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Evaluation of insulin secretion through Intravenous Glucose Tolerance Test (IVGTT) in normoglycaemic lean individuals and in obese subjects with normoglycaemia or varying degrees of dysglycaemia

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Research Article

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Evaluation of insulin secretion through Intravenous Glucose Tolerance Test (IVGTT) in normoglycaemic lean individuals and in obese subjects with normoglycaemia or varying degrees of dysglycaemia

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Abstract

We investigated the insulin secretion and glucose dynamics using intravenous glucose tolerance test (IVGTT) in obese subjects with varying metabolic profiles. We enrolled 68 subjects divided into 5 groups of obese individuals: normal glucose tolerance (Ob-NGT, n=29), impaired fasting glucose (Ob-IFG, n=2), impaired glucose tolerance (Ob-IGT, n=16), Ob-IGT/IFG (n=6), Ob-T2D (n=6), and one control group of lean subjects with NGT (Lean-NGT, n=9). Through IVGTT, we evaluated the acute insulin response (AIR) and insulin sensitivity was assessed using the calculated sensitivity index (CSi). β-cell function was determined using the Disposition Index (DI). Ob-T2D subjects exhibited a marked reduction in the first-phase insulin secretion (FPIS) compared with both Lean-NGT and Ob-NGT. In contrast, Ob-IGT, Ob-IFG and Ob-IFG/IGT subjects retained FPIS but demonstrated a pronounced enhancement of the second phase insulin secretion relative to both Ob-T2D and Ob-NGT. The secretion curves for Ob-NGT closely mirrored those of Ob-IGT in terms of overall pattern. We observed a significant difference between obese groups and Lean-NGT group in CSi (P=0.000). Similarly, DI was markedly reduced in obese subjects compared with Lean-NGT and showed a progressive decline with worsening glucose tolerance within the obese cohort (P=0.000). These findings highlight several alterations in insulin sensitivity and secretion in obese individuals relative to Lean-NGT controls.

Keywords: obesity; diabetes; metabolic disease; IVGTT; insulin secretion

1 Introduction

Obesity and type 2 diabetes (T2D) are strongly associated, sharing pathophysiological mechanisms. Indeed, some authors consider them as a single entity referred to as "diabesity" [1], a term first introduced by Sims et al in 1973 [2]. It is estimated that by 2025 more than 300 million people will suffer from T2D associated with obesity [3]. In this context insulin plays a key role in both diabetes and obesity. Moreover, it has been proposed that increased β-cell insulin secretion in obesity may promote insulin resistance, originating a vicious circle [4], although it may also represent an adaptive response to counteract the impaired insulin sensitivity [5]. Understanding insulin secretion in obesity has important clinical implications, as it is linked to the risk of developing T2D. The most important stimulus for insulin

secretion is post-prandial glucose concentration, which is amplified by the effects of fatty acids, amino-acids and hormones produced in the gastrointestinal tract [6]. Studies using the euglycemic clamp and in vitro experiments on pancreatic islets, have shown that glucose induces a 'triggering' phase, named first-phase insulin secretion (FPIS), and an 'amplifying' phase of insulin secretion, named second phase of insulin secretion (SPIS) [7]. Interest in the biphasic kinetics model has been supported by several clinical studies reporting that the dysfunction of FPIS is an initial sign of β-cell dysfunction in subjects with prediabetes, such as those with impaired glucose tolerance (IGT) or in the early stages of T2D [8,9]. In states of overnutrition, such as obesity, β-cell mass expands its insulin secretory capacity through hypertrophy, neogenesis, and proliferation [10] to preserve glucidic homeostasis and overcome insulin resistance. However, a persistently hyperglicemic state leads to the activation of a variety of stressor, oxidative stress, amyloidal stress, inflammatory stress, mitochondrial stress, which contribute to β-cell dysfunction [11-13]. In obese subjects with varying degrees of glucose tolerance, β-cell function has been found to vary in relation to insulin sensitivity (IS) [14]. However, in the absence of metabolic syndrome, defect in FPIS is generally not observed in obesity, but emerge in subjects with progressive decline of glucose tolerance, in such cases, overt T2D is linked to insulin secretory defect rather than to obesity itself [15]. The gold standard to evaluate insulin secretion is euglycemic hyperinsulinemic clamp, but its complexity has led to the widespread of this test, such as the oral glucose tolerance test (OGTT) and the intravenous glucose tolerance test (IVGTT) [16,17].

To date, there are limited data on β cell function in normal glucose tolerant (NGT) obese subjects and in obese subjects with different degrees of glycemic control. The aim of this pilot study was to assess insulin secretion and glucose response using IVGTT in obese subjects compared with lean NGT subjects. In particular, we sought to determine whether insulin secretion is already altered in obesity and to explore differences between normal weight and obese individuals with different metabolic profiles.

2 Materials and Methods

2.1 Subjects and design of the study

The study group consisted of 68 Caucasian subjects (26 men and 42 women) consecutively recruited at the outpatient clinic on obesity disorders of the University Hospital "Renato Dulbecco" of Catanzaro (Italy) (diagnostic and therapeutic network for obesity - PDTA Obesità), Department of Medical and Surgical Science of the University of "Magna Graecia" of Catanzaro. Subjects, with a mean age of43,5±12,6 years, were excluded if they had previous diagnosis of diabetes mellitus type 1, diabetes mellitus type 2, kidney failure (eGFR< 60 ml/min/1.73 m2), severe liver failure, heart failure, myocardial infarction or recent stroke (within the previous 30 days), known inflammatory disease, history of any malignant disease or pathologies or drugs able to modify glucose metabolism. All participants underwent anthropometrical evaluation and complete physical examination. Body weight was measured using a professional mechanical balance in the morning, in the fasting state, after bladder emptying, without shoes and wearing only light clothing (underwear). Height was measured using a flexible measuring tape with 0.5 cm precision. Abdomen and hip circumferences were measured with the same device: abdominal circumference was measured at navel height, while the hip one was detected following the horizontal line passing through the iliac ridges. Body mass index was calculated using the formula weight (kg)/height (m2) and body composition was estimated by bioelectrical impedance (STA/BIA Akern, Milan, Italy). Blood pressure (BP) was measured using a calibrated manual sphygmomanometer and a stethoscope on the brachial artery at the antecubital area of the left elbow with participants supine after five minutes of quit rest and in a comfortable environment. Three measurements were performed by the same operator at 2 minute intervals from each other and the mean of the 3 values will be recorded in the study database. Systolic and diastolic BP values were recorded at the first appearance (phase I) and disappearance (phase V) of Korotkoff sounds, respectively. After an overnight fasting, each study participants underwent to biochemical characterization including a 75g OGTT in which plasma glucose and insulin concentrations were measured at baseline and at30, 60, 120 and 180 min after the glucose ingestion. Glucose tolerance status was defined on the basis of OGTT according to the American Diabetes Association (ADA) criteria [18]. Based on glucose tolerance subjects were divided into 5 groups: Ob-NGT(n=29), Ob-IFG (n=2), Ob-IGT (n=16), Ob-IGT/IFG (n=6), Ob-T2D (n=6) and the control group Lean-NGT (n=9).

In all subjects, insulin sensitivity was evaluated using the Matsuda index, also called ISI (insulin sensitivity index), calculated as follows:

$$ISI(Matsuda) = \frac{10000}{\sqrt{G0 \, x \, I0 \, x \, Gmean \, x \, Imean}}$$

Where G0 and I0 correspond respectively to fasting glucose (mmol/l) and basal insulinemia (mu/l) while Gmean and Imean correspond to the average of blood sugar and insulinemia respectively during OGTT. The β-cell function has been evaluated through the Stumvoll index, using the formula, calculated from 120 min-OGTT:

$$0.226 - \left(0.0032 \times BMI\left[\frac{kg}{m^2}\right]\right) - \left(0.0000645 \times insulin\,120\left[\frac{\mu U}{ml}\right]\right) - \left(0.00375 \times glucose\,90\left[\frac{mmol}{l}\right]\right)$$

Early insulin secretion was evaluated from the OGTT data as the ratio from incremental area under the curve of insulin (ΔIns[AUC]) and the incremental area under the curve of glucose (ΔGluc[AUC]) during the 0- to 30-min of the OGTT [19,20].

Subsequently, all individuals underwent IVGTT, that consists of a rapid (usually 4 min) infusion of a standard glucose dose:

$$\frac{weight\,(kg) \times 0,3}{0,363\,gr}$$

Given that the participants had severe obesity, we used a maximum glucose infusion dose of 35 gr. Glucose, insulin and C-peptide concentrations were measured every 2 minutes in the first 10 minutes and then 20', 30', 40', 50', 60'.

In the last years, several abbreviated protocol of IVGTT have been developed, we adopted the short IVGTT proposed by Tura et al. [21]. In this protocol estimation of AIR corresponds at first 10 min after glucose infusion [in the text AIR = first-phase insulin secretion (FPIS)]and the insulin sensitivity is calculated using the "calculated sensitivity index" (CSi), which is derivedon plasma insulin/glucose measurements collected over 50 min. CSi has shown a good degree of correlation with M of Clamp and capable to discriminate between subjects with different degree of glucose tolerance [21]. While in the OGTT surrogate parameters of insulin secretion are represented by first phase of Stumvolland the ratio of the total area under the insulin curve to the total area under the glucose-curve (ratio of AUC insulin/ glucose or AUCI/G-R) [22]. In obese subjects it has been observed that the impairment of insulin sensitivity alone will lead to glucose intolerance if accompanied by a decrease in GE and by an insulin secretion inadequate for the degree of insulin resistance [23].

For IS we used CSi whose mathematical formula is:

$$CSI = \alpha \frac{KG}{\Delta\, AUC\, INS/T}$$

Where α is the constant, KG the slope of the glucose curve, InsAUC is the area below the insulin curve, and T is the time interval between 0 and 50 (=40 minutes). CSi was expressed as 10-4 min-1 [pmol/l]-1.

AIR during the IVGTT was calculated as the incremental area under the curve (AUC) for insulin during the first 10 min of the IVGTT using the trapezoidal rule, the FPIS corresponds to InsAUC from 0 to 10 minutes after the administration of glucose, while the second phase from 10 minutes to 50 minutes.

To evaluate β-cell function, DI was calculated as:

$$DI = CSI \times AIRg$$

The protocol was approved by local ethics committee and informed written consent was obtained from all participants. All investigations were performed in accordance with the principles of the Declaration of Helsinki.

2.2 Biochemical parameters

Blood levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured by enzymatic methods (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin levels were determined via chemiluminescence tests (Roche Diagnostics GmbH, Mannheim, Germany). Creatinine levels were measured exploiting the Jaffe method. The estimation of glomerular filtration rate (eGFR) was calculated using the new CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. The high sensitivity C-reactive protein (hs-CRP) was measured by an automated immunoturbidimetric method (Cardio Phase hs-CRP, Milan, Italy).

2.3 Statistical Analysis

Continuous variables are reported as mean ± standard deviation (SD) while categorical variables are reported as percentages and they were compared by $\chi 2$ test. Comparison of anthropometric parameters, serum values and metabolic characteristics among the obese subjects' groups, divided according to the degree of glucose tolerance, and the control group (lean-NGT) were assessed by one-way ANOVA for continuous variables, with Bonferroni post hoc correction for multiple comparisons. A P value < 0.05 was considered statistically significant. Correlation between continuous variables were calculated with Pearson correlation coefficient (r). Statistical analyses were performed using SPSS software Version 26.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

3 Results

The study cohort of 68 subjects was divided into 1 control group consists of normoglycemic lean individuals (Lean-NGT, n=9) and 5 groups of individuals with obesity and different degrees of glycemic control: obese with normal glucose tolerance (Ob-NGT, n=29), obese with impaired fasting glucose (Ob-IFG, n=2), obese with impaired glucose tolerance (Ob-IGT, n=16), obese with combined defect impaired fasting/impaired glucose tolerance (Ob-IFG/IGT, n=6) and obese with type 2 diabetes (Ob- T2D, n=6). Among these 68 subjects, 26 were male and 42 were female.

Clinical characteristics and laboratory values of cohort of subjects in study are listed in Table 1.

Table 1. *Physical characteristics and fasting metabolic profile in obese (Ob) subjects with NGT, IGT, IFG, IGT/IFG, T2D and in lean subjects. Data are expressed as mean ± DS. The mean difference between groups is calculated by ANOVA and is significant at the 0.05 level.*

	Ob-NGT	**Ob-IFG**	**Ob-IGT**	**Ob-IFG/IGT**	**Ob-T2D**	**Lean-NGT**	***P* between groups (ANOVA)**
n	29	2	16	6	6	9	
Sex (M/F)	8/20	1/1	8/9	2/4	2/4	5/4	0.821
Age (years)	41.08± 12.7	53±2.8	45.86± 13	55.5± 13.9	51.2± 6.9	34.78± 6.8	0.023
BMI (kg/m^2)	44.28±7	36.22± 1.2	41.74± 6.47	51.43± 7	45.46±9	23.93± 2.28	0.000
HC (cm)	126.44±14.1	116±0.0	133.5±20.3	149.75±17.6	132±17.6	98.88±4.3	0.000
WC (cm)	123.06±13.5	126±0.0	133.5±20.28	146.25±18	125.25±19.5	87.00± 6.7	0.000
W\H ratio	0.97±.0.11	1.08±0.0	0.98±0.12	0.94±0.05	0.95±0.10	0.89±0.07	0.407
Fat mass (%)	45.47±7.59	39.5±14.85	38.52±9.7	54±4.24	42.98±9.51	23.4±6.64	0.000
Lean mass (%)	54.97±8.17	60.5±14.85	61.48±9.68	46±4.24	57.02±9.51	76.55±6.64	0.000
Fasting glucose	84.21±8.36	107.00±1.4	87.56±9.5	105.83±4	113.5±10.6	87.67±5.24	0.000

(mg/dl)							
2h glucose (mg/dl)	118.48±16.18	125.5±19.1	159.94±14	177.67±24	217.8±14.1	99.00±15.5	0.000
Fasting insulin (µU/ml)	14.20±6.74	13.85±0.7	23.17±25.78	21.64±8,5	15.77±10.2	7.24±1.62	0.130
HbA1c (%)	5.62±0.33	5.65±0.64	5.94±0.41	6.12±0.5	6.4±0.35	5.06±0.11	0.000
IGF-1 (ng/ml)	111.71±36,3	185.1± 41	123.73±18.4	120.35±80	107.2±44,0	198.88±33	0.001
CRP (mg/dl)	8.11±5.85	3.23±0.00	7.51±3.68	13±10.49	12.67±9.90	0.77±0.56	0.002
Uric acid (mg/dl)	5.86±1.31	6.15±2.05	6,04±1.57	5.83±0.9	6.18±1.31	3.81±0.98	0.003
Creatinine (mg/dl)	0.72±0.13	0.95±0.22	0.76±0.15	0.76±0.18	0.76±0.24	0.72±0.1	0.397
Total Cholesterol (mmol/l)	193,93±28,9	182,5±3,54	188±28,78	182,5±15,5	177,83±30,4	186,06±38,5	0.715
HDL-c (mmol/l)	50.31±13.59	44±14.14	46.31±8.25	50.83±9.15	41.67±8.55	52.22±10.62	0.430
LDL-c (mmol/l)	118.88±26.9	109.1±12.6	116.7±25.97	104.23±12	99.83±20.9	122.33±29	0.454
Triglycerides (mmol/l)	129.96±77.9	140±36.77	128.63±46.5	107.33±38	182.17±92.6	79.89±35.25	0.101
AST (UI/L)	20.28±8.22	24.5±6.36	19,75±5.68	15.33±3.56	27.83±12.12	17.78±5.89	0.074
ALT (UI/L)	27.69±16.06	31±28.28	25.94±13.21	17.33±3.62	41.83±16.99	21.44±15.21	0.088
SBP (mmHg)	133.8±14.18	135±7.07	134.4±6,63	127±16.47	118.5±8.09	113±9.17	0.000
DBP (mmHg)	82.17±8.79	82.5±3.54	84.38±8.92	74.83±14.3	75.5±6.44	70±10.68	0.006
PP	54.18±13.41	52.5±10.61	56.63±16,34	55.17±22.7	43±12.65	43±6.82	0.155

We detected a statistically significant different between control group and obese population in terms of: BMI (P=0.000), fat mass % (P=0.000) and lean mass % (P=0.000), waist circumference (P=0.000), hip circumference (P=0.000),systolic blood pressure (P=0.000), IGF-1 (P=0.001), CRP (P=0.002), uric acid (P=0.003) (Table 1).The obese population was homogeneous for BMI and fat mass, in fact the Bonferroni test showed no statistically significant differences between the various groups of obese subjects. No statistically significant differences in fasting insulin have been observed between the various groups of obese subjects. As expected, there were statistically significant differences between the various groups regarding fasting glucose (P=0.000), 2h glucose (P=0.000) and HbA1c (P=0.000). The difference, as expected, in obese population was greater between the Ob-NGT group and the Ob-T2D group (Table 1). Moreover, we compared graphically the mean of AUC of insulin curves during IVGTT among differentgroups (Figure 1).

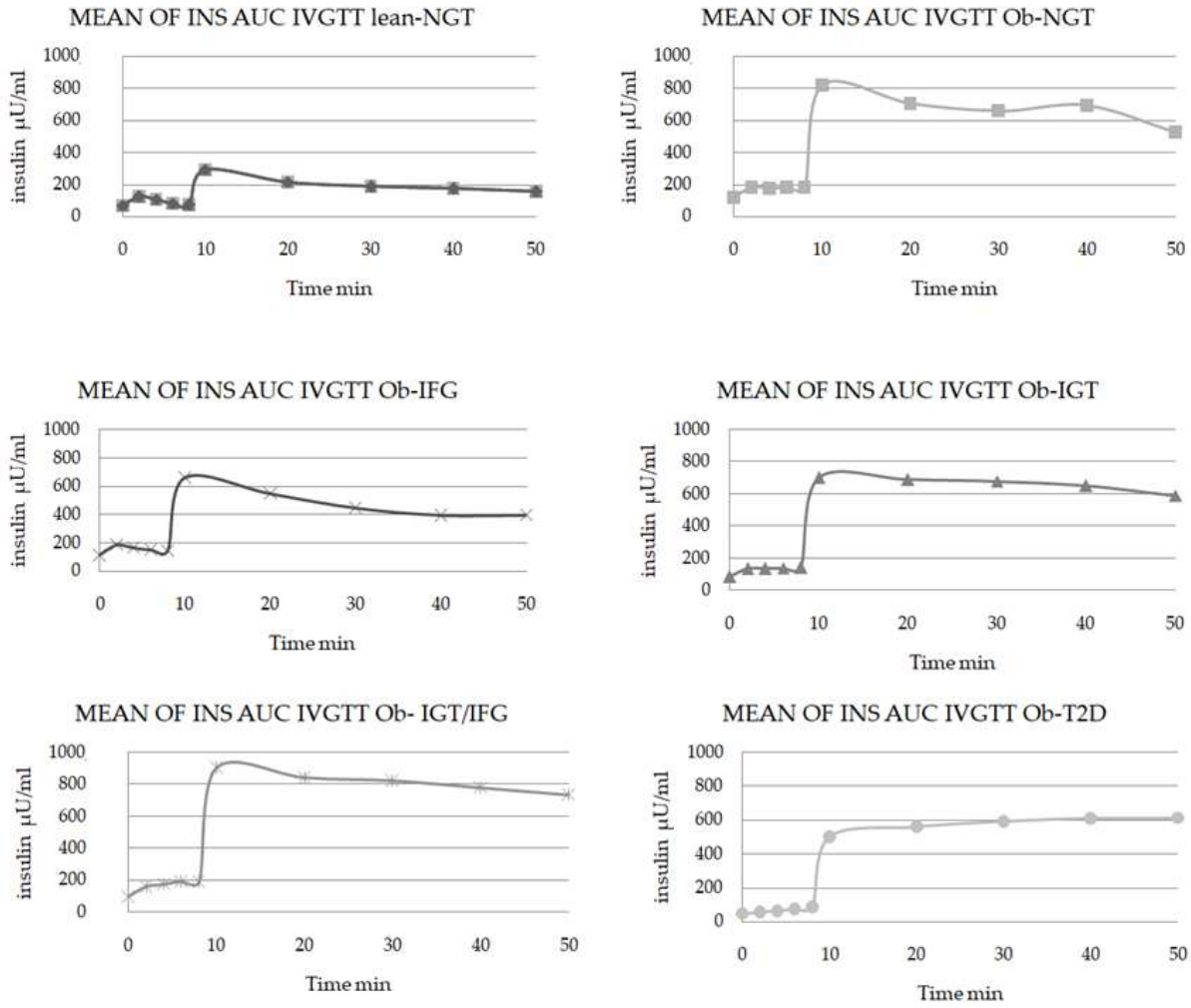


Figure 1. *The figure shows the means of the AUCs of the insulins obtained from IVGTT (measured by the integration of insulin concentrations over a period of time, using the trapezoidal rule method) between groups. Time is represented on the x-axis, individual points represent sample points. Insulin concentrations are represented on the ordinate axis. AUC $_{0\text{-}10}$ rapresents the FPSI and AUC $_{10\text{-}50}$ rapresents the second phase. As expected, compared to the Lean –NGT curve, the Ob-T2D curve demonstrated a loss of the first-stage spike, while the curve of the Ob -NGT seemed similar to that of the Ob-IGT. Insulin secretion in the Ob group seemed increased for all degrees of glucose tolerance alteration.*

As expected, subjects with obesity and T2D showed a loss of the FPIS. Conversely, in obese subjects with IGT and those with IFG/IGT (combined defect), there was no reduction in the FPIS compared to group of obese with NGT and an increase in the second phase of secretion when compared to both T2D and NGT. Moreover, the curve of the Ob-NGT were similar to that of the Ob-IGT in term of pattern. The AIR during the IVGTT, expressed as InsAIR AUC IVGTT (µU/ml*min), did not differ between the groups of individuals (Table 2). No significant differences were found between the control group and the other groups, nor between the various groups of obese people subjects, by performing a post-hoc analysis with the Bonferroni test. There was no statistically significant difference between the first phase of Stumvoll, calculated from the OGTT, HOMA IR and the Matsuda index across all groups (Table 2).

Table 2. *Study of insulin secretion and insulin-sensitivity. Data are expressed as mean ± DS. The mean difference between groups is calculated by ANOVA and is significant at the 0.05 level.*

	Ob-NGT	Ob-IFG	Ob-IGT	Ob-IFG/IGT	Ob-T2D	Lean-NGT	*P* between groups (ANOVA)
n	29	2	16	6	6	9	
Stumvoll first phase	1617.33±567	1628.86±1074	1894.64±1759	1488.17±969	948.47±625	976.31±330	0.275
AIRg	76.92±46.17	56.6±43.27	70.17±60.44	66.58±60.16	23.1±19.65	46.85±26.08	0.176
Homa IR	3.02±1.66	3.66±0.03	5.19±6.17	5.59±2.20	4.55±3.07	1.57±0.38	0.106
MI	76.96±40.47	47.18±2.64	70.01±57.45	36.05±19.27	56.44±58.2	139.06±71	0.006
CSi	1.61±1.55	1.53±0.78	1.71±1.19	1.05±1.59	1.57±0.91	8.44±7.31	0.000
DI	122.01±74.4	69.9±22.34	96.49±59.89	51.13±40.84	27.85±23.46	292.58±138	0.000

Since insulin secretion is dependent on insulin sensitivity, we evaluated CSi and DI, calculated by IVGTT. Our data showed a statistically significant difference between the obese groups and lean group in CSi (ANOVA test, Table 2). The post hoc analysis by Bonferroni confirmed a significant difference between the control group and all groups of obese subjects, both NGT and with glucose tolerance alteration. On the contrary, no significant differences were found between the various groups of obese people subjects (post-hoc analysis with the Bonferroni test) (Figure 2).

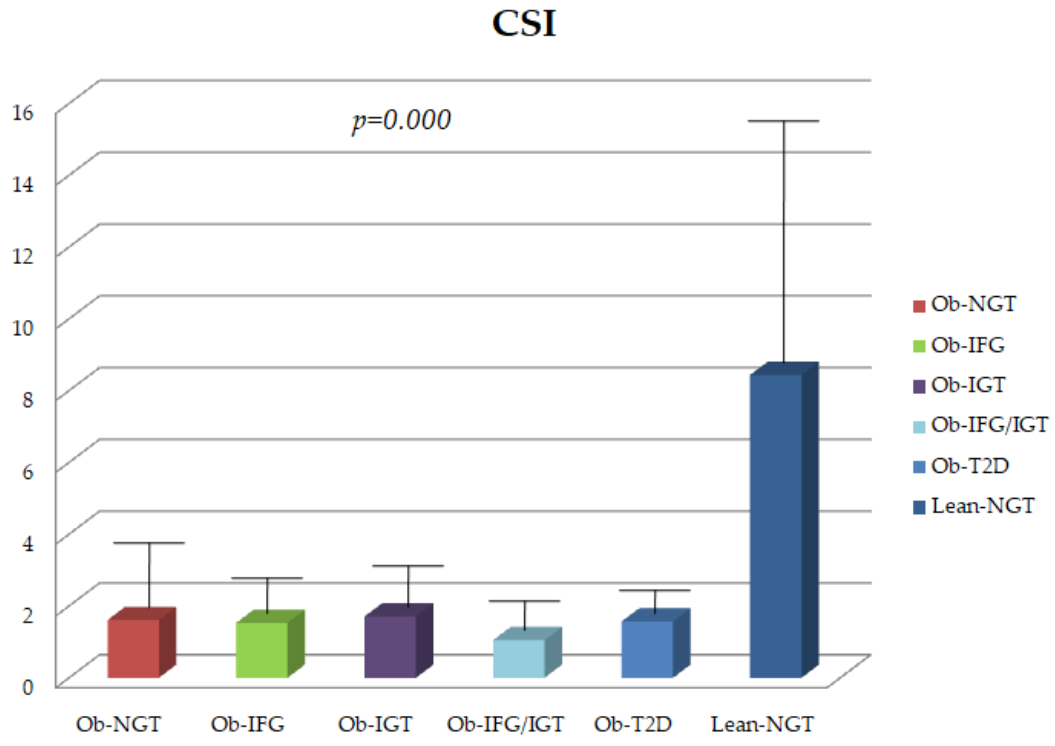


Figure 2. *CSi between the groups in the study. CSi was reduced in the obese population compared to lean subjects, P=0.000 (ANOVA test). There were no statistically significant differences between the obese groups (Bonferroni test).*

Interestingly, group of Ob-NGT had a CSi significantly reduced compared to lean-NGT. Similarly, Disposition Index was significantly reduced in obese subjects compared with lean group (ANOVA test, Table 2). In the group of obese subjects, the DI decreased with worsening of the degree of glucose tolerance but post hoc analysis by Bonferroni not showed a significant difference between the various groups of obese subjects (Figure 3).

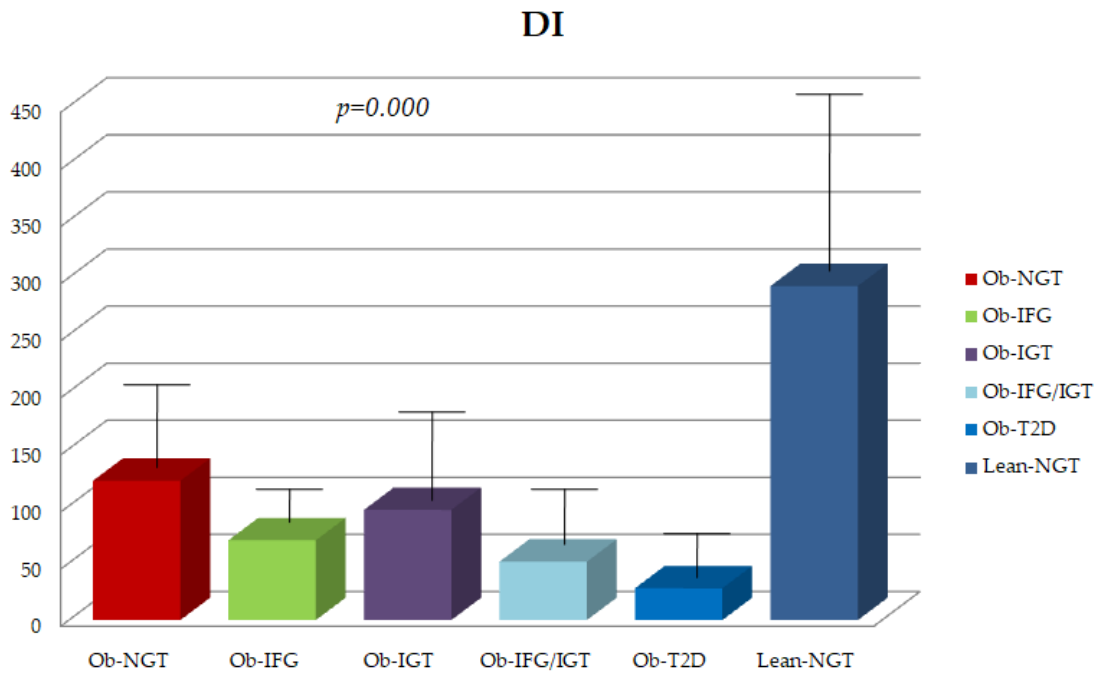


Figure 3. *Differences of DI between the groups in the study. As expected, the DI is greater in lean subjects compared with obese subjects P=0.000, (ANOVA).There were no statistically significant differences between the obese groups (Bonferroni test).*

The post hoc analysis by Bonferroni highlighted that DI is significantly lower in the Ob-NGT group compared to the Lean-NGT group and the difference was significant, P=0.000.

Despite having a normal glucose homeostasis, Ob-NGT still seemed to have an altered β-cell function. Moreover, we calculate Pearson's correlation coefficient (r) between DI and BMI. Correlation quantifies the strength of the linear relationship between these two variables. Pearson's test demonstrated a negative correlation of BMI and DI (Pearson's coefficient: -0.565, P = 0.000), in particular the increase of the BMI would correspond a worsening of the DI (r2= 0.330) (Figure 4).

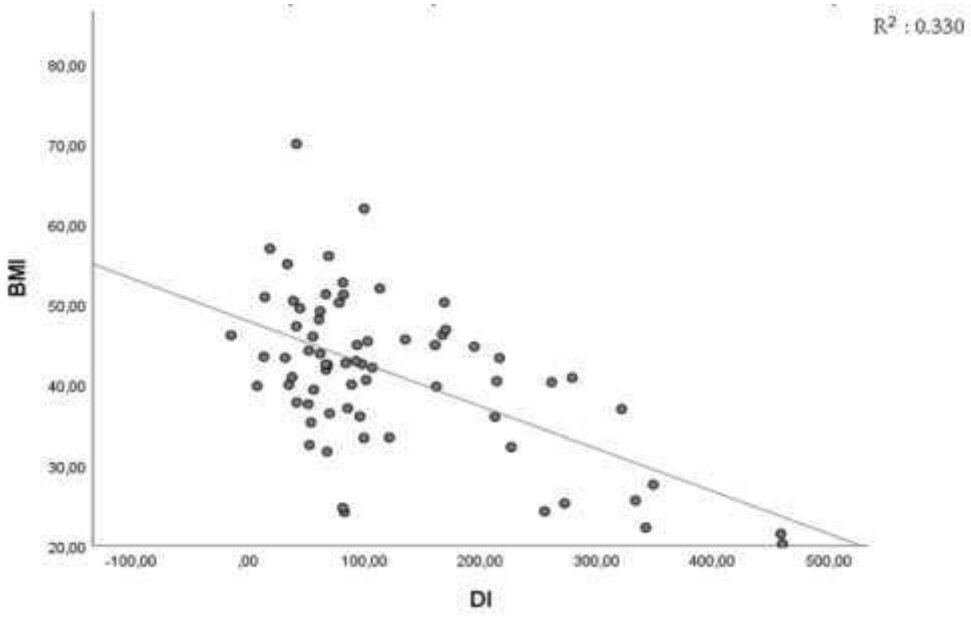


Figure 4. *Scatter plot for Pearson's correlation between BMI and DI. There is a negative correlation between two variables, P=0.000*

4 Discussion

In this study, using IVGTT and OGTT we identified several alterations in the insulin secretion and/or insulin resistance in subjects with obesity when compared with lean subjects with normal glucose tolerance. In our study, as expected, we observed a reduction of FPIS, estimated by AIR during an IVGTT, in the group of obese subjects with T2D when compared with Ob-NGT subjects and with Lean-NGT subjects (23.1±19.65 vs. 76.92±46.17 and 2 vs. 3.1±19.65 46.85±26.08, respectively) although the differences were not statistically significant. FPIS, defined as the initial rapid release of insulin after a glucose spike, is known to be impaired in T2D and this early defect in □-cell function may represent the first detectable abnormality in individuals predisposed to T2D. In fact, in some individuals with T2D, the FPIS is often diminished or absent even before the development of insulin resistance [24-28]. To date, there are limited and inconclusive data on β cell function in NGT obese subjects and in obese subjects without T2D. Several evidences suggest that obese subjects often exhibit a delayed or blunted FPIS, meaning that insulin release is neither rapid or quantitatively sufficient in the early phase after glucose ingestion. In our study, we found that the first-phase insulin response to glucose during IVGTT was progressively reduced in subjects with obesity and IGT or IFG/IGT (combined defect), when compared with Ob-NGT. Our data are consistent with those observed by Bacha F. et al.[29]. In this study, performed in obese youth with normal glucose tolerance, IGT and T2D was reported that the first-phase insulin response to glucose and DI progressively decreased with the degree of impairment of glucose tolerance, with a more profound defect in obese subjects with T2D involving both the first- and second-phase insulin responses [29]. Accordingly, also in our study, we found a progressive decrease in DI with the degree on impairment of glucose tolerance. Furthermore, in our study, in obese subjects with IGT and those with IFG/IGT there was no a significant reduction in the FPIS when compared to group of obese with NGT. Interestingly, we observed that also Ob-NGT have an increased insulin secretion when compared to the Lean-NGT group, although the difference was not statistically significant. Polonsky K.S. et al. [30], reported that insulin secretion is increased in obesity in response to i.v. glucose and is highly correlated with BMI suggesting that insulin secretion increases as the degree of obesity increases [30]. Senio S. et al. [31], also hypothesize that the increase in insulin secretion in obese subjects may be not due to □-cells hyper-responsiveness but rather to the larger functional β cell mass [31]. In addition, the increased insulin secretion could also be expression of the fact that in subjects with obesity, pattern of insulin secretion could be different than in non-obese subjects, due to high insulin resistance. In fact, most patients of the obese population had a BMI ≥ 40 kg/m2 and with a marked insulin resistance, with insulinemia values that increased, both during OGTT and during IVGTT, by more than 25 times the basal limit and that did not follow a normal distribution in the sample. In our study, subjects in Ob-NGT group have higher mean values of basal insulin than the control group, even if the difference does not reach statistical significance. As regards insulin sensitivity, calculated by the IVGTT and expressed as CSi, we found statistically significant difference between the control group and the other groups and in the obese population between the Ob-NGT group and the Ob-T2D group.

Matsuda index, a surrogate measure of insulin sensitivity, differed significantly between the control group and the other groups, and between the Ob-NGT group and the Ob-T2D group. The DI, calculated as the product of insulin sensitivity and insulin secretion, reflects the β-cell compensatory capacity to overcome insulin resistance. Moreover, it is important to underline that in our study the lean NGT group have a similar FPIS in absolute terms than the glucose intolerant groups but a DI that is at least 2.5 times higher than any of the other groups. Moreover, DI was inversely correlated with BMI, indicating that higher BMI was associated with reduced β-cell compensation.

Our data are consistent with those reported by the literature which show that in an over nutrition state, such as obesity, β-cell mass increases insulin secretory capacity to maintain the glucose homeostasis and win the insulin resistance via hypertrophy, neogenesis and proliferation [10]. However, chronic hyperglycemic state leads to the activation of a variety of stressor, oxidative stress, amyloidal stress, inflammatory stress, mitochondrial stress, which contribute to dysfunction of β-cell [11-13]. Both insulin-resistance and impaired β-cell function play a critical role in T2D and in the pre-diabetes [32], including subjects with IGT, IFG and combination of both IGT and IFG [33]. Several clinical studies report that the dysfunction of the FPIS is one of the earliest signs of β-cell dysfunction in subjects with IGT or in the early stages of T2D [8,9]. Moreover, β-cell function in T2D progressively decreases, starting with the reduction until the total disappearance of the first phase (0-10 min) of insulin secretion, followed by the reduction of the SPIS (10-60 min) [27, 34]. In a Japanese study, insulin secretion and sensitivity in subjects with and without obesity with different stages of glucose tolerance was investigated. Authors found that the insulin secretion pattern in the group NGT obese subjects was similar to that observed in Caucasians with IGT [35]. In obesity in the absence of metabolic syndrome defect in FPIS was not found, but it emerged in subjects with progressive decline of glucose tolerance, then overt T2D was linked to insulin secretory defect [15]. A study, conducted on a Caucasians without diabetes cohort, shown that BMI was correlated with both FPIS and second phase insulin secretion, using hyperglycemic clamp [36]. Similarly, another study used IVGTT demonstrated that FPIS was associated with BMI in non-diabetic subjects [37]. Moreover, a study carried out in obese Asian indicated that the first and second phases of insulin secretion (evaluated with hyperinsulinemic euglycemic clamp), were significantly decreased in obese T2D compared to non-diabetic obese subjects, while if compared to the lean T2D, authors showed an alteration due to both insulin resistance and secretion [38].

In summary, our data confirm an alteration of insulin secretion in obese subjects with diabetes, characterized by loss of FPIS, but without significant reduction of the second phase of insulin secretion, across all degrees of dysglycaemia, compared to the control group. Notably, obese but NGT subjects also demonstrated an altered insulin secretion curve that is similar to that found in obese IGT subjects. In obesity it is widely accepted dogma that excess adiposity causes insulin resistance with hyperinsulinemia as a result, both through altered insulin clearance and increased secretion [24, 39, 40, 41]. Whether this hyperinsulinemia is a cause or consequence of obesity remains to be clarified.

5 Conclusion

In conclusion, our study demonstrates that FPISprogressively declines in obese individuals with IFG, IGT or IFG/IGT, compared with Ob-NGT. Regarding insulin sensitivity, CSi was significantly different between the control group and all obese groups, and, within the obese cohort between the Ob-NGT group and the Ob-T2D group. Furthermore, the DIin Ob-NGT subjects was markedly lower than in lean NGT. In conclusion, although preliminary, our data indicate multiple alterations in insulin sensitivity and/or secretion in obese subjects when compared with Lean-NGT subjects. These findings underscore the need for targeted clinical studies to optimize preventive therapy strategies.

6 Limitation

There are a number of potential limitations to our study; however, the most relevant is the small sample size income subgroups, which introduces heterogeneity into the data. Furthermore, all the participants included in the study were of white ethnicity. Consequently, these results may not be generalizableto other ethnic populations, as previous studies have reported ethnic differences in β-cell function and insulin-resistance.

7 Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

8 Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Maria Chiara Pelle, Isabella Zaffina, Maria Resilde Natale, Vincenzo Trapanese, Federica Giofrè, Clara Vatalaro, Melania Melina, Francesca Cosentino, Ludovica Arturi, Franco Arturi. The first draft of the manuscript was written by Maria Chiara Pelle, Isabella Zaffina and Franco Arturi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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12 Consent to participate

Informed consent was obtained from all individual participants included in the study.

Abbreviations

Acute insulin response	AIR
Acute-insulin-response-to-glucose	AIRg
Alanine aminotransferase;	ALT
Aspartate aminotransferase;	AST
Bio electrical impedance	BIA
Body Mass Index;	BMI
Blood pressure	BP
Chronic Kidney Disease Epidemiology Collaboration equation	CKD-EPI
C-reactive protein;	CRP
Calculated sensitivity index	CSi
Disposition Index	DI
Estimated glomerular filtration rate	eGFR
First-phase insulin secretion	FPIS
Glicated Hemoglobin;	HbA1c
High density lipoprotein cholesterol;	HDL-c
Insulin-like growth factor-1;	IGF-1
Intravenous glucose tolerance test	IVGTT
Low density lipoprotein cholesterol;	LDL-c
Obese with impaired fasting glucose	Ob-IFG
Obese with impaired glucose tolerance	Ob-IGT
Obese with normal glucose tolerance	Ob-NGT
Oral glucose tolerance test	OGTT
Diagnostic Therapeutic Assistance Pathway	PDTA
Pearson coefficient	r
Standard deviation	SD
Insulin sensitivity index	SI
Second phase of insulin	SPIS
Type 2 diabetes	T2D
Waist circumference;	WC
Gamma glutamiltransferase	γGT
Ratio from incremental area under the curve of insulin	ΔIns[AUC]

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