have to be maintained at levels that are statistically significant higher than in normal weight patients with NODM. The reason why β cells need to produce more insulin in overweight T2DM patients is the increased insulin resistance of these individuals [19]. In our study overweight T2DM patients had a mean value of HOMA-IR of 3.55, significantly higher than the cut-off value of HOMA-IR of 2.5 suggestive for the presence of insulin resistance in Caucasian population [20]. Interestingly the mean value of HOMA-IR in normal weight NODM patients was 2.65 also above the HOMA-IR cut-off value of 2.5 specific for insulin resistance, although we would expect normal weight individuals to be non-insulin resistant. These findings confirm early results from literature regarding the existence of metabolic unhealthy normal weight individuals that develop T2DM and who are characterized by insulin resistance, lipid anomalies, high blood pressure and increased risk of cardiovascular diseases [21-25]. In both groups the β cell function determined by the calculation of HOMA-β was decreased compared to the cut-off values of 72.6 in non-diabetic individuals [26].

Lower values of insulin secretion in patients recently diagnosed with T2DM reveal the depletion of insulin in these individuals, but this depletion is more pronounced in normal weight NODM patients where HOMA-β index was statistically significant lower than in overweight NODM patients. β cell dysfunction is an important element of T2DM development, data from literature showing that normal weight individuals tend to have a high frequency of impaired β cell function [27]. In our study between ISI index and HOMA-R there was an inverse proportional relationship, normal weight NODM individuals who had a lower HOMA-IR index had a higher value of ISI index, while overweight NODM individuals who had a higher level of HOMA-IR had a lower value of ISI index. This association between an increased BMI, an increased insulin resistance and decreased insulin sensitivity has been reported by previous studies [28-30].

Also, the decreasing in insulin sensitivity is a proven factor for deteriorating glucose metabolism [31]. In the overweight group, the value of ISI index was significantly lower than the cut-off value of 4.5, values below this value representing decreased insulin sensitivity [32]. IGI index that represents the early insulin secretion, was significantly above the cut-off value of 0.5 [33], the insulin secretion response was higher in overweight individuals, but not statistically significant; both groups had a good insulin secretion early response after glucose administration.

In our study, β-cell function correlated statistically significant with HbA1c, a good level of β-cell function corresponded to lower values of HbA1c. Similar findings have been reported in other studies [34], where a decreased glucose control was associated to substantial reduction in β cell function. Insulin resistance did not correlate with HbA1c in our study, as in other studies where HOMA-IR higher values were not associated with a decreased glucose status [35]. HbA1c correlated with insulin sensitivity assessed by ISI index, interestingly low values of HbA1c corresponded to low values of ISI index. Data from literature report exactly the opposite, a negative correlation between HbA1c and ISI index, but the correlation was modest [36]. IGI index correlated with HbA1c in normal weight NODM patients but not in overweight NODM patients. Previous studies report that islets secretory capacity assessed by IGI index correlates significantly with HbA1c but ISI does not correlate with HbA1c [37].

Conclusions

The presence of normal BMI in the moment of T2DM diagnosis is associated with a significantly decreased level of insulin resistance, a significantly increased level of insulin sensitivity, a significantly worse β cell function and a non-significantly decreased early insulin secretion when compared with overweight patients.

References

1.KASUGA, M. J. Clin. Invest., **116**, nr. 7, 2006, p. 1756.
2.KADOWAKI, T., YAMAUCHI, T., KUBOTA, N., HARA, K., UEKI, K., TOBE, K., J. Clin. Invest., **116**, nr. 7, 2006, p. 1784
3.OLONSKY, K.S., GIVEN, B.D., HIRSCH, L., SHAPIRO, E.T., TILLIL, H., BEEBE, C., GALLOWAY, J.A., FRANK, B.H., KARRISON, T., VAN CAUTER, E., J. Clin. Invest., **81**, nr. 2, 1988, p. 435.
4.KAHN, S.E., PRIGEON, R.L., MCCULLOCH, D.K., BOYKO, E.J., BERGMAN, R.N., SCHWARTZ, M.W., et al. Diabetes, **42**, nr. 11, 1993, p. 1663.
5.GUJA, C., BOTNARIU, G., CERGHIZAN, A., DINCA, M., POPA, A., SUCIU, G., Diab. Res. Clin. Pract., **120**, nr. Suppl. 1, 2016, p. S101.
6.MOTA, M., POPA, S.G., MOTA, E., MITREA, A., CATRINOIU, D., CHETA, D.M., GUJA, C., HANCU, N., IONESCU-TIRGOVISTE, C., LICHIARDOPOL, R., MIHAI, B.M., POPA, A.R., ZETU, C., BALA, C.G., ROMAN, G., SERAFINCEANU, C., SERBAN, V., TIMAR, R., VERESIU, I.A., VLAD, A.R., J. Diabetes, **8**, nr. 3, 2016, p. 336. DOI: 10.1111/1753-0407.12297
7.POPA, S., MOTA, M., POPA, A., MOTA, E., SERAFINCEANU, C., GUJA, C., CATRINOIU, D., HANCU, N., LICHIARDOPOL, R., BALA, C., ROMAN, G., RADULIAN, G., TIMAR, R., MIHAI, B., J. Endocrinol. Invest., **39**, nr. 9, 2016, p. 1045. DOI: 10.1007/s40618-016-0470-4
8.SZASZ, F., LEVAI, C., NAVOLAN, D., FARCAS, S., ANDREESCU, N., BIRSASTEANU, F., MEHEDINTU, C., IONESCU, C.A., BOHILTEA, R., CARABINEANU, A., NEMESCU, D., SIMU, S., STOIAN, D., Rev. Chim.(Bucharest), **69**, no. 2, 2018, p. 529.
9.BERCEANU, C., TETILEANU, A.V., OFITERU A.-M., BRATILA, E., MEHEDINTU, C., VOICU, N.L., SZASZ, F.A., BERCEANU, S., VLADAREANU, S., NAVOLAN, D.-B., Rom. J. Morphol. Embryol., **59**, nr. 1, 2018, p. 175.
10.GUJA, C., BOTNARIU, G., CERGHIZAN, A., DINCA, M., POPA, A., SUCIU, G., Diabetes mellitus as cardiovascular disease. International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications, 2016, p. 255. 2nd International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications, INTERDIAB, Bucharest, Romania.
11.ROMAN, G., BALA, C., CRETEANU, G., GRAUR, M., MOROSANU, M., AMORIN, P., PIRCALABOIU, L., RADULIAN, G., TIMAR, R., CADARIU, AA. Acta Endocrinol.-Bucharest., **11**, nr. 1, 2015, p. 64. DOI: 10.4183/aeb.2015.64
12.GUJA, C., IOACARA, S., CATRINOIU, D., NEGRISANU, G., POPA, A. Interdisciplinary Approaches in Diabetic Chronic Kidney Disease, 2015, p. 230. 1st International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications, INTERDIAB, Bucharest, Romania.
13.HUSSEIN, S., POPA, A.R., ALBU, M.S., Diabetologia, **44**, nr. Suppl. 1, 2001, p. A138.
14.SAISHO, Y., World. J. Diabetes, **6**, nr. 1, 2015, p. 109.
15.COLEMAN, N.J., MIERNIK, J., PHILIPSON, L., FOGELFELD, L., J. Diabetes Complications, **28**, nr. 4, 2014, p. 500.
16.BALASUBRAMANYAM, A., YAJNIK, C.S., TANDON, N., Trans. Endocrinol. Metab., **2**, 2011, p. 43.
17.TAYLOR, R., HOLMAN, R.R., Clin. Sci. (Lond)., **128**, nr. 7, 2015, p. 405.
18.SINGH, B., SAXENA, A., World J. Diabetes, **1**, nr. 2, 2010, p. 36.
19.PANAG, K.M., KAUR, N., GOYAL, G., Int. J. Appl. Basic Med. Res., **4**, nr. Suppl 1, 2014, p. S41.
20.YAMADA, C., MORIYAMA, K., TAKAHASHI, E., J. Diabetes Investig., **3**, nr. 4, 2012, p. 384.
21.LEE, S.H., HAN, K., YANG, H.K., et al. Nutr. Diabetes, **5**, nr. 4, 2015, p. e149. doi:10.1038/nutd.2014.46
22.DIACONU, C.C., DRAGOI, C.M., BRATU, O.G., NEAGU, T.P., PANTEA STOIAN, A., COBELSCHI, P.C., NICOLAE, A.C., IANCU, M.A., HAINAROSIE, R., STANESCU, A.M.A., SOCEA, B., Farmacia, 2018, **66**, nr. 3, 2018, p. 408.
23.DIACONU, C.C., MANEA, M., IANCU, M.A., STANESCU, A.M.A., SOCEA, B., SPINU, D.A., MARCU, D., BRATU, O.G., Rev. Chim.(Bucharest), **69**, no. 5, 2018, p. 1071.
24.DIACONU, C.C., STANESCU, A.M.A., PANTEA STOIAN, A., TINCU, R.C., COBILINSCHI, C., DRAGOMIRESCU, R.I.F., SOCEA, B., SPINU, D.A., MARCU, D., SOCEA, L.I., BRATU, O.G., Rev. Chim.(Bucharest), **69**, no. 6, 2018, p. 1367.
25.RADULESCU, D., BALCANGIU STROESCU, A., PRICOP, C., GEAVLETE, B., NEGREI, C., BRATU, O., GINGHINA, O., VACAROIU, I., Rev. Chim.(Bucharest), **68**, no. 1, 2017, p. 52.
26.GHASEMI, A., TOHIDI, M., DERAKHSHAN, A., HASHEMINIA, M., AZIZI, F., HADAEGH, F., Acta Diabetol., **52**, nr. 5, 2015, p. 905.
27.MENEILLY, G.S., ELLIOTT, T., TESSIER, D., HARDS, L., TILDESLEY, H., Diabetes Care, **19**, nr. 12, 1996, p. 1320.
28.ZHAO, Q., LAUKKANEN, J.A., LI, Q., LI, G., Clin. Interv. Aging, **12**, 2017, p. 745. doi:10.2147/ CIA.S130014
29.MOISA, C., HOAGHIA, M.-A., SIMEDRU, D., CADAR, O., Studia Universitatis Babes-Bolyai, Chemia, **61**, nr. LXI, 2016, p. 441.
30.ABDEL-DAIM, M.M., ZAKHARY, N.I., ALEYA, L., BUNGAU, S.G., BOHARA, R.A., SIDDIQI, N.J., Oxidat. Med. Cell. Longev., **2018**, 2018, ID 2098123, 2 pages. https://doi:10.1155/2018/2098123
31.MITSUI, R., FUKUSHIMA, M., NISHI, Y., UEDA, N., SUZUKI, H., TANIGUCHI, A., et al., Metabolism, **55**, nr. 1, 2006, p. 53.
32.RADIKOVA, Z., KOSKA, J., HUCKOVA, M., KSINANTOVA, L., IMRICH, R., VIGAS, M., et al., Exp. Clin. Endocrinol. Diabetes, **114**, nr. 5, 2006, p. 249.
33.HAECKEL, R., RABER, R., WOSNIOK, W., Clin. Chem. Lab. Med., **44**, nr. 7, 2006, p. 817.
34.HOU, X., LIU, J., SONG, J., et al., J. Diabetes Res., **2016**, 2015, p. 8797316.
35.ONAL, Z.E., ATASAYAN, V., GURBUZ, T., HEPKAYA, E., NUHOGLU, C., Afr. Health Sci., **14**, nr. 3, 2014, p. 533.
36.LI, G., HAN, L., WANG, Y., et al. BMJ Open. **8**, nr. 8, 2018, p. e020665. doi:10.1136/bmjopen-2017-020665
37.WIESLI, P., SCHAFFLER, E., SEIFERT, B., SCHMID, C., DONATH, M.Y., Swiss Med. Wkly., **134**, nr. 37-38, 2004, p. 559.

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