SUMMARY

Background. To relate lipoprotein profile, conventional lipid and glycemia along with abdominal fat to explore the possible risk factors associated with the progression from a non-diabetic, asymptomatic state to IGT, IGF and type-2 DM in Maltese Canadian population.

Methods. We conducted analysis of 780 subjects using the ADA and WHO criteria to classify subjects into groups based on: 1) normal glucose tolerance with FBG <6.0 and 2hBG <7.5; 2) isolated IFG (FBG 6.1-6.9 and 2hBG <8.0); 3) isolated IGT (FBG >6.1 and 2hBG 8-12); and 4) combined IFG/IGT (FBG 6.1-6.9 and 2hBG 8-12). We compared the three groups for glycemia, insulin secretion, and insulin sensitivity based on their apolipoprotein levels and abdominal fat.

Results. The subjects with higher apolipoprotein levels (12.8 (IGT) vs. 8.9 mg/dL (normal), P<0.001), WHR (0.91 (IFG) vs. 0.89 (normal), P<0.01) and higher abdominal fat were found to be suffering from IGF and IGT and were highly insulin resistant. There were significant differences in the 2-h glucose (5.2 for NGT vs. 9.1 for IGT and 13.4 mmol/L for T2DM, P<0.001) and insulin (37.1 vs. 89.3 vs. 53.2, μU/mL).

Conclusions. Poor glycemia was associated with potentially more atherogenic lipoprotein profiles and led to the worsening of insulin resistance and insulin secretory dysfunction. The higher apolipoprotein C-III and B levels, BMI and abdominal fat were strongly associated with metabolic abnormalities and shown to be predictors of diabetes.

Key words: metabolic syndrome, impaired fasting glucose, impaired glucose tolerance, insulin resistance, type 2 diabetes

LIPID AND APOLIPOPROTEIN PREDICTORS OF PROGRESSION FROM ASYMPTOMATIC STATE OR IMPAIRED GLUCOSE TOLERANCE TO TYPE 2 DIABETES MELLITUS

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Abbreviations: 2hBG, 2-hour glucose after oral load • FBG, fasting blood glucose • NGT, normal glucose tolerance • IFG, impaired fasting glucose • IGT, impaired glucose tolerance • ADA, American Diabetes Association • MS, metabolic syndrome • IR, insulin resistance • ISI, insulin sensitivity index • OGTT, oral glucose tolerance test • WHR, waist-to-hip ratio • BMI, body mass index • VFA, visceral fat area • SFA, subcutaneous fat area • WHO, World Health Organization
INTRODUCTION

There is growing evidence showing that type 2 diabetes can be prevented or delayed by lifestyle changes or by lowering blood glucose concentration with drugs (1-5). So far, most of these prevention programs have targeted subjects with impaired glucose tolerance (IGT), as IGT was originally defined to identify people at a high risk of developing diabetes; it has now been recognized as an important intermediate stage in the natural history of type 2 diabetes and has been associated with an increased risk of cardiovascular disease and mortality (6-13).

The American Diabetes Association (ADA) recommends screening for IGT or impaired fasting glucose (IFG) in men and women aged ≥45, particularly those who are overweight or obese. ADA and the World Health Organization (WHO) (14-22) have revised the diagnostic criteria for diabetes and glucose intolerance. A new category of IFG when fasting blood glucose (FBG) is 6.1-6.9 mmol/L has been added to IGT. When only disturbance in glucose metabolism is measured, IGT remains undetected in many subjects (6-13,23-31).

Insulin resistance and impaired secretion concur towards diabetes and glucose intolerance, but it is unclear which defect arises first and which relates to either IFG or IGT, which reflect different alterations in glucose homeostasis (23-31). Few studies have shown that subjects with IFG have hyperinsulaemia and/or worsening of insulin resistance, and those with IGT have defective secretion in response to glucose loading (26-31). Although screening for these hyperglycemic states will inevitably find cases of undiagnosed diabetes, benefit from their early detection and treatment has not been directly documented. Thus, the characteristic metabolic abnormalities of IFG compared with IGT remain to be elucidated. Since 1956, the impact of body fat distribution on the occurrence of metabolic abnormalities in obese people has received increased attention (32-41). Nowadays, abdominal fat accumulation is seen as a key event for the pathogenesis of the metabolic syndrome (MS) (35,38-42). However, the impact of visceral fat on mortality of patients with MS is not completely known. Also, data concerning the cutoff values for CT-determined intra-abdominal fat associated with high cardiovascular risk are not widely available.

The major goal of our study was to evaluate the diagnostic power of FBG with respect to 2hBG (second-hour glycemia after oral load), assuming that the latter can be considered a reference indicator of metabolic disease. This study was designed specifically to offer the opportunity for analyzing the relationship of FBG and 2hBG with insulin resistance and secretion in isolated IFG, isolated IGT, and combined IFG and IGT, and to derive a numerical score based on readily available clinical information to predict the presence of IGT. This study was also aimed to evaluate the association of measurements of generalized (expressed by BMI) and visceral (visceral fat area) adiposity with typical abnormalities of metabolic syndromes.

RESEARCH DESIGN AND METHODS

The study was performed at the Diabetes Care and Research Clinic in Toronto affiliated with the St. Joseph Hospital and University of Toronto. Institutional review boards at the St. Joseph Hospital and University of Toronto approved the study protocol. After age and sex stratification to match the Canadian Census data, a sample of 780 was drawn randomly from the total population of 9300. All subjects selected had no previous history of diabetes. We excluded subjects with known type 2 diabetes or other abnormalities defined by ADA and WHO diagnostic criteria. We enrolled 778 subjects, 426 male and 778 female, aged between 35 and 85 years. None was taking medications affecting glucose or insulin metabolism and based on the results of physical examination and routine laboratory examinations, all were classified as healthy. We pre-established the use of ADA and WHO criteria (14-22) to classify subjects into groups based on glycemic values expressed in mmol/L: 1) normal glucose tolerance (NGT) with FBG <6.0 and 2hBS <7.8; 2) isolated IFG (FBG 6.1-6.9 and 2hBG <7.8); 3) isolated IGT (FBG <6.1 and 2hBG 7.8-11.1); and 4) combined IFG/IGT (FBG >6.1-6.9 and 2hBG >7.8-11.1).
Inclusion criteria were the absence of clinical anomalies; physical examination without reasonably major clinical abnormalities; normal electrocardiogram (ECG); HbA1c ≤ 7%; and body mass index (BMI) less than 38 kg/m². Safety parameters included acceptable general physical examination, medical history, ECG, urinalysis and complete biochemical profile at screening. Vital signs were determined and clinical chemistry was assessed at screening during and at the end of the study.

After an initial screening for general physical health, the subjects underwent a 75-g oral glucose tolerance test (OGTT). Venous blood samples were drawn at baseline and during the test duration every 30 min for 120 min for determination of insulin, C-peptide and glucose levels. On the morning of OGTT, we measured BMI (kg/m²) and waist-to-hip ratio (WHR) as an index of body fat distribution. We calculated pancreatic β-cell function and insulin resistance (IR) from fasting glucose and insulin concentrations.

Laboratory analysis: all clinical laboratory determinations were performed by a central laboratory (St. Joseph’s Hospital, University of Toronto, Canada).

- Glucose: plasma glucose was determined using the hexokinase method.
- Insulin: immunoreactive insulin was determined by double antibody radioimmunoassay. Interassay coefficient of variation (CV) was 3.3% at 300 pmol/l.
- C-peptide: C-peptide was determined by radioimmunoassay. Inter-assay CV was 4.2% at 1.32 nmol/l.

Triglycerides and HDL and LDL cholesterol: triglycerides and HDL and LDL cholesterol were determined by enzymatic methods.

HbA1c: HbA1c was determined by the standard HPLC methods.

All samples were analyzed in duplicate.

Subcutaneous (SFA) and visceral (VFA) fat areas were obtained by using a combination of Futrax infrared and Skin Fold Caliper systems.

Statistical methods: Results were expressed as mean ± standard deviation unless stated otherwise. Normality of distribution was tested using Shapiro-Wiks test. Analysis of variance (ANOVA) was used to test differences between three or more groups, with area-under-the-curve (AUC) as a response variable, with post-hoc analysis. Statistical analysis also included unpaired Student’s t-test or Mann-Whitney U test to evaluate differences between groups. Pearson and Spearman coefficients were used to test correlation between variables. Independent associations of variables with MS were analyzed by logistic regression. Receiver operating characteristic (ROC) curve analysis was used to establish cutoff points for a number of variables associated with the occurrence of MS. All statistical analyses were performed with STATISTICA 6.0 (StatSoft). The level of significance was set at P<0.05.

The Mann-Whitney U test was used to evaluate differences between groups, whereas Friedman’s test (variance analysis) followed by Wilcoxon’s paired test were used to evaluate differences within groups. P<0.05 was considered significant. All statistical analyses were performed with STATISTICA 6.0 (StatSoft). Data are presented as mean ± SEM.

The sample of 778 participants consisted of 426 male and 354 female subjects aged between 35 and 85 years and BMI ranging between 17.5 and 38.0 kg/m². Overweight was defined as BMI between 25 and 29.99 kg/m², and obesity as BMI >30 kg/m². We arranged the factors offering greatest predictive ability to distinguish between IFG, IGT and type 2 diabetes classification in descending order: fasting glucose, insulin levels, C-peptide, triglycerides, age, systolic blood pressure, height, HDL-C, waist circumference, ethnicity, and sex. For practical purposes, risk functions were developed with and without lipid variables. We assessed hypertensive medication use and parental history of diabetes by interview and obtained physical examination under fasting condition and with an empty bladder. We performed a standard 75-g OGTT after an overnight fast. Waist girth was measured at the umbilical level. Blood pressure was determined as the mean of two standardized
measurements; hypertension was defined as blood pressure $\geq 140/90$ mm Hg and/or the use of anti-hypertension medication.

The 1980 WHO Malta Study demonstrated the prevalence of type 2 DM to be 6.6% and of IGT 7.7%.

In 1991 survey, there were 780 stratified randomly selected subjects, Toronto residents of Maltese origin, aged $\geq 15$, with data collected on: (a) biochemical, (b) demographic, (c) anthropometric, (d) hereditary, (e) nutrition and (e) lifestyle parameters. On five-year follow up phase to investigate the incidence, comparative analysis showed no difference between the native and expatriate population in terms of prevalence rate and nutritional profile, thus placing in question the contribution due to dislocation factors such as immigration, changes in climate, culture and lifestyle.

Significant relationships were seen between: (a) plasma insulin and various biochemical end points (correlation coefficient and significance for triglycerides; $r=0.3 P<0.001$, HDL; $r=-0.19 P<0.001$, apolipoproteins b; $r=0.14 P<0.001$ and C3; $r=0.12 P<0.005$, uric acid; $r=0.27 P<0.001$, systolic BP; $r=0.25 P<0.001$), and (b) diagnostic status (type 2 DM, IGT, normal) and anthropometry (waist/hip; mean 0.95 for type 2 DM, 0.092 for IGT, 0.89 for normal group, all mutually differentiated at $P<0.05$). Lifestyle factors such as level of physical activity and occupation played no significant role in type 2 DM prevalence in the study sample.

Elevated serum lipids (TG; $P<0.002$, TC/HDL; $P<0.038$), HBA 1c; $P<0.001$ and increased intake of dietary fat at the expense of lower carbohydrate intake; $P<0.031$, were associated with progression to type 2 DM.

In conclusion, the prevalence rate for type 2 DM and IGT held no difference among the native Maltese population and subjects of Maltese origin living in the Toronto area.

The 5-year follow up phase of the Toronto study in 1996 revealed a high incidence rate of IGT 10.6% and type 2 DM 6.9% resulting in final prevalence rates of 13.2% for IGT and/or 19.1% for type 2 DM after the original sample aging by 5 years.

These increasing prevalence rates for IGT and DM T2 may suggest an epidemic proportion of diabetes. It should be noted that the subjects with IGT were highly insulin resistant and had higher WHR, and apolipoprotein C3 was higher in IGT subjects as opposed to normal subjects (14.3 mg/dL IGT vs. 11.2 mg/dL IGT, $P<0.002$).

Correlation coefficients between BMI, SFA, or VFA and a number of variables are shown in Table 1. VFA was found to correlate with metabolic parameters characteristic of metabolic syndromes, such as triglyceride, HDL cholesterol and uric acid levels, as well as 2-h post-glucose load glyceremia, insulinemia, and insulin resistance index. BMI showed significant correlation with fasting plasma glucose, HDL cholesterol, uric acid levels, percent of body fat mass, waist and hip circumferences, and VFA and SFA. When groups with matched BMIs were compared, those with higher VFA (more centralized adiposity) showed higher waist circumference, 2-h plasma glucose and insulin levels, glucose AUC, triglyceride and uric acid levels. BMI was correlated with both components of adipose tissue – subcutaneous ($P<0.01$) and VFA ($P<0.02$). In contrast, VFA was correlated with 2-h glucose and insulin levels ($P<0.03$ both), triglycerides, HDL cholesterol and uric acid (5<0.05 all). Subjects with high VFA matched for BMI showed greater plasma glucose AUC (761±133 vs. 589±118 mmol/L, $P<0.03$), 2-h insulin (889±509 IGT vs. 599±387 μU/mL, $P<0.01$), and uric acid levels (0.33±0.07 vs. 0.26±0.06 mmol/L, $P<0.05$) than subjects with low VFA. Variables of interest in the association with MS, e.g., waist circumference, VFA, and 2-h insulin were shown to be independently associated with MS. Using the average AUC values of 107 cm for waist circumference, 162 cm$^2$ for VFA and 90.8 μU/mL for 2-h insulin were found to be the best indicators of metabolic syndromes. Insulin levels and waist circumference were concordant with metabolic syndrome diagnosis in 81% of patients ($P<0.001$ both), whereas the concordance of VFA was 73% ($P<0.006$). Taken into consideration the three variables, the presence of at least two resulted in a degree of concordance of 76% ($P<0.001$).
CONCLUSIONS

According to the ADA diagnostic criteria from 1997 (14-22), impaired glucose homeostasis can be defined not only by 2hBG of 7.8-11.1 mmol/L but also by FBG of 6.1-6.9 mmol/L.

The diversity between IFG and IGT groups involves both insulin secretion and resistance. It has been known that subjects with type 2 diabetes have both β-cell dysfunction and insulin resistance. Defective insulin action is the major identifiable defect in subjects at risk for type 2 diabetes, whereas β-cell dysfunction seems to become abnormal only when FBG is elevated. Nevertheless, defective insulin secretion may be present before the onset of overt diabetes.

The data showed insulin secretion to be defective in IFG as compared with IGT subjects, whereas in the isolated IGT group, impaired insulin sensitivity was more apparent. Defects in insulin resistance or secretion have different effects on fasting and postprandial glucose metabolism. In our study, in fact, subjects with IFG had significantly higher FBG and 2-h insulin levels than IGT patients. Therefore, they would need to secrete more insulin to control their fasting glycemia. Subjects with IGT showed significantly higher 2hBG and 2-h insulin levels than those with IFG (Figs. 1 and 2). They also had significantly higher insulin resistance, as demonstrated by their lower insulin sensitivity. In other words, the excessive insulin secretion of these patients is not sufficient to control their 2hBG. This demonstrates the presence of marked insulin resistance.

Insulin resistance has been shown as a link between intra-abdominal adiposity and metabolic abnormalities. Several lines of evidence have supported a more deleterious role of visceral fat relative to

<table>
<thead>
<tr>
<th>Clinical characteristics of study subjects</th>
<th>NGT</th>
<th>Isolated IFG</th>
<th>Isolated IGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45.5±12.7</td>
<td>49.9±10.2</td>
<td>47.7±10.3</td>
</tr>
<tr>
<td>M/F</td>
<td>310/230</td>
<td>47/30</td>
<td>60/39</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4±2.2</td>
<td>29.8±3.6</td>
<td>32.6±5.8</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87±0.07</td>
<td>0.91±0.09</td>
<td>0.94±0.09</td>
</tr>
<tr>
<td>NBP/HBP</td>
<td>410/130</td>
<td>37/40</td>
<td>79/20</td>
</tr>
<tr>
<td>Systolic (mm Hg)</td>
<td>118±2</td>
<td>130±5</td>
<td>140±8</td>
</tr>
<tr>
<td>Diastolic (mm Hg)</td>
<td>71±2</td>
<td>80±2</td>
<td>84±5</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.3±0.3</td>
<td>6.0±0.7</td>
<td>6.4±0.9</td>
</tr>
<tr>
<td>2-h Glucose (mmol/L)</td>
<td>5.2±0.4</td>
<td>9.1±0.5</td>
<td>13.4±1.2</td>
</tr>
<tr>
<td>Fasting insulin (μU/mL)</td>
<td>10.7±5.6</td>
<td>12.7±6.3</td>
<td>19.8±10.4</td>
</tr>
<tr>
<td>2-h insulin (μU/mL)</td>
<td>54.3±37.9</td>
<td>44.8±21.6</td>
<td>130.1±68.1</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/L)</td>
<td>998±338</td>
<td>1410±415</td>
<td>1598±498</td>
</tr>
<tr>
<td>2h C-peptide (pmol/L)</td>
<td>354±1251</td>
<td>405±1431</td>
<td>579±1832</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5±0.1</td>
<td>5.9±0.1</td>
<td>6.4±0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.28±0.09</td>
<td>1.97±0.24</td>
<td>2.22±0.22</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.09±0.10</td>
<td>5.26±0.10</td>
<td>5.45±0.11</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.28±0.04</td>
<td>1.12±0.03</td>
<td>1.20±0.05</td>
</tr>
<tr>
<td>FFAs (pmol/L)</td>
<td>0.45±0.21</td>
<td>0.51±0.23</td>
<td>0.60±0.24</td>
</tr>
<tr>
<td>Apolipoprotein C3 (mg/dL)</td>
<td>11.2±0.11</td>
<td>12.1±0.11</td>
<td>14.3±0.18</td>
</tr>
<tr>
<td>Abdominal obesity (waist circumference) (cm)</td>
<td>101±12</td>
<td>113.3±11.3</td>
<td>123±12</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>101±14</td>
<td>142.7±60.5</td>
<td>182.3±47.9</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>42.5±5.4</td>
<td>44.1±4.4</td>
<td>49.6±7.4</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>75</td>
<td>26</td>
<td>31</td>
</tr>
</tbody>
</table>

There are no data on subcutaneous fat area (SFA) (Futrax infrared and skinfold caliper system) and area under the curve (AUC) for the analysis of variance (ANOVA).
subcutaneous fat (31-42). A recent study found correlation of the subcutaneous abdominal fat with characteristic features of insulin resistance (42). Thus, the assessment of adipose tissue distribution and its relationship with morbidity is of great interest in order to identify subjects at high cardiovascular risk.

In the present study, VFA was associated with metabolic abnormalities such as reduced HDL cholesterol; elevated triglyceride and uric acid levels; and disturbed insulin sensitivity and markedly elevated insulin resistance. Our findings are in agreement with other reports that emphasize the importance of visceral adipose tissue for the genesis of insulin resistance syndrome. After stratifying subjects according to similar BMI or similar VFA, we found that the discriminatory power of VFA was markedly better than BMI since the former identified patients with worse lipid profile and higher uric acid and worst post-challenge glucose levels, which are well-recognized cardiovascular risk factors. In agreement with the others, our data support the indication of measurements of fat distribution to assess cardiovascular risk.

Our findings in patients with metabolic syndromes, i.e. elevated BMI accompanied by higher VFA and insulin levels, corroborate the hypothesis that insulin may be the factor underlying metabolic abnormalities. Waist circumference, VFA and post-challenge insulin levels were found to be independent predictors of MS. This study showed that a combination of an anatomical measurement, such as waist circumference (high sensitivity) and/or VFA (high specificity) with a metabolic parameter such as 2-h insulin determination improved diagnostic concordance and could be useful to identify subjects at high risk of developing metabolic syndrome.

The findings of the present study also suggest that IFG and IGT subjects represent two distinct populations with altered glucose metabolism. Thus, both FBG and 2hBG are useful diagnostic tools, since their combined use allows for stratification of subjects with impaired glucose homeostasis into three specific subgroups with different metabolic abnormalities: isolated IFG, isolated IGT, and combined IFG/IGT. This distinction may help clinicians in choosing strategies to prevent cardiovascular disease and diabetes progression by introducing early necessary lifestyle changes and therapeutic interventions.

In conclusion, it is meaningful to compute the diagnostic power of FBG, the test recommended by the ADA as a simplified estimate of impaired glucose tolerance. However, the information yielded by FBG and 2hBG is complementary and provides a more complete picture of the patient’s metabolic state, useful in identifying the appropriate treatment. Reliable diagnostic conclusions and clinical decisions can be reached safely if the information on FBG along with 2hBG, visceral fat, adiposity, BMI and insulin sensitivity data are combined together.
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