

**Inflammation markers and metabolic characteristics of subjects with one-hour plasma glucose levels**

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*Objective:* To assess the association of 1-h plasma glucose (1hPG) and inflammation with normal glucose tolerance (NGT) and pre-diabetes (pre-DM).

*Research Design And Methods:* A cohort of 1062 subjects was enrolled. After oral glucose load (OGTT), we compared NGT and pre-DM subjects above and below the 1hPG cut point (155 mg/dl). Fibrinogen and leucocytes count (WBC) for subclinical inflammation, lipid ratios, insulin sensitivity (Matsuda Index), were determined.

*Results:* NGT and pre-DM patients 1hPG>155 mg/dl showed a significant increase of inflammatory markers and lipid ratios (for all,  $p<0.05$ ). In age-sex-BMI-adjusted analysis, 1hPG is associated with a significant higher WBC count and fibrinogen ( $p<0.05$ ). Patients with elevated 1hPG showed a highly significant lower insulin sensitivity than subjects below 1hPG ( $p<0.01$ ).

*Conclusions:* Elevated 1hPG in NGT and pre-DM subjects is associated to subclinical inflammation, high lipid ratios and insulin resistance. Therefore, 1hPG >155 mg/dl could be considered a new “marker” for cardiovascular risk.

**P**re-diabetes (pre-DM) identifies subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) at high risk for type 2 diabetes (DM2); moreover, it is associated to insulin resistance (IR), subclinical inflammation and cardiovascular diseases (CVD) (1-4). Recently, 1-hour hyperglycaemia (1hPG) during glucose tolerance test (OGTT) with a cut point of 155 mg/dl has been indicated as a further risk factor for DM2 (5, 6) and showed early carotid atherosclerosis (7). Aim of this study is to evaluate the metabolic characteristics and inflammation markers in subjects with normal glucose tolerance (NGT) and pre-DM with or without 1hPG>155 mg/dl.

## RESEARCH DESIGN AND METHODS

We examined a consecutive series of 1062 subjects with no history of diabetes, CVD, with any malignant disease, liver or chronic kidney failure or inflammatory diseases and with any drugs interfering with glucose or lipid metabolism. All subjects gave their written informed consent before study participation. Anthropometrical data and blood pressure were measured. After overnight fasting, a 75-g OGTT with blood samples at 0, 30, 60, 90' and 120 minutes was performed. Plasma glucose level, triglycerides (Tg), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and serum uric acid were automatically measured (Beckman Instruments, Brea, USA), as well as fibrinogen and leucocytes (WBC) count as subclinical inflammation markers. Plasma insulin were determined by a standard assay (Roche Diagnostics GmbH, Mannheim, Germany). Insulin sensitivity (IS) was evaluated by Matsuda index (8) calculated as  $10,000/\text{square root of [fasting glucose (mg/dl) x fasting insulin (}\mu\text{U/ml)] x [mean glucose x mean insulin during OGTT]}$ . Lipid ratios as

Tg/HDL-C >3.5 and TC/HDL-C >5 were considered as predictors of CVD risk (9).

According to American Diabetes Association (ADA) criteria (10), we considered NGT and pre-DM categories; patients with diagnosis of DM2 were excluded. The cut point of 1hPG during OGTT >155 mg/dl was applied, subdividing all patients in four groups, below and above the 1hPG cut off: NGT-high, NGT-low, pre-DM-high and pre-DM-low. Diagnosis of metabolic syndrome (MS) was performed according to NCEP-ATP III criteria (11). Statistical analysis was performed using SPSS 15.0 software. We used ANCOVA analysis to compare differences between selected groups in means and the Bonferroni test to assess differences between selected groups. Adjustment for age and sex was made in all analyses. Statistical significance was considered with  $P < 0.05$ .

## RESULTS

Of 1062 patients studied, 507 (47.7%) were NGT and 555 (52.3%) pre-DM; among NGT subjects 122 (24.0%) had 1hPG >155 mg/dl during OGTT, while 433 (78.0%) of pre-DM patients showed elevated 1hPG. Glucose 30' and 120' were significantly higher in NGT-high and pre-DM-high vs NGT-low and pre-DM-low patients ( $p < 0.05$ ), while glucose 30', 60' and 120' were highly significantly elevated in subjects with 1hPG >155 mg/dl vs NGT-low and pre-DM-low ( $p < 0.01$ ). NGT-high and pre-DM-high patients showed a significant increase ( $p < 0.05$ ) of fibrinogen level and WBC count with respect to NGT-low and pre-DM-low subjects; all subjects with any history of CVD were excluded from the analysis (tab. 1). NGT-high and pre-DM-high subjects were older, female and had higher BMI in comparison to NGT-low and pre-DM-low patients; therefore a logistic regression analysis adjusted for age, sex and BMI was

applied. After adjusted analysis, fibrinogen concentration and WBC count remained significantly associated with gender ( $P<0.001$ ), age ( $P<0.001$ ), BMI ( $P<0.05$ ) and with 1hPG ( $P<0.001$ ). Tg/HDL-C ratio was significantly increased in NGT-high vs NGT-low subjects, in pre-DM-low vs NGT-low individuals, in pre-DM-high vs NGT-low subjects, and between pre-DM-high and NGT-high patients (for all,  $p<0.05$ ). Higher significantly levels of TC/HDL-C ratio were found in NGT-high vs NGT-low subjects, in pre-DM-low vs NGT-low individuals, in pre-DM-high patients vs NGT-high, NGT-low and NGT-high subjects (for all:  $p<0.05$ ). Significant increased concentrations of uric acid were observed in NGT-high than NGT-low subjects ( $p<0.05$ ), in pre-DM-high vs pre-DM-low, NGT-low and NGT-high patients (for all,  $p<0.05$ ). A highly significant lower IS was found between pre-DM-high vs pre-DM-low and between NGT-high vs NGT-low subjects ( $p<0.01$ ). Overall MS prevalence was 43.5%; considering those subjects with 1hPG>155 mg/dl, 100% fulfilled MS criteria, but 31.0% patients without MS diagnosis revealed 1hPG >155 mg/dl.

## CONCLUSIONS

Pre-diabetes is associated with a high risk for DM2, subclinical inflammation, early atherosclerosis and CVD. Moreover, it was shown that NGT subjects with 1hPG>155 mg/dl had a fivefold DM2 risk than NGT subjects with 1hPG below the cut off of 155 mg/dl (5). In this study, NGT-high subjects showed increased WBC count and fibrinogen levels, signs of subclinical inflammation, as patients with pre-diabetes and a significant worsening of lipid profile than NGT-low patients. The mechanism that links elevated 1hPG to subclinical inflammation is probably

due to hyperglycemia that acutely increases circulating cytokine concentrations by oxidative mechanisms, and this effect is more pronounced in patients with impaired glucose regulation (3).

Subjects with 1hPG>155 mg/dl showed significantly lower IS (Matsuda Index). As previously observed, MS is strongly associated with decreased IS (12), therefore the link observed between elevated 1hPG and insulin resistance (IR) could be explained by the high prevalence of MS in patients with elevated 1hPG.

We decided to measure inflammation levels by fibrinogen and WBC count without considering the measure of C-reactive protein (CRP). It is important to underline that the measurement of CRP concentration requires a specific high sensitivity assay method that is not available in all laboratories; moreover, several data have shown that elevated levels of CRP were associated to increased CVD, but recently, analysis of genetic polymorphisms in the CRP gene in Caucasian subjects showed that these polymorphisms were not associated with an increased risk of ischemic vascular disease (13), increasing the doubts about the reliability of such a measurement in clinical practice. A high WBC count is associated with increased CVD related morbidity and mortality in several populations and clinical settings; it may turn out to be a less expensive and more readily available risk CVD marker than other currently available risk factors (14, 15).

In conclusion, our data show the relevance of 1hPG >155 mg/dl as an important cut-off related to subclinical inflammation, lipid disorders and IR; therefore, this threshold could be seriously taken in consideration to identify subjects at high CVD risk.

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**Table 1. Demographic and clinical characteristics of patients enrolled**

| Variables                       | NGT low    | NGT high     | Pre-DM low                  | Pre-DM high               |
|---------------------------------|------------|--------------|-----------------------------|---------------------------|
| Male/female                     | 136/249    | 56/66        | 52/70                       | 206/227                   |
| Age (yrs)                       | 37.8±14.1  | 45.3±13.4*   | 45.7±12.4 <sup>  </sup>     | 49.7±12.5 <sup>†§</sup>   |
| BMI (kg/m <sup>2</sup> )        | 33.0±6.6   | 34.1±7.5     | 34.1±6.6 <sup>  </sup>      | 36.2±7.3 <sup>†§</sup>    |
| Waist circumference (cm)        | 104.4±13.9 | 107.9±14.7   | 104.6±13.3                  | 111.2±13.6 <sup>†§</sup>  |
| Glucose 0' (mg/dl)              | 89.0±6.3   | 92.1±5.2*    | 102.9±8.0                   | 106.4±9.0 <sup>††</sup>   |
| Glucose 30' (mg/dl)             | 130.1±22.3 | 157.6±19.3*  | 146.5±23.0                  | 172.9±23.8 <sup>††</sup>  |
| Glucose 60' (mg/dl)             | 118.2±21.1 | 174.0±17.9** | 132.8±16.2                  | 194.0±27.6 <sup>††</sup>  |
| Glucose 120' (mg/dl)            | 99.2±19.4  | 112.9±19.5*  | 119.5±27.2                  | 146.0±29.4 <sup>††</sup>  |
| SBP (mm Hg)                     | 135.4±19.0 | 140.9±20.4*  | 141.9±21.0 <sup>  </sup>    | 146.5±21.9 <sup>§#</sup>  |
| DBP (mm Hg)                     | 83.2±11.2  | 85.8±11.4    | 86.8±12.5 <sup>  </sup>     | 89.0±12.6 <sup>§#</sup>   |
| Uric acid (mg/dl)               | 4.0±1.0    | 4.5±1.2*     | 4.2±0.9                     | 4.8±1.3 <sup>†§</sup>     |
| Fibrinogen (mg/dl)              | 347.7±85.3 | 360.8±80.1*  | 369.3±71.5 <sup>†‡  #</sup> | 379.0±77.0 <sup>†§#</sup> |
| WBC count (x10 <sup>9</sup> /L) | 6.0±1.2    | 6.8±1.4*     | 7.1±1.3 <sup>†  </sup>      | 7.8±1.5 <sup>†§#</sup>    |
| Insulin sensitivity             | 4.8±2.5    | 3.2±1.7**    | 3.8±1.9 <sup>  </sup>       | 2.7±1.3 <sup>†≈</sup>     |
| Tg/HDL-C                        | 2.6±2.2    | 3.4±2.7*     | 3.5±3.0 <sup>  </sup>       | 4.3±3.6 <sup>§#</sup>     |
| TC/HDL-C                        | 3.9±1.4    | 4.6±1.4*     | 4.9±1.5 <sup>  </sup>       | 5.3±1.5 <sup>†§#</sup>    |

data are expressed as mean±SD.

\*: p<0.05 vs NGT-low;

\*\* : p<0.01 vs NGT low;

!!: p<0.01 vs NGT-low;

≈: p<0.01 vs NGT-low;

†: p<0.05 vs Pre-DM-low;

††: p<0.01 vs vs Pre-DM-low;

‡: p<0.05 vs NGT-high;

§: p<0.05 vs NGT-low;

||: p<0.05 vs NGT-low;

#: p<0.05 vs NGT-high.

SPB: systolic blood pressure; DBP: diastolic blood pressure; WBC count: total leucocytes count; Tg/HDL-C: triglyceride/HDL cholesterol ratio; TC/HDL-C: total cholesterol/HDL cholesterol ratio.