One-hour post-load plasma glucose levels associated with decreased insulin sensitivity and secretion and early makers of cardiometabolic risk

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Running title: 1-h post-load glycemia in obese youth

Word count: 2945

Tables: 3

Figure: 2

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ABSTRACT

Purpose: Obese adults with normal glucose tolerance (NGT) but with 1-hour post-load plasma glucose (1hPG) ≥155mg/dL are at higher risk of developing type 2 diabetes (T2D) and cardiometabolic complications. Little information is available for the pediatric population, where recently, a lower cut-off, 132.5 mg/dL, has been suggested as being more sensitive to identify subjects at risk of T2D.

Our aim was to assess whether obese Caucasian youth with 1hPG ≥132.5mg/dL have worse insulin sensitivity and secretion and a worse cardiometabolic profile compared to obese youth with 1hPG <132.5mg/dL.

Methods: Medical records of 244 (43% male; age: 11.1±2.7years) overweight/obese children and adolescents, who had undergone an oral glucose tolerance test (OGTT), were retrieved. Anthropometric and biochemical data were collected from the hard copy archive. Indexes of insulin resistance (HOMA-IR), insulin sensitivity (WBISI) and insulin secretion (Insulinogenic Index, Disposition Index) were calculated.

Results: Of the 244 records analyzed, 215 fulfilled criteria for NGT and had complete biochemical data. Among NGT patients, 42 (19.5%) showed 1hPG ≥132.5mg/dL (high-NGT), while the remaining had 1hPG <132.5mg/dL (low-NGT). The high-NGT group showed a higher male prevalence (59.5 vs 37%), lower Disposition Index (0.54[0.39-0.71] vs 0.79[0.47-1.43]) and WBISI (0.24[0.18-0.35] vs 0.33[0.23-0.50]) than the low-NGT group. High-NGT subjects also showed a trend towards lower HDL-cholesterol and higher TG/HDL ratio (2.13[1.49-3.41] vs 1.66[1.24-2.49]).

Conclusions: In overweight/obese NGT Caucasian youth a 1hPG ≥132.5mg/dL was able to identify those with impaired insulin sensitivity and secretion and a trend towards a worse cardio-metabolic profile, a group likely at risk for future T2D.

Key words: 1-h plasma glucose, obesity, children, adolescents, insulin sensitivity, insulin secretion, cardio-metabolic risk

INTRODUCTION

Over the last decades, the prevalence of childhood obesity has increased worldwide and this has been mirrored by an increasing number of cases of youth-onset type 2 diabetes (T2D), particularly in the US (1,2).

T2D is characterized by reduced insulin sensitivity and secretion (3), and represents a key determinant of cardiovascular morbidity and mortality in the adult population (4–6). Recent data indicate that especially obese youth with T2D are at increased risk for the development of early signs of vascular complications, thus highlighting the importance of preventing this condition (7,8).

Epidemiological data suggest that about 60% of adults with T2D had either impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), two conditions known as pre-diabetes, when assessed 5 years prior to diagnosis (9). However, the other 40% of T2D adults had normal glucose tolerance (NGT) at baseline, indicating that the use of IFG or IGT categories may overlook a considerable proportion of subjects who will develop T2D (9). These observations led to several studies investigating alternative models for predicting T2D. Recent reports have identified plasma glucose concentration at 1-h ≥155 mg/dl during the oral glucose tolerance test (OGTT) as a strong predictor of future risk for T2D in adults of different ethnic backgrounds, independently of glucose tolerance status (9–13). In addition, this glucose cutoff identifies subjects with a worse cardio-metabolic profile, characterized by high blood pressure, dyslipidemia, liver steatosis, early signs of atherosclerosis, as well as an increased mortality (14–20).

Data in the pediatric population are mainly limited to obese American and Hispanic children, where the 155 mg/dl cutoff has been related to a worse insulin sensitivity and secretion (21,22). Limited information is available for Caucasian children and adolescents. Of interest, a recent study performed in obese Caucasian youth suggested that a lower cut-off point of 132.5 mg/dl might perform even better in identifying individuals with a high risk for developing T2D among those with NGT, having a better sensitivity than the 155 mg/dl cutoff and a similar specificity (23).

The aims of the present study were to assess whether NGT obese Caucasian youth with 1-h plasma glucose concentration ≥132.5 mg/dl have worse insulin sensitivity and secretion along with a worse cardio-metabolic profile compared to obese with 1-h post-load glycemia <132.5 mg/dl, and to assess factors associated with high-NGT condition.

METHODS

Study population

The study was based on a retrospective review of the hard copy archive of the Pediatric Endocrinology Clinic of the Department of Pediatrics (University of Chieti, Italy) for the period between November 2011 and February 2016.

Records of Caucasian children and adolescents (age 4-18 years) affected by overweight (body mass index [BMI] ≥ 85th and < 95th percentiles for age and sex) or obesity (BMI ≥ 95th percentile for age and sex), who had undergone an OGTT, were selected.

Anthropometric data (height, weight, BMI, waist circumference, pubertal staging and blood pressure measurements) and biochemical data (lipid profile including total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides) were obtained from the hard copy archive.

None of the patients had chronic diseases (diabetes, thyroid or other endocrine disorders, hereditary diseases or systemic inflammation) and none were taking any medication. Data on family history of T2D and cardiovascular disease were also retrieved from the archive.

The study was conducted in compliance with ethical principles based on the Declaration of Helsinki. Formal consent for this study was not required, since it was confined to data collected as part of a routine assessment of obese children and data were entered into the study database in an anonymized and unidentifiable way.

Clinical data

All children and adolescents affected by overweight/obesity were seen by the same Pediatric Endocrinology team (University of Chieti, Italy), with the supervision of a senior physician, using standard methods.

Height was measured to the nearest 0.1 cm with Harpenden stadiometer (Holtein, Wales, UK). For this measurement, patients stood straight, with feet placed together and flat on the ground; heels, buttock and scapulae against the vertical backboard; arm loose and relaxed with palms facing medially and the head positioned in the Frankfurt plane. Body weight was measured to the nearest 0.1 kg with a calibrated scale. BMI, used as index of adiposity, was calculated as the weight in kilograms divided by the square of the height in meters.

A flexible tape was used to measure waist circumference to the nearest 1 mm at the mid-point between the lower ribs and the pelvic bone. Three waist circumference measurements were taken at the midst of each respiratory cycle.

Height, weight and BMI standard deviation scores (SDS) were calculated based on the age and sex reference values for the Italian population (24). The waist to height ratio was also calculated in order to have an age- and sex-independent index (25).

In all patients, pubertal stage was defined on the basis of breast development in girls and genital development in boys, based on Tanner's criteria (26).

Systolic (SBP) and diastolic (DBP) blood pressure were measured twice, after 10 min rest, using standard methods at the non-dominant arm, by using a calibrated sphygmomanometer.

Oral glucose tolerance test

All study participants had undergone a 2-hour OGTT. All OGTT were performed at the Pediatric Endocrine Clinic by the same nurses, using a standard protocol. In details, after an overnight fasting, a baseline sample for measurements of glucose and insulin was collected. Afterwards, flavored glucose in a dose of 1.75 g/kg body weight (up to a maximum of 75 g) was given orally

and blood samples were obtained every 30 min up to 120 min for the measurement of glucose and insulin. Samples were transferred to the local laboratory soon after each collection to be processed. Glucose tolerance status was classified according to the criteria of the World Health Organization (WHO). (27)

Indexes of insulin resistance and secretion

The Homeostasis Model Assessment of fasting insulin resistance (HOMA-IR), a measure of insulin resistance, was computed as follows: HOMA-IR = [fasting insulin (μIU/ml) × fasting glucose (mmol/ml)]/22.5 (28)

The Whole Body Insulin Sensitivity Index (WBISI) was calculated with the formula: WBISI = [10 000/(fasting glucose × fasting insulin × mean glucose × mean insulin concentration)$^{1/2}$], where mean glucose and mean insulin are the averaged glucose and insulin concentrations; glucose and insulin are expressed in mg/dl and μIU/ml, respectively. (29)

The insulin secretion was estimated by means of the Insulinogenic Index, defined as IGI = [insulin (t30) – insulin (t0)/glucose (t30) – glucose (t0)], where glucose is expressed in mg/dl and insulin is expressed in μIU/ml and by HOMA-β, computed as follows: HOMA-β = [20 × fasting insulin (μIU/ml)/fasting glucose (mmol/l) – 3.5]. (28,30)

The Disposition Index was calculated with the formula: DI = insulinogenic index/HOMA-IR. (31)

Statistical analysis

Data were reported as mean ± standard deviation (SD) or median [interquartile range], unless otherwise stated. Not normally distributed variables were log-transformed before data analysis. Patients with NGT were divided into two groups, based on 1-h plasma glucose, during OGTT: high-NGT with a 1-h plasma glucose ≥132.5 mg/dl and low-NGT with a plasma glucose <132.5 mg/dl. Differences between high-NGT and low-NGT subjects in continuous variables were tested by an

unpaired *t*-test, whereas differences in categorical variables where assessed by χ^2 test or Fisher's exact test. Analysis of covariance was applied to adjust for potential confounding factors. Logistic regression analysis was performed to assess factors associated with the high-NGT category and results are presented as odds ratio [95% confidence interval (C.I.].

When comparing the main study outcomes (insulin sensitivity/secretion, cardiometabolic parameters), multiple testing was controlled for using the false discovery rate (FDR) method, using 0.05 as the criterion). All calculations were made with the computer program Statistical Package for the Social Science (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Description of the sample

The records of 244 overweight or obese children and adolescents with a mean age of 11.1 ± 2.7 years (range 4-18) were retrieved and two subjects, who had incomplete biochemical data (insulin results), were excluded. Of the remaining 242 patients, 104 were males (43%) and 111 subjects (46%) were prepubertal. Ten (4.1%) subjects had IGT, while 17 (7.0%) met the criteria for IFG and they were excluded from subsequent analysis. No patient presented with overt diabetes.

Among 215 subjects with NGT, 42 (19.5%) were classified as high-NGT and 173 (80.5%) as low-NGT (Figure 1).

The high-NGT and low-NGT groups were comparable in terms of family history of T2D (42.9% vs 50.3%) and cardiovascular disease (45.2% vs 56.6%).

Characterization of NGT patients with 1HPG ≥ 132.5

The high-NGT and low-NGT groups were similar for age, weight, BMI and pubertal stages (Table 1). For a subgroup of the study population (n 124), waist circumference was also available and there were no significant differences between the high-NGT (n 32, 85.8 ± 8.2 cm) and the low-NGT (n 92, 86.0 ± 12.6 cm) groups (p=0.9) in this parameter or in the waist circumference/height ratio (0.57

± 0.06 vs 0.59 ± 0.06, p=0.28). A statistically significant difference was observed for sex distribution between the two groups, with a higher prevalence of males in the high-NGT group (*p*= 0.009)).

High-NGT subjects had significantly higher fasting glucose and, as expected, higher 1-h and 2-h post-load plasma glucose levels. High-NGT youth had higher 1-h and higher 2-h post-load insulin levels (Table 2).

A significant difference in insulin sensitivity was shown between the two groups by using WBISI, with values significantly lower in high-NGT (0.24[0.18-0.35]) than low-NGT youth (0.33[0.23-0.50]), p=0.003. Similarly, the Insulinogenic Index was significantly reduced in high-NGT subjects (1.82[1.24-2.55] vs 2-62[1.46-5.06] *p* = 0.063). The Disposition Index was significantly lower in the high-NGT group (0.54[0.39-0.71] vs 0.33[0.23-0.50], p=0.028 (Figure 2). The above comparisons were adjusted for sex, pubertal stages and BMI SDS and for multiple comparisons.

In contrast, there were no significant differences in HOMA-IR (3.37[2.62-4.66] vs 3.31 [2.23-5.01], p=0.58) and HOMA-beta (173.0 [136.8- 243.0] vs 203.7[128.3-321.4], p=0.47) between the high-NGT and low-NGT groups.

The high-NGT and low-NGT groups had similar values of total cholesterol, LDL-cholesterol, triglycerides, GOT and GPT. In contrast, a trend toward lower HDL-cholesterol the high-NGT group (adjusted *p*= 0.007)) and TG/HDL ratio (higher in the high-NGT group (adjusted *p*= 0.07)) (Table 2). Again these comparisons were adjusted for sex, pubertal stages and BMI SDS and for multiple comparisons. No significant differences were observed in SBP and DBP between the two groups (Table 2).

Factors associated with the high-NGT condition were sex (male), insulinogenic index, WBISI and baseline glycemia. (Table 3). All but baseline glycemia remained independently associated to the high-NGT condition in a multiple regression model.

DISCUSSION

This clinical-based study of Caucasian obese youth clearly demonstrated that a considerable proportion presented a 1-h plasma glucose greater than 132.5 mg/dL, which was associated not only with decreased insulin sensitivity and secretion but also with early signs of cardio-metabolic dysfunction, as indicated by a trend towards reduced levels of HDL and increased TG/HDL ratio.

Over the last decades, concomitantly with the epidemics of obesity and its cardio-metabolic complications (1,2), there has been growing interest in the identification of early abnormalities in glucose metabolism predictive of future risk of T2D (32). In this context, a lot of interest has been focused on IGT and IFG, both considered as 'pre-diabetes' conditions (33), but subsequent studies, performed in the adult population, have shown that these categories are not optimal predictor of T2D (33,34). This has led to efforts to identify more sensitive and specific markers of T2D risk and to the finding that 1-h post-load plasma glucose ≥155 mg/dl during the OGTT is a good predictor for the progression to diabetes (9–11,13). Indeed, several lines of evidence have highlighted that high-NGT subjects have reduced insulin secretion and increased insulin resistance along with higher risk of developing cardiovascular disease and increased mortality rates (10–13,15–19,35). In the pediatric population, there are limited data on the value of 1-h post-load glucose, and they are data mainly based on studies performed in American and Hispanic obese youth (21,22). In a cross-sectional study, Tfayli et al (21) showed that, in a group of 113 overweight and obese youth, those with a post-load glucose above 155 mg/dl had a reduced disposition index, by approximately 41%, compared to their peers with a lower post-load glucose. More recently, in a prospective study, Kim et al not only confirmed these findings but also highlighted a predictive role of the 155 mg/dl cutoff for β-cell deterioration and progression to prediabetes (22). There is limited information on the value of 1-h post-load glucose for Caucasian children and adolescents. The study by Tfayli et al (21) included only 65 Caucasian children, whereas more recently, Manco et al (23) performed a cross-sectional study in a large Caucasian pediatric population, where it was found that a lower cutoff, equal to 132.5 mg/dl, was able to identify NGT children and adolescents at risk of diabetes,

with a higher sensitivity and specificity. The latter findings suggest that this lower cutoff might help in detecting glucose abnormalities at an earlier stage in youth in order to implement preventative strategies.

In the present study, we found that almost 20% of obese NGT children and adolescents showed a 1-h post-load glucose ≥132.5 mg/dl, a percentage similar to that reported in adult studies (33,34).

In addition, we found that high-NGT children and adolescents showed worse glucose and insulin profile during the OGTT compared to low-NGT individuals. In line with previous studies, the high 1-h post-load glucose was associated with reduced insulin sensitivity, as indicated by lower values of WBISI. The Disposition Index, which reflects the ability of pancreatic islets to compensate for insulin resistance, was around 32% lower in youth with high NGT, suggesting an inadequate β-cell compensation, and therefore a very early metabolic marker for an increased risk of future diabetes development (3).

Previous studies have shown a worse cardio-metabolic profile associated with high 1-h plasma glucose. In particular, adults with NGT and 1-h glucose above 155 mg/dl showed high blood pressure and lipid levels, and increased prevalence of liver steatosis and increased inflammatory markers along with subclinical signs of cardiovascular disease, such as increased intima-media thickness, vascular stiffness and left ventricular hypertrophy (15–19). More recently, a large prospective study in adults has also highlighted an increased mortality risk associated with high 1-h glucose levels (35).

In the present study, we could not detect any association between 1-h plasma glucose and classic feature of metabolic syndrome, in contrast to adult studies. However, it needs to be highlighted that, the high-NGT group showed a trend towards decreased HDL levels a higher TG/HDL ratio. The latter is a recently emerged marker of cardiovascular risk and previous studies have shown that, in obese adults and children, the TG/HDL-C ratio is strongly associated with insulin resistance and early signs of cardiovascular complications, such as increased intima-media thickness (36,37). Therefore, in our young study population the TG/HDL ratio might represents an early cardio-

metabolic abnormality related to the high post-load glucose This finding adds to the previous association between 1-h plasma glucose above 132.5 mg/dL and risk of T2Din the pediatric population (23), by suggesting a wider link with cardio-metabolic complications.

An interesting finding of the present study was that boys were at higher risk of having 1-h plasma glucose above 132.5 mg/dl, even though girls were more represented in our study population. This is in line with findings from adult studies, where a higher percentage of males was detected among the high-NGT category (18,38). The increased metabolic risk for boys might also be related to intrinsic characteristics of our Caucasian population. This is also supported by findings from another study from our group, where even the genetic risk of having a higher BMI was more evident in boys than in girls (39).

Some limitations of the present study need to be acknowledged. In particular, the retrospective study design limited the clinical/biochemical data we could analyze. However, all obese children and adolescents included in the study were assessed in the same center using standardized techniques and that gives validity to the study findings. Another study limitation is related to the assessment of a single OGTT, given the known variable reproducibility of this test. However, the study reflects what generally happens in the clinical setting, where the OGTT is a test, which can be easily performed, but it is repeated only when abnormal findings are detected. The findings of this study highlight how an OGTT could be a useful means in the clinical setting and not only in the research setting to identify youth at potential increased risk of T2D.

The results of the present study emphasize the importance of an early diagnosis of prediabetes, which is related to the possibility of an early lifestyle intervention. Indeed, several lines of evidence have highlighted that lifestyle interventions prevent or delay the progression from IGT (or IFG) condition to that of overt diabetes. However, it is unknown whether high-NGT youth may benefit from lifestyle intervention similarly to IGT individuals. Unfortunately, the respective nature of this study design did not allow the estimation of the predictive role of the specific 1-h cutoff used.

Therefore, longitudinal studies are needed to investigate whether or not this early impairment in insulin sensitivity and secretion will translate into higher rates of T2D in the future.

In conclusion, the present study suggests that, at least in the clinical setting, a high 1-h post-load glycemia might be an early metabolic signal for an enhanced risk of developing diabetes. Although this finding needs to be confirmed by future studies, obese children with 1-h plasma glucose response during the OGTT exceeding 132.5 mg/dL may require more vigilant follow-up and lifestyle recommendation in order to avoid further weight gain and promote weight lost need to be strongly encouraged.

Conflict of interest: none to declare

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Table 1. General characteristics of low-NGT and high-NGT youth

Variables	**Low-NGT**	**High-NGT**	***p* value**
N (%)	173 (80.5%)	42 (19.5%)	
sex (male/female)	64 (37%)/109 (63%)	25 (59.5%)/17 (40.5%)	0.009
Prepubertal/pubertal/postpubertal	92 (53.2%)/40 (23.1%)/41 (23.7%)	19 (45.2%)/14 (33.3%)/9 (21.3%)	0.68
Age (years)	10.8 ± 2.7	11.0 ± 2.7	0.59
Weight (SDS)	2.21 ± 0.72	2.15 ± 0.78	0.66
Height (SDS)	0.66 ± 1.06	0.64 ± 1.33	0.92
BMI (SDS)	2.24 ± 0.50	2.24 ± 0.55	0.98
Overweight/Obese	22 (12.7%)/151 (87.3%)	5 (11.9%)/37 (88.1%)	0.56

Data are expressed as mean ± SD.
NGT: normal glucose tolerance. SDS: standard deviation score. BMI: body mass index.

Table 2. Glucose metabolism data and cardiometabolic profile in the study population.

Variables	Low-NGT	High-NGT	*p value**	Benjamini-Hochberg P-value
HbA1c (mmol) **HbA1c (%)**	35.2 ± 3.71 5.37 ± 0.34	36.6 ± 3.75 5.49 ± 0.34	 0.06	
C-peptide	2.37 ± 0.98	2.53 ± 1.04	0.88	
Glucose T0 (mg/dl)	90.2 ± 7.30	92.9 ± 5.9	0.25	
Glucose 60' (mg/dl)	104.9 ± 16.5	143.0 ± 18.7	<0.001	<0.001
Glucose 120' (mg/dl)	101.9 ± 14.5	114.4 ± 13.9	<0.001	<0.001
Insulin T0	15.0[9.92-22.5]	14.5[10.7-20.4]	0.77	
Insulin 60'	61.8[37.0-110.0]	120.9[70.5-158.9]	<0.001	<0.001
Insulin 120'	61.0[33.9-94.1]	94.5[56.7-143.6]	0.007	0.029
Total cholesterol	162.4 ± 30.8	161.3 ± 34.0	0.96	
HDL-cholesterol	48.0 ± 9.93	43.7 ± 9.38	0.026	0.07
LDL-cholesterol	94.3 ± 26.4	95.8 ± 30.0	0.67	
Triglycerides (mg/dl)	80.0[63.0-108.0]	94.5[69.7-124.7]	0.098	
Tg/HDL	1.66[1.24-2.49]	2.13[1.49-3.41]	0.029	0.07
GOT (U/L)	29.3 ± 9.6	30.5 ± 10.1	0.78	
GPT (U/L)	34.8 ± 17.9	36.7 ± 18.0	0.88	
SBP	115.2 ± 14.8	114.2 ± 13.1	0.28	
SBP SDS	0.97 ± 1.22	0.76 ± 1.02	0.22	
DBP	69.2 ± 8.90	69.2 ± 8.85	0.81	
DBP SDS	0.67 ± 0.74	0.64 ± 0.73	0.89	

Data are expressed as mean ± SD or median[interquartile range].
DBP: diastolic blood pressure; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; HDL: high-density lipoprotein; HbA1c: glycosylated hemoglobin; LDL: low-density lipoprotein; NGT: normal glucose tolerance; Tg: triglycerides; SBP: systolic blood pressure; SDS: standard deviation score;

*p values adjusted for sex, pubertal stage and BMI SDS

Table 3. Factors associated with high-NGT in the study population

	Odds ratio [95%C.I.]	**p value**	**Odds ratio [95% C.I.]**	**p value**
Age	1.036 [0.913-1.174]	0.59		
Sex (male)	2.505 [1.257-4.989]	0.009	2.628 [1.197-5.767]	0.016
Pubertal stage	1.088 [0.723-1.626]	0.69		
BMI SDS	1.010 [0.523-1.950]	0.98		
WBISI (log)	0.199 [0.051-0.776]	0.020	0.026 [0.004-0.160]	<0.001
Insulinogenic index (log)	0.372 [0.156-0.885]	0.025	0.092 [0.024-0.35]	<0.001
Glycaemia T0	1.065 [1.007-1.127]	0.028	1.053 [0.988-1.1.21]	0.116
Insulin T0 (log)	1.063 [0.292-3.867]	0.926		

Data are expressed as odds ratios [95% confidence intervals] and associated p values.
WBISI: Whole Body Insulin Sensitivity Index; Wht: waist circumference height ratio

Figure legend

Figure 1. Study cohort Flowchart

Figure 2. Indexes of insulin resistance and secretion: low-NGT vs high-NGT

Data are on the log10 scale and are expressed as means, 95% confidence interval. DI: disposition index; NGT: normal glucose tolerance; WBISI: Whole Body Insulin Sensitivity Index. Benjamini-Hochberg P-value are reported

Figure 1

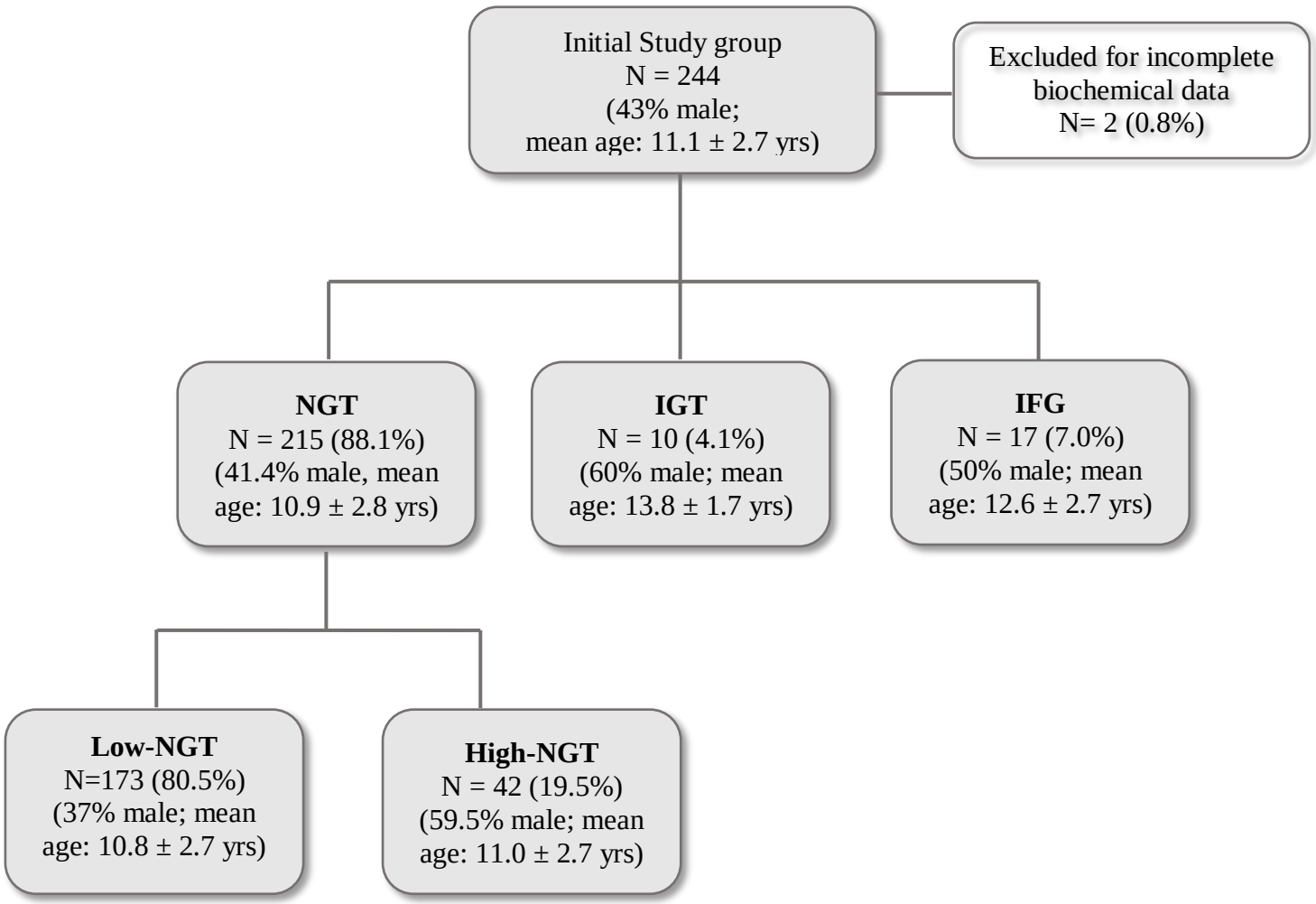
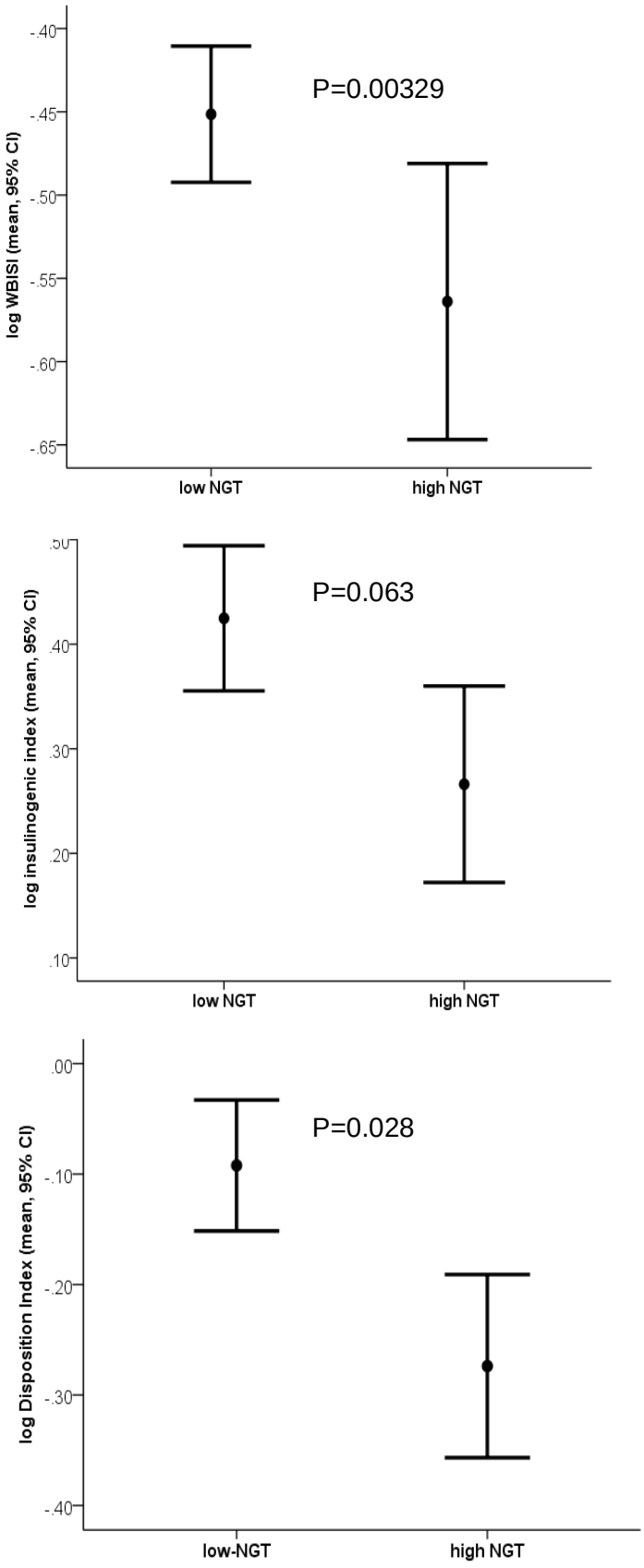

Initial Study group
N = 244
(43% male;
mean age: 11.1 ± 2.7 yrs)
Excluded for incomplete biochemical data
N= 2 (0.8%)
NGT
N = 215 (88.1%)
(41.4% male, mean age: 10.9 ± 2.8 yrs)
IGT
N = 10 (4.1%)
(60% male; mean age: 13.8 ± 1.7 yrs)
IFG
N = 17 (7.0%)
(50% male; mean age: 12.6 ± 2.7 yrs)
Low-NGT
N=173 (80.5%)
(37% male; mean age: 10.8 ± 2.7 yrs)
High-NGT
N = 42 (19.5%)
(59.5% male; mean age: 11.0 ± 2.7 yrs)

Figure 2.


log WBISI (mean, 95% CI)
-.40
-.45
-.50
-.55
-.60
-.65
P=0.00329
low NGT
high NGT
log insulinogenic index (mean, 95% CI)
.40
.30
.20
.10
P=0.063
low NGT
high NGT
log Disposition Index (mean, 95% CI)
.00
-.10
-.20
-.30
-.40
P=0.028
low-NGT
high NGT