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OBESITY INFLUENCE ON INSULIN ACTIVITY AND RESTING METABOLIC RATE IN TYPE 2 DIABETES

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Abstract

Background and Aims. *Diabetes mellitus and obesity modify the resting metabolic rate (RMR) in opposite directions. This study aimed to evaluate the relationship between RMR, insulin activity and adipocytokines in type 2 diabetes patients with associated obesity.* ***Material and Method.*** *Anthropometric and biochemical measurements, bioimpedance body composition, C peptide, insulin, proinsulin, adiponectin, leptin, indirect calorimetry measured RMR were determined in 306 (53% male, 54.9% obese) diabetic patients.* ***Results****. RMR, C peptide, insulin, proinsulin, Homeostasis Model Assessment (HOMA) IR, HOMA %B, leptin increased and adiponectin decreased with body mass index (BMI). BMI had a statistical significant higher effect size on fat mass (FM), leptin (0.652-0.339 eta squared), moderate positive effect size on fat-free mass (FFM), insulin, C peptide, RMR, HOMA %B (0.228 - 0.155 eta squared) and lower effect size on HOMA IR, adiponectin, proinsulin-to-adiponectin ratio, proinsulin (0.098-0.062 eta squared). RMR had positive correlations with FFM (r=0.633), BMI (r=0.300), proinsulin-to-adiponectin ratio (r=0.218), HOMA1-IR (r=0.176), proinsulin (r=0.151), insulin (r=0.144), fasting plasma glucose (r=0.132), fat mass (r= 0.131), triglycerides (r = 0.119), proinsulin-to-insulin ratio (r= 0.117) and negative with age (r=-0.368), HDL cholesterol (r=-0.284), adiponectin(r=-0.282) and leptin (r=-0.178).* ***Conclusions*** *RMR was correlated with HOMA IR and insulin resistance biomarkers, but not with HOMA %B and C peptide.*

key words: *resting metabolic rate, type 2 diabetes, insulin resistance, proinsulin, adipocytokines, HOMA*

Background and Aims

The "continuum" of diabetes begins with an initial defect of insulin action followed by a decrease of beta cell function. When fasting plasma glucose (FPG) increases above diabetic level it is considered that the level of beta cells secretion is decreased with 80% and insulin resistance is at maximum level [1]. The balance between these pathogenic mechanism and other

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factors such as the excess and distribution of fat tissue, its pro inflammatory state or secreted adipokines is particular for each clinical situation and explains the metabolic diversity of type 2 diabetes (T2DM) [2,3]. The metabolic changes characteristic for T2DM are the relative insulin deficiency, increased endogenous glucose output (EGO), lipid oxidation and decreased glucose oxidative and non oxidative disposal.

Increased energy expenditure has been reported by different authors in hyperglycaemic and insulin deficient states [4,5]. Poor glycemic control was estimated to increase the resting metabolic rate (RMR) with 8% [4]. RMR is defined as the energy requirement for maintaining the metabolic processes in awake state and thermo neutral environment [6]. Adipose tissue has lower energy consumption in comparison with skeletal musculature so its accumulation increases RMR but decreases the ratio RMR/ kg total body weight [7].

C peptide, insulin, proinsulin, proinsulin-to-adiponectin ratio, proinsulin-to-insulin ratio and HOMA indices were proposed and analyzed by different authors for measuring insulin resistance and beta cell dysfunction [1,8-11]. The initial defect of insulin action induces an over secretion of beta cells with higher levels of insulin, C peptide and proinsulin. C peptide is a better indicator of beta cell function than insulin because has a steady concentration and no hepatic clearance. Proinsulin is increased in type 2 diabetes as a consequence of insulin demand with insufficient processing or premature release of insulin from the secretory granules, but also as a result of its decreased clearance in comparison with insulin. The homeostasis model assessment (HOMA) is a validated mathematical model that determines an index for insulin resistance from FPG and insulin level. The initial linear equation of HOMA1-IR published by Matthews was followed by the software programme of HOMA2-IR considered more accurate by its physiological adjustments. HOMA2-IR is a non linear estimation that takes into account hepatic and peripheral insulin resistance, renal glucose output and an estimate proinsulin secretion level [8]. HOMA1-IR and HOMA2-IR indices were identified as having different cut-off points for insulin resistance in healthy subjects and also in those with metabolic syndrome [12].

Leptin and adiponectin are adipocytokines involved in energy homeostasis. Leptin increases with fat mass accumulation, activates glucose metabolism and modifies insulin sensitivity. Leptin correlates with BMI, fat mass, insulin, proinsulin, C peptide, HOMA IR and HOMA %B. Decreased leptin levels were reported in diabetes compared with healthy subjects in the fasting state or after stimulation [13-15]. Adiponectin increases energy expenditure, insulin action of suppressing hepatic glucose production, free fatty acids (FFA) oxidation, glucose uptake and disposal and counteracts the proinflammatory effect of TNF alfa [16] High adiponectin levels are associated with insulin sensitivity and has a protective cardiovascular effect [17].

The aim of this study was to evaluate the relationship between RMR and insulin activity parameters in relation to fat tissue accumulation in a cohort of newly diagnosed patients with type 2 diabetes.

Material and Methods

The study included 306 (53% male) patients successively diagnosed with type 2 diabetes in the outpatient department of the National Institute of Diabetes, Nutrition and Metabolic Diseases “Prof. N.C. Paulescu” between 2012-2014. The study was approved by the local Ethic Committee and was a part of a grant of the Romanian National Authority for Scientific

Research, CNCS-UEFISCDI. All the subjects agreed and signed an informed consent.

Exclusion criteria were: type 1 diabetes, symptoms or signs of acute and chronic infection, malignancies, significant pulmonary disease, excessive alcohol consumption, prior antidiabetic treatment, thyroid dysfunctions. All subjects were invited to perform study procedures in the following order: blood sampling, anthropometric measurements, bioelectrical impedance measurements and resting metabolic rate determination.

Patients were instructed to fast and drink only water for 10 hours before tests and refrain from smoking and intense physical activity in the previous 24 hours before the test. COBAS INTEGRA 400 PLUS Hitachi Roche (Roche Diagnostic, Basel, Switzerland) device was used for HbA_{1c}, blood glucose, HDL cholesterol, total cholesterol and triglycerides measurement. Serum insulin, proinsulin, adiponectin, leptin levels were determined by ELISA method using commercial kits from DRG Instruments GmbH, Germany (EIA 1560, EIA 4177, EIA 2395, EIA 2935, EIA 1293). HOMA1-IR index was computed with the formula: HOMA1-IR = fasting plasma insulin (µU/ml) x fasting plasma glucose (mg/dl)/405 while HOMA1-%B according to the formula HOMA1-%B = (360 x insulin (µU/ml)/glucose (mg/dl) -63)% [18]. The HOMA2-IR and HOMA2-%B index were computed with the v2.2.2.computer model HOMA Calculator [19]. Proinsulin-to-insulin molar ratio % was computed as the [proinsulin (pmol/L)/ insulin (pmol/L)] x 100. Proinsulin-to-adiponectin ratio was computed as proinsulin (pmol/L)/ adiponectin (µg/ml). Weight and height were measured with subjects wearing thin clothes and without shoes. Body composition was determined by bioimpedance using the TANITA BC- 601 analyzer [20].

Indirect calorimetry was performed with face mask system from Quark CPET COSMED. RMR was computed from gas measurement using the abbreviated Weir equation [21,22]. The device was warmed up for 10 min and calibrated for each determination with a certified gas (16.2% O_2, 5.02%CO_2, 78.7% N_2 produced by Air Liquide Healthcare America Corporation, USA). Examination was performed in the morning, after 30 minutes of rest with the patient relaxed and awaked, in a thermo neutral environment. The first initial 5 minutes of registration were discharged. The recording length was 20 minutes after reaching the steady state. Respiratory quotient (RQ) is the ratio between CO_2 produced and O_2 consumed (VCO_2/VO_2). RQ indicates also the ratio of oxygen to carbon from a nutrient molecule, a low RQ equal to 0.7 characterizes lipids oxidation and a high RQ of 1.0 is associated with glucose oxidation.

Weight status was evaluated using the BMI according to the World Health Organization recommendations [23]. According to BMI, patients were divided in 5 subgroups: 1- normal weight (<24.9 kg/m^2), 2 - overweight (25-29.9 kg/m^2), 3 - obesity class 1 (30-34.9 kg/m^2), 4 - obesity class 2 (35-39.9 kg/m^2) and 5 – obesity class 3 or severe obesity (> 40 kg/m^2).

Statistical analysis. We used SPSS Statistic 22 software for data analysis. The data were tested for normality with the Kolmogorov-Smirnov test. Data were reported as mean ± standard deviation (SD) or percentiles score as appropriate. Comparisons were done using Mann-Whitney and Kruskal Wallis test. Correlations between variables were performed with Spearman's test. A p-value < 0.05 was considered statistically significant.

Results

The study group consisted of 145 (47%) females and 161 (53%) males, age 58.83±10.27years (mean±SD) for total group. Regarding weight status, 29 (9.4%) patients had normal weight, 109 (35.6%) patients were overweight and 168 (54.9%) patients were obese. According to the degree of obesity, the number of patients was: class 1 obesity - 104 (34%) patients, class 2 obesity - 47 (15.3%) patients and class 3 obesity - 17 (5.5%) patients.

There were no significant differences between genders for BMI, FPG, HbA_{1c}, total cholesterol, triglycerides, C peptide, insulin, HOMA indices and respiratory quotient (RQ). Females had significantly higher age, HDL cholesterol, leptin, adiponectin and fat mass, while males had significantly higher height, weight, proinsulin level, proinsulin-to-insulin ratio, proinsulin-to-adiponectin ratio and FFM. The characteristics of study group are presented in Table 1.

Table 1. Study group anthropometrical, biochemical, calorimetric and bioimpedance data

	Females (n=145)	Males (n=161)	p-value
Age (years)	60.55±10.11	57.27±10.2	<0.05
Height (m)	160.7±6.3	173.5±7.1	<0.05
Weight (kg)	82.01±18.5	92.5±15.1	<0.05
BMI (kg/m²)	31.7±6,6	30.7±4.5	ns
FPG (mg/dl)	167±64.3	176.2±70.9	ns
HbA_{1c} (%)	7.5±1.8	7.76±2	ns
Cholesterol (mg/dl)	221.5±55.6	213.9±48.4	ns
HDL cholesterol (mg/dl)	49.3±12.2	42.1±10.6	<0.05
Triglycerides (mg/dl)	159.7±100	185.5±106	ns
C peptide (ng/ml)	5.1±3.9	4.9±3.3	ns
Insulin (pmol/L)	96.45±69	98.62±73	ns
Proinsulin (pmol/L)	5.14±6.8	8.04±9.8	<0.05
Proinsulin-to-insulin molar ratio %	5.6±7	8.8±9	<0.05
Proinsulin-to-adiponectin ratio	0.96±1.42	2.85±4.59	<0.05
Leptin (ng/ml)	27.88±19.9	10.4±10.5	<0.05
Adiponectin (µg/ml)	10.92±9.3	5.74±5,1	<0.05
HOMA1-IR	5.64±4.49	6,62±8.41	ns
HOMA2-IR	2.1±2	2.38±2.7	ns
HOMA1-%B	61.9±45	57.4±40.6	ns
HOMA2-%B	54.8±35.6	51.14±30.9	ns
RMR (Kcal)	1385.88±295.4	1737.84±401.9	<0.05
RQ	0.78±0.06	0.79±0.05	ns
Fat Mass (kg)	34.62±12.6	27.59±9.7	<0.05
Fat Free Mass (kg)	46.37±7.7	64.53±11.7	<0.05

All data are mean ±SD; compared with Mann Whitney test; ns – not statistical significant

For both genders, significant differences between BMI subgroups were observed for C peptide, insulin, leptin, HOMA indices, RMR, FM and FFM as pointed out in Table 2. Additionally, in males statistical significant difference between BMI groups was also observed for proinsulin, adiponectin, proinsulin-to-adiponectin ratio.

Male had increased level of C peptide and insulin for overweight and obese and increased level for proinsulin, proinsulin-to-insulin ratio, proinsulin-to-adiponectin ratio, RMR, FFM for all BMI subgroups in comparison with the female group. There were no significant differences in BMI subgroups for FPG, HbA1c and RQ.

Table 2. Insulin activity, cytokines, calorimetric and bioimpedance data according to the BMI subgroups

Females					
BMI subgroups	1 (n=19)	2 (n=44)	3 (n=45)	4 (n=25)	5 (n=12)
C peptide (ng/ml)*	4.79±4.8	3.8±2	5.06±4.3	5.65±2.3	8.95±5.9
Insulin (pmol/l)*	77.04±71	73.2±38	99.66±72	131.05±75	128.06±93
Proinsulin (pmol/l)**	3.22±4.1	4.99±9.4	5.21±4.7	5.28±5.6	8.19±7
Proinsulin-to-insulin molar ratio %**	5.53±8	6.59±10	5.28±4	3.97±2	7.31±6
Proinsulin/adiponectin**	0.71±1.3	0.73±1.1	1.09±1.5	1.18±1.5	1.28±1.3
Leptin (ng/ml)*	11.45±12.2	17.63±11.7	29.73±14.9	38.62±15.8	62.19±22.4
Adiponectin (µg/ml)**	15.48±10.3	12.23±10.1	8.57±7	9.57±10.5	10.57±6,5
HOMA1-IR*	4.4±0.8	4.6±0.4	5.7±0.5	8±1.3	6.2±1,1
HOMA2-IR*	2.7±1	1.7±0.1	2.1±0.2	2.8±0.3	2.5±0.5
HOMA1-%B*	52.9±12.6	45.2±4.7	63.3±10	76.4±7.2	101.8±25.7
HOMA2-%B*	49.5±8.4	43.8±3.9	54.2±5.7	65.86±5.2	82.7±14.8
RMR (kcal)*	1264.5±248	1250.5±197	1382.5±254	1643.3±284	1550.3±417
FM (kg)*	19.6±1.1	27.8±0.6	35.8±0.7	45.3±1.1	56.5±6
FFM (kg)*	40.2±1	43±0.5	47.6±0.8	52.4±1	50.9±5
Males					
BMI subgroups	1 (n=10)	2 (n=65)	3 (n=59)	4 (n=22)	5 (n=5)
C peptide (ng/ml)*	3.27±1.7	3.96±2.7	5.40±3.1	5.94±2.8	11.33±6.3
Insulin (pmol/l)*	52.41±28	79.18±44	103.6±49	157.2±144	126.5±80
Proinsulin (pmol/l)*	4.34±6.15	5.43±5.8	8.96±10.5	14.22±15.2	11.49±8.7
Proinsulin-to-insulin molar ratio %**	7.33±6	8.12±9	8.75±10	10.33±10	15.24±17
Proinsulin/ adiponectin*	1.5±1.3	1.7±0.2	3.4±0.7	4.2±0.9	5.8±3.2
Leptin (ng/ml)*	3.69 ±4.07	5.7±5	11.9±10.2	17.84±11.4	34.39±14.7
Adiponectin (µg/ml)*	11.9±2.8	5.9±0.5	4.9±0.5	4.8±0.8	3.6±1.6
HOMA1-IR*	3.3±0.5	5±-0.3	6.3±0.5	11.5±4.2	9.3±2.6
HOMA2-IR*	2.5±1.3	1.8±0.1	2.3±0.1	3.9±1.3	2.8±0.7
HOMA1-%B*	32.6±6.1	46.9±4.6	62.4±4.5	88.2±11.1	41.4±18.5
HOMA2-%B*	34.6±6.7	43.3±3.6	55.4±3.6	72.2±9	40.8±13.6
RMR (kcal)*	1408.9±231	1663.5±325	1733±392	1970.6±408	2394.4±582
FM (kg)*	13.6±2	21.5±0.6	31.2±0.5	38.9±1.1	41.5±10.5
FFM (kg)*	52.5±6.2	61±0.8	66.5±0.9	68.9±1.3	90.5±15.8

All data are mean ±SD; *- statistical significant difference (p<0.05) between groups according to Kruskal Wallis test; **- not statistical significant

The eta squared as a measure of effect size was determined (Table 3). As expected, the highest effect size of BMI was on FM, leptin and FFM. BMI had a moderate positive effect size on C peptide, insulin, HOMA %B and RMR and a lower effect size on proinsulin, proinsulin-to-adiponectin ratio, adiponectin and HOMA IR. The effect size of BMI was higher for HOMA %B than HOMA IR for both indices.

Correlation indices for RMR with the analyzed parameters are shown in Table 4. For total group and males subgroup, RMR correlates positively (in the descending order for correlation indices) at a significant level with FFM, BMI, proinsulin-to-adiponectin ratio, HOMA IR, proinsulin, FPG, FM and negatively with age, HDL cholesterol, adiponectin and leptin. For females subgroup RMR correlates only with age (negatively) and BMI, FM and

FFM (positively). No correlation was found between RMR and HbA1c, C-peptide, HOMA %B and RQ. We found a statistical significant difference for RQ depending of the FPG (FPG < 180mg/dl and > 180 mg/dl) only for the male subgroup.

Table 3. Effect size of BMI on analyzed parameters

	Total	Females	Males
C peptide	0.160[1]	0.155	0.178
Insulin	0.162	0.158	0.177
Proinsulin	0.052	ns[2]	0.062
Proinsulin/adiponectin	0.058	ns	0.086
Leptin	0.339	0.46	0.405
Adiponectin	0.063	ns	0.075
HOMA1-IR	0.082	0.078	0.096
HOMA2-IR	0.094	0.096	0.098
HOMA1-%B	0.132	0.156	0.15
HOMA2-%B	0.102	0.125	0.12
RMR	0.122	0.228	0.153
Fat mass	0.652	0.757	0.673
Fat Free mass	0.132	0.505	0.255

1- Eta squared for Kruskal Wallis test; 2 - not significant effect size

Table 4. Correlation indices for RMR

	Total (n=306)	Male (n=165)	Female (n=141)
Positive correlation			
Fat free mass	0.633[1]	0.474	0.508
BMI	0.300	0.323	0.431
Proinsulin/adiponectin	0.218	0.195	ns[2]
HOMA1-IR	0.176	0.206	ns
HOMA2-IR	0.171	0.176	ns
Proinsulin	0.151	0.168	ns
Insulin	0.144	ns	ns
FPG	0.132	0.193	ns
HbA1c	ns	0.197	ns
Fat mass	0.131	0.255	0.404
Triglycerides	0.119	ns	ns
Proinsulin/insulin	0.117	ns	ns
Negative correlation			
Age	–0.368	–0.367	–0.329
HDL-cholesterol	–0.284	–0.197	ns
Adiponectin	–0.283	–0.172	ns
Leptin	–0.178	ns	ns

1– statistical significant correlation indices with Spearman p<0.05; 2 – ns - not statistical significant correlation

HOMA1-IR and HOMA2-IR 25th, 50th, 75th, 90th percentiles scores were higher in males vs. females regardless of the used model (Figure 1).

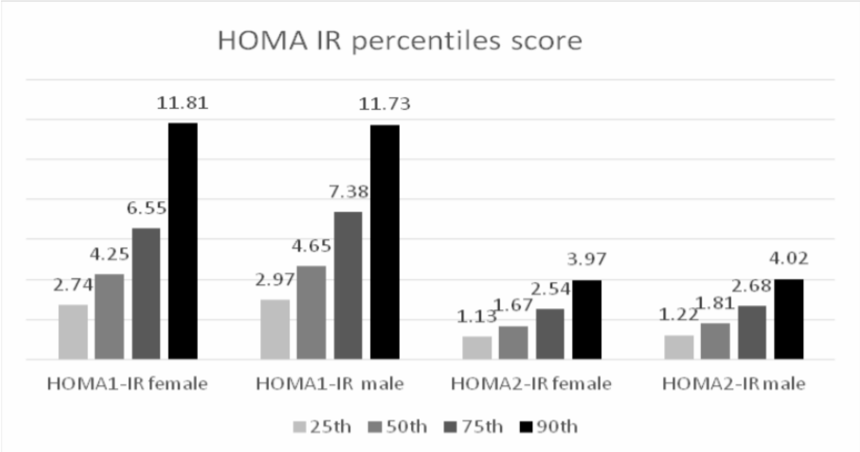

Figure 1. *Comparative percentiles score for HOMA1 IR and HOMA 2 IR*

Discussions

This study investigated the relation between RMR, insulin activity and adipose tissue in type 2 diabetes subjects. Obesity prevalence in this cohort was 54.9% vs. 31,9% the prevalence of obesity reported for subjects between 20-70 years of age in PREDATORR study for Romanian population [24]. Even our study group was older, this increased obesity prevalence in a diabetes cohort underlines the frequent association of diabetes with obesity.

For similar FPG and HbA_{1c}, increasing BMI was associated with increased C peptide, insulin, proinsulin, proinsulin-to-insulin ratio, proinsulin-to adiponectin ratio, HOMA1-IR and HOMA %B indices, leptin, RMR and FM. The mean value for C peptide, insulin, proinsulin had an upward trend related to BMI, but this increase was not linear and continuous. In comparison with class 2 obesity, the class 3 obesity subgroup associated increased C peptide, proinsulin, proinsulin-to-insulin ratio, proinsulin-to-adiponectin ratio, leptin, HOMA %B indices and FM, with relatively reduced insulin, adiponectin, HOMA IR, RMR and FFM. For this subgroup the relatively dissociation between higher C peptide level and lower insulin level could be probably explained by the increased release of proinsulin and insulin hepatic clearance.

In the analysis by gender, the differences in anthropometric parameters, HDL cholesterol, RMR, FM, FFM could be considered in agreement with known physiology. Male subgroup was similar to female subgroup regarding BMI, FPG, HbA_{1c}, cholesterol, triglycerides, C peptide, insulin and HOMA indices. Higher levels of proinsulin were calculated for all BMI subgroups in males vs. females, but the difference was statistically significant only for class 1 and 2 obesity subgroups. In previous studies, the proinsulin value reported for obese and diabetes subjects were 10% to 22% of immunoreactive insulin secretion [25-27]. The increasing level of proinsulin with BMI was reported also for other insulin resistant conditions such as polycystic ovary syndrome [11]. As C-peptide and insulin had no significant differences between genders, a significant increased of proinsulin with a proinsulin-to-insulin ratio in males could plead for an increased beta cell dysfunction for this study group.

The mean value of adiponectin decreased and the mean value of leptin increased with BMI. In the female subgroup, the mean value for adiponectin was similar with reported data for lean non diabetic female subjects (10.9±9.3 vs. 10-30µg/ml[3]) and decreased in the previous estimated level by 4.3 µg/mL from healthy to overweight and obese. In males, the decrease of adiponectin between BMI subgroups was higher than the estimated value of 1.9 µg/mL [28].

For both genders, the mean value for leptin in class 3 obese subjects was considerably higher than the expected value for lean subjects (62.19±22.4 in females and 34.39±14.7 in males vs. 2-8 ng/ml [3]). It was shown that leptin increases energy expenditure by increasing the uncoupling protein 1 (UCP-1) and sympathetic nervous system stimulation. Leptin is thought to directly increase glucose uptake and fatty acid oxidation in skeletal muscle.

As expected, RMR increased with BMI subgroups for both genders. In females there was a decrease of mean RMR between normal weight and overweight subgroups, respectively between class 2 and class 3 obesity subgroups. These decreases were not statistically significant and were associated with a decrease of mean insulin and HOMA2-IR. In males a relatively decrease of insulin and HOMA2- IR was found for severe obesity vs. obesity class 2, but it was not associated with the RMR decrease. Male severe obese study subgroup had a significant increase of FFM in relation with class 2 obesity subgroup.

The significant correlation between RMR and FFM, FM, BMI, age was expected from prior data. This study exposed also the RMR significant positive correlation with proinsulin, proinsulin-to-adiponectin ratio, FPG, HOMA IR and negative correlation with adiponectin and HDL cholesterol. RMR did not correlate with insulin secretion markers as HOMA %B and C peptide.

Conclusions

RMR was significantly correlated with insulin resistance biomarkers (with positive correlation indices for proinsulin, proinsulin-to-adiponectin ratio, HOMA IR and negative correlation indices for adiponectin, and HDL cholesterol), but not with insulin secretion biomarkers (C peptide and HOMA %B).

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Conflict of interest: None of the authors had any conflict of interest

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