

**UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA  
DOCTORAL SCHOOL**

# **DOCTORAL THESIS**

***The Importance Of 1 Hour Glucose During  
The Oral Glucose Tolerance Test In The  
Evaluation Of Insulin Secretion And Insulin  
Resistance***

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### KEY WORDS:

*OGTT, 1 hour glucose, insulin resistance, insulin secretion, cardiovascular risk*

# INTRODUCTION

Diabetes mellitus (DM) is currently considered to be an important public health problem. This statement is reinforced by the rapid increase in the number of patients with DM in the recent decades and we are now able to speak of a "pandemic" of diabetes globally. Another cause for concern is the fact that DM is still under-diagnosed as the number of patients diagnosed with diabetes is in reality almost doubled by that of patients with undiagnosed diabetes or pre-diabetes

## PART I STATE OF KNOWLEDGE

### 1. DIAGNOSIS OF DM

#### Criteria for type 2 DM diagnosis:

- fasting blood glucose  $\geq 126$  mg / dL (7 mmol / l) in 2 different tests *or*
- glycated hemoglobin (HbA1c)  $\geq 6.5\%$  determined by a certified method *or*
- 2 hours plasma glucose during oral glucose tolerance test (OGTT)  $\geq 200$  mg/dl (11,1 mmol/l) *or*
- random plasma glucose  $\geq 200$ mg/dl (11,1mmol/l) in subjects with symptoms of hyperglycemia

#### The role of the OGTT in the diagnosis of type 2 DM

There is still some controversy regarding the role of the OGTT versus fasting glucose in practice. The OGTT presents the inconvenience of a higher cost, a lower reproducibility and a small discomfort for the patients. However, there are multiple advantages, as compared to the diagnosis of type 2 DM only by fasting blood glucose. Active detection is required in patients with DM and pre-diabetes (impaired fasting glucose: IFG, impaired glucose tolerance: IGT), given that early intervention is vital for these patients.

#### The role of 1 hour glucose in assessing cardio-metabolic risk

Recently, the idea of an excess of metabolic and cardiovascular risk in patients currently considered to be glucose tolerant but showing elevated 1 hour glucose during the OGTT was emphasized.

It has been hypothesized that subjects with normal glucose tolerance and 1 hour glucose during the OGTT as high as that of subjects with IGT may have an intermediate phenotype of glucose metabolism characterized by alteration of insulin

sensitivity and impaired glucose sensitivity of the  $\beta$  cells, with increased risk of developing DM.

## 2. INSULIN RESISTANCE

Insulin resistance is defined as the reduction of the responsiveness to normal circulating levels of insulin. Insulin resistance is demonstrated to be the common attribute of obesity, type 2 DM, dyslipidemia (DLP), cardiovascular disease and other components of the metabolic syndrome (MS) and it is an important link in the pathophysiology of these disorders.

### Markers of insulin resistance

➤ **Clinical markers of insulin resistance:**

- ❖ Obesity. Body mass index (BMI)  
 $BMI = \text{weight (kg)} / \text{height}^2 \text{ (m}^2\text{)}$
- ❖ Abdominal obesity: Waist circumference (WC), Hip circumference (HC), waist to hip ratio (WHR)

Parameter	High risk of metabolic complications	Very high risk of metabolic complications
WC (cm) (women)	80- 88	$\geq 88$
WC (cm) (men)	94- 102	$\geq 102$
WHR (women)	-	$\geq 0,85$
WHR (men)	-	$\geq 0,90$

- ❖ Waist to height ratio (normal value  $<0.5$ )
- **Biological markers of insulin resistance:**
- ❖ Homeostatic Model Assessment (HOMA)  
 $HOMA\ 1-IR = (I \times G) / 22,5$ ; I = fasting insulin (mU/l), G= fasting glucose (mmol/l)
- ❖ The quantitative insulin sensitivity check index (QUICKI)  
 $QUICKI = 1 / [\log(I) + \log(G)]$ ; I = fasting insulin (microunits/ ml), G = fasting glucose (mg/ dl)
- ❖ Fasting insulin. Fasting glucose to insulin ratio  
 Fasting glucose to insulin ratio values of  $\leq 4,5$  have 95% sensitivity and 84% specificity in diagnosing insulin resistance.
- ❖ Adiponectin  
 Adiponectin is an insulin-sensitizing multifunctional protein with pleiotropic effects. For this reason it is considered to be one of the key molecules involved in the pathogenesis of the MS.

- ❖ The Reaven Index (TG to HDL-chol ratio)

The TG to HDL ratio  $\geq 3,5$  best correlates with insulin resistance and the atherogenic phenotype of small and dense LDL particles, thus precisely identifying dyslipidemic, insulin resistant subjects, with high risk of cardiovascular events.

### **3. THE IMPORTANCE OF EARLY INTERVENTION IN PREVENTING OR DELAYING THE ONSET OF DIABETES**

Studies have shown that early intervention, even in the stage of IGT and IFG can reduce progression to type 2 DM as well as the associated cardiovascular risk.

## **PART II**

### **PERSONAL CONTRIBUTION**

#### **1. THE IMPORTANCE OF THE THEME**

The theme highlights a group of patients with a special metabolic profile, to whom current standards of care and prevention do not apply.

#### **2. STUDY OBJECTIVES**

##### **2.1. Main objectives:**

- Highlighting a new category of impaired glucose metabolism, represented by elevated 1 hour glucose during the OGTT.
- Assessing insulin resistance and insulin secretion in this category of subjects compared to subjects with normal glucose tolerance and subjects with IGT.

##### **2.2. Secondary objectives:**

- Assessing cardiovascular risk in subjects with elevated 1 hour glucose and comparing it to the cardiovascular risk of glucose normal tolerant subjects and subjects with IGT
- Assessing the prevalence of MS in subjects with elevated 1 hour glucose and comparing it to the prevalence of MS in subjects with normal glucose tolerance and IGT.

#### **3. MATERIAL AND METHODS**

The study was conducted between January and August 2015. The target population were subjects that had not been previously diagnosed with DM or IFG and which presented to evaluate their metabolic status.

Subjects were completely evaluated at baseline. After applying the inclusion and the exclusion criteria and signing the informed consent, all subjects were performed 75 g glucose OGTT and were sampled into 3 groups:

<b>GROUP</b>	<b>1A</b>	<b>1B</b>	<b>2</b>
Fasting glucose	< 110 mg/dl	<110 mg/dl	<110 mg/dl
1 hour glucose	< 155 mg/dl	≥ 155 mg/dl	regardless
2 hours glucose	< 140 mg/dl	< 140 mg/dl	140-200 mg/dl

We studied 75 sex and age-matched subjects, 25 subjects (13 men and 12 women) for each of the 3 groups. According to the literature, we established the cutoff value of 1 hour glucose at 155 mg / dl.

Cardiovascular risk was calculated according to the SCORE charts, applicable for high risk European countries and the presence of the MS was objectified using NCEP ATP III criteria.

#### **4. STATISTICAL ANALISYS**

Data processing used the Microsoft Excel software (Microsoft Corp, Redmond, WA, USA) together with XLSTAT suite for MS Excel (Addinsoft SARL, Paris, France), IBM SPSS Statistics 20.0 software (IBM Corporation, Armonk, NY, United States).

#### **5. RESULTS**



##### **The groups' characteristics:**

There were no statistically significant differences between the subjects of the 3 groups in terms of: distribution by sex, age, personal history of DLP. Regarding the smoking status there were no statistically significant differences between group 1A and 1B (p Fisher's exact = 0.044) or between group 1B and 2 (p Fisher's exact = 0.044). Also, there were significant differences in terms of personal history of high blood pressure as follows: there were no statistically significant differences between group 1A and 1B (p Fisher's exact = 0.587), while between group 1B and 2 the difference was significant (p Fisher's exact = 0.587).



##### **The glycemc control**

In terms of the HbA1c, we analyzed the mean values of this parameter in the three groups using the ANOVA test and we obtained a p value of  $7.26 \times 10^{-8}$  (statistically highly significant). We used the test Fisher's LSD (Fisher's Least Significant Difference) in order to identify the pairs that differed and observed that Group 1A had

a significant lower HbA1c than group 1B ( $p=0.024$ ) and group 1B had a significant lower HbA1c than group 2 ( $p=0.032$ ).

➤ **Clinical markers of insulin resistance**

For all the quality parameters that we analyzed we used Chi square test for an overall comparison of the three groups and Fisher exact test to compare Group 1B and Group 2.

Regarding the BMI, we found no significant differences between the three groups ( $p$  Chi square =  $0.361 > 0.05$ ) nor between group 1B and group 2 ( $p$  Fisher's exact =  $0.778 > 0.05$ )

Neither the parameters that assessed insulin resistance through the abdominal obesity showed any statistically significant differences: for the WC,  $p$  Chi square was  $0.521$  and  $p$  Fisher's exact =  $0.463$  and for WHR  $p$  Chi square =  $0.319$  and  $p$  Fisher's exact =  $0.289$ .

Regarding the waist circumference to height ratio, there were no statistically significant differences:  $p$  Chi square =  $0.376$  and  $p$  Fisher's exact =  $0.349$ .

➤ **Biological markers of insulin resistance**

- ❖ Plasma triglycerides (TG) (a value of  $\geq 150$  mg / dL was considered significant to insulin resistance)

Although the numerical difference between group 1A and the other two groups was very high, it did not present statistical significance, probably due to the small number of patients in the study groups, the Chi square test result  $p$  was  $0.110$ . In group 1B and group 2 the distributions were identical, so we could not put in question any statistically significant difference.

- ❖ HDL- cholesterol

No statistically significant differences were detected ( $p$  Chi square= $0.804$ ,  $p$  Fisher's exact =  $0.776$ ).

- ❖ The Reaven Index (TG to HDL ratio) (a value of  $\geq 3$  was considered significant to insulin resistance)

In this case the Chi square test  $p$ -value of  $0.074$  was very close to the maximum allowed for statistical significance,  $p = 0.05$ . When comparing groups 1B and 2 by Fisher's exact test, we obtained a statistically non-significant value:  $p = 0.396$ .

- ❖ Fasting insulin (a value of  $\geq 16.3$  uU/ml was considered significant to insulin resistance)

In this case there were highly statistically significant differences between the 3 groups (p Chi square = 0.0006893 p <0.001), also group 1B and group 2 did not differ significantly (p Fisher's exact = 0.780).

- ❖ Fasting glucose to insulin ratio (a value of <4 was considered significant to insulin resistance)

We objectified a significant difference when comparing the control group with the other two groups (p Chi square = 0.04), while group 1B and group 2 did not show any statistically significant differences (p Fisher's exact= 0.725).

- ❖ HOMA-IR Index (a value of  $\geq 2.5$  was considered significant to insulin resistance)

We detected a highly statistically significant difference (p Chi square= 0.000078) between the 3 groups, while between group 1B and group 2 there was no significant difference (p Fisher's exact = 0.742).

- ❖ QUICKI Index (a value of < 0.357 was considered significant to insulin resistance)

We achieved a statistically significant difference between the control group and the other two groups (p Chi square = 0.014), while between group 1B and group 2 there was no significant difference (p Fisher's exact = 0.349)

- ❖ Adiponectin (a value of < 7 mg/dl was considered significant to insulin resistance)

There were no significant differences between the 3 groups (p Chi square = 0.790) or between group 1B and group 2 (p Fisher's exact = 0.769).

#### ➤ **Assessing insulin secretion (HOMA % B Index)**

In terms of the mean values of HOMA% B the ANOVA test returned a p-value of 0.143 > 0.05, indicating a significant difference between the three groups. Although apparently, there are important numerical differences between the three means, the statistically non-significant result can be explained by the high variability within each of the three groups (standard deviation is almost as high as the mean).

#### ➤ **Assessing the cardiovascular risk**

When we compared the mean value of the cardiovascular risk between the three groups we identified a significant overall difference, with the ANOVA test result of p = 0.034. After continuing the analysis using the post-hoc Fisher LSD test, in order to find the matching pairs that present significant differences, we found that the mean value for patients in group 1A differ significantly both to the patients in group 1B and

to the patients in group 2. Surprisingly, we found that for patients in group 1B the cardiovascular risk was higher than for patients in group 2.

➤ **The prevalence of the metabolic syndrome**

When we compared the three groups through the association with the MS, we found no statistically significant differences:  $p$  Chi square = 0.320 and  $p$  Fisher's exact = 0.754.

## **5. DISCUSSION**

**Regarding the clinical markers of insulin resistance**, although many studies have analyzed and communicated statistically significant associations between the parameters describing obesity and in particular abdominal obesity and insulin resistance associated to subjects with elevated 1 hour glucose, our study did not reveal this association.

**Regarding the biological markers of insulin resistance**, our findings confirmed data published by large studies that investigated the correlation between insulin resistance objectified by biological markers (fasting insulin, fasting glucose to insulin ratio, HOMA IR, Quick) and elevated 1 hour glucose.

**Regarding HbA1c** our results demonstrated a statistically significant difference between the group of patients with 1-hour glucose above 155 mg / dl and the group with IGT compared to the group with 1 hour glucose below 155 mg / dl.

**As regards the assessment of insulin secretion** using HOMA% B index, the results did not show a statistically significant difference between the 3 groups. This can be explained by the high variability of the data obtained in each group.

**Regarding the cardiovascular risk** assessed according to the SCORE charts applicable to high risk European countries, we observed a statistically significant difference in the sense of an increased risk in subjects with elevated 1 hour and 2 hours glucose compared to patients in the control group ( $p = 0.048$ ). Moreover, the cardiovascular risk of patients with elevated 1 hour glucose, that are currently considered to be normal glucose tolerant, proved to be higher than that of subjects with IGT.

Our data do not identify a statistically significant difference in the **prevalence of the MS** in subjects with elevated 1 hour glucose compared to subjects from the other two groups.

### **The innovative elements of this study are:**

It is the only national study that investigated this parameter: 1 hour glucose during OGTT and identified a new category of impaired glucose metabolism, with distinct metabolic traits (insulin resistance, high cardiovascular risk). This category is not currently recognized and it doesn't receive any prophylactic measures.

Our study identified a higher cardiovascular risk in the group of patients with elevated 1 hour glucose compared to subjects with IGT and this excessive risk was not correlated with a higher prevalence of the MS in this group of subjects

The statistically significant association between elevated 1 hour glucose and HbA1c has never been communicated in the literature.

### **5. CONCLUSIONS**

- Elevated 1 hour glucose (above the threshold of 155 mg / dl) was associated with parameters of insulin resistance (fasting insulin, fasting glucose to insulin ratio, HOMA IR index, QUICKI index) to the same extent they were associated with elevated 2 hours glucose (patients with IGT). This correlation was not observed in normal glucose tolerant patients with 1 hour glucose below 155mg / dl.
- Elevated 1 hour glucose was found to be associated with a higher mean HbA1c
- Elevated 1 hour glucose correlated with an increased cardiovascular risk, higher than the cardiovascular risk of patients with IGT
- The increased cardiovascular risk in patients with 1 hour glucose  $\geq$  155 mg / dl could not be attributed to an increased prevalence of the MS.
- Subjects with elevated 1 hour glucose could benefit from lifestyle optimization interventions similar to those applied to patients with IGT or IFG in order to reduce the cardiovascular risk and the risk of progression to DM.
- 1 hour OGTT is easier to perform and could find its place in epidemiological studies and even in the screening and diagnosis of DM.

