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Influence of the Gly1057Asp variant of the insulin receptor substrate 2 (IRS2) on insulin resistance and relationship with epicardial fat thickness in the elderly

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ABSTRACT

Insulin receptor substrate 2 (IRS2) plays a crucial role in the regulation of insulin signaling. Several polymorphisms of the gene encoding IRS2 have been identified. The variant causing Gly1057Asp substitution is relatively frequent in humans and its impact on insulin sensitivity seems to be dependent on age and body weight. The aim of our study was to evaluate the relationships between Gly1057Asp variant and insulin sensitivity assessed by HOMA, and adiposity evaluated by measurement of epicardial fat (EpiF) thickness in the elderly. We studied 87 subjects, 42 men and 45 women, mean age ± SD: 74.23 ± 7.24 years. In the subjects carrying the Gly1057Asp variant of the *IRS2* gene we found higher HOMA index values (3.40 ± 1.14 vs. 2.21 ± 1.25, $p<0.001$) and increased epicardial adipose tissue (11.77 ± 1.65 vs. 10.43 ± 1.93 mm, $p<0.001$) compared to wild type subjects. Univariate linear regression analyses evidenced that HOMA index was correlated with BMI (beta = 0.152, $p<0.001$), fasting plasma glucose (beta = 0.018, $p=0.002$), LDL cholesterol (beta = 0.008, $p=0.024$), total cholesterol (beta = 0.007, $p=0.039$), weight (beta = 0.054, $p<0.001$), presence of Gly1057Asp variant (beta = 1.185, $p<0.001$) and EpiF thickness (beta = 0.540, $p<0.001$). In multivariate analysis HOMA index was still associated with the presence of the Gly1057Asp variant of the IRS-2 gene (beta = 0.568, $p=0.002$) and with EpiF thickness (beta = 0.414, $p<0.001$). Furthermore, a statistically significant positive correlation between EpiF thickness and HOMA was found ($r=0.773$, $p<0.001$) and this was not different between wild type control subjects and carriers of Gly1057Asp variant of the *IRS2* gene ($p=0.718$). Similar results were obtained in comparing subjects with normal fasting glucose levels. In conclusion, in our elderly subjects the presence of the allelic variant Gly1057Asp of *IRS2* gene was associated to the degree of insulin resistance assessed by HOMA index and with EpiF thickness, independently from the extent of obesity, suggesting its contribution to global cardiometabolic risk.

1. Introduction

Insulin resistance is a prominent feature of obesity and type 2 diabetes and may be related to mutations in the insulin receptor (IR) or to defects in insulin action at the postreceptor level. IR substrate (IRS) genes coding for key proteins in insulin transduction may be involved, and allelic variants in the insulin receptor substrate gene *IRS2* may play a functional role on insulin resistance (Sun et al., 1995). The connection between insulin resistance, hyperinsulinemia, and coronary heart disease has been established by several transverse, prospective, and experimental studies (Ducimetiere et al., 1980; Welborn and Wearne, 1997).

The crucial role played by IRS2 in the regulation of insulin signaling is widely demonstrated by studies on transgenic animal models (Nandi et al., 2004). Knock-out mice for the *IRS2* gene (−/−) show many features of human type 2 diabetes, including increases in peripheral insulin resistance and non-compensatory expansion of β-cells compartment (Withers et al., 1998, 1999).

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In humans several polymorphisms of the gene encoding IRS2 have been identified, and among these the variant causing Gly1057Asp substitution is frequent (Bodhini et al., 2007; Fritsche et al., 2001; Laukkanen et al., 2004; Ouederni et al., 2009; Stefan et al., 2003).

In a Chinese population and in northern Europe populations the Gly1057Asp variant neither showed association with the common form of type 2 diabetes, nor appeared to affect any clinical and biochemical feature in both glucose-tolerant and type 2 diabetic subjects, and did not seem to have any impact on insulin secretion and insulin sensitivity (Bernal et al., 1998; D'Alfonso et al., 2003; Wang et al., 2001).

On the other hand, in an Italian population the Gly1057Asp variant was found to be associated with a lower prevalence of type 2 diabetes in middle-aged lean subjects, and with a tendency toward an increased prevalence of type 2 diabetes in overweight subjects, and Asp/Asp type 2 diabetic patients exhibited increased fasting C-peptide concentrations, most likely in relationship with lower insulin sensitivity (Mammarella et al., 2000).

The discrepancy among the results of the various studies may be related to regional differences in the distribution of this genotype and to differences in the age of the study populations, suggesting that the potential effect of this *IRS2* gene variant develops as a result of age-related modifications in insulin sensitivity and is not detectable in early adult life (Almind et al., 1999).

The ageing process in humans usually involves the occurrence of a state of insulin resistance, as well as a change in body composition, with increased fat mass (adiposity) at the expense of muscle mass (sarcopenia). Both of these conditions are risk factors for the onset of various metabolic disorders closely related to disability, morbidity and mortality in the elderly (Facchini et al., 2001), and reduced *IRS2* expression may contribute to accelerated atherosclerosis in the metabolic syndrome (González-Navarro et al., 2008).

One of the best predictors of cardiovascular risk is represented by epicardial fat (EpiF) thickness, and transthoracic echocardiography is a reliable methodology to measure the amount of fat surrounding the heart. EpiF stores triglycerides to supply free fatty acids for myocardial energy production, produces adipokines which are disposed directly into the coronary circulation through a mechanism of "vasocrine-signaling," protects coronary arteries against the torsion induced by the arterial pulse wave and cardiac contraction, and facilitates coronary artery remodeling (Iacobellis and Willens, 2009; Sacks and Fain, 2007). EpiF constitutes 20% of total heart weight, covers 80% of the heart's surface and is an index of cardiac and visceral adiposity. Epicardial and abdominal adipose tissues have recently been demonstrated to play inflammatory roles in coronary atherosclerosis and like visceral abdominal fat, EpiF thickness, measured by echocardiography, is increased in obesity (Cheng et al., 2008; Rabkin, 2007). Increased waist circumference is a surrogate measure of visceral abdominal obesity and a marker of an adverse metabolic profile associated with high cardiovascular risk, but may be confounded by large amounts of subcutaneous fat, particularly in severely obese people. On the other hand, waist circumference seems to quantify subcutaneous fat better than visceral fat, and might be a not reliable measure in older individuals, so that a more reliable and affordable measure of visceral obesity would be useful in epidemiological studies and clinical settings (Iacobellis et al., 2005; Seo et al., 2009). The gold standard technique to accurately measure visceral adiposity is represented by whole body magnetic resonance imaging, but clinical imaging studies have demonstrated a strong direct correlation between epicardial adipose tissue and abdominal visceral adiposity and the reliability of direct measurement of EpiF thickness via echocardiography as a marker for visceral adiposity (Iacobellis et al., 2003a,b).

In a previous study in old-aged subjects we have validated EpiF thickness measurement by transthoracic echocardiography and abdominal fat thickness measurement by dual-energy X-ray absorptiometry (DEXA), and we have found a strong linear correlation between the two techniques (Stramaglia et al., 2010).

Autopsy studies have evidenced that epicardial fat increases until age 20–40 years and the amount of epicardial fat seems not dependent on age (Corradi et al., 2004; Fei et al., 2010), but echocardiographic studies have evidenced a strong linear relationship between EpiF thickness and age (Mazzoccoli et al., 2012).

The aim of our study was to investigate the relationships between the presence of the polymorphism Gly1057Asp of *IRS2* gene, insulin sensitivity, and thickness of epicardial adipose tissue in a population of elderly subjects.

2. Methods

2.1. Sample Size

A total sample of 86 subjects was considered necessary to detect an effect size of 0.80 (large effect size) with an alpha level of 0.025 (which accounts for the two endpoints to analyze, i.e. HOMA index and epiF thickness, and keeps the overall alpha level to 0.05) and a statistical power greater than 0.90.

2.2. Subjects and biochemical parameters

A total of 87 consecutive subjects, 45 men (51.7%) and 42 women (48.3%), were recruited from the Division of Internal Medicine, IRCCS Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy. The subjects had one of the four listed reasons for consulting the Division of Internal Medicine: gastroesophageal reflux disease, peptic ulcer disease, irritable bowel syndrome, or subacute and chronic low back pain. The study was approved by the local Scientific and Ethical Committee, the enrolled subjects gave their written informed consent and the investigation conformed to the principles outlined in the Declaration of Helsinki. Clinical history was obtained from all subjects, including age, gender, personal medical history, drug use, drug abuse and addiction, smoking and alcohol consumption, physical exercise. Subjects were rejected for inclusion if they had either a diagnosis or evidence upon physical examination of cardiovascular disease, overt diabetes mellitus (defined at baseline as fasting glucose levels of 126 mg/dl or higher, nonfasting glucose levels of 200 mg/dl or higher, or a history of or treatment for diabetes), arterial hypertension (defined as systolic arterial pressure >140 mmHg and diastolic arterial pressure >90 mmHg or a history of or treatment for arterial hypertension), psychiatric disorder, cancer, infectious disease, surgical procedures in the last 180 days, hypo- or hyperthyroidism, liver, kidney or heart failure or condition of being chronically ill and did not refer pharmacological therapy or treatment with chemotherapy or hormonal agents.

In all enrolled subjects weight in kilograms and standing height in centimeters were measured at the clinic examination by standard protocols. Level of overweight was measured by body mass index (BMI).

Blood pressure was measured using the same protocol. Three measurements were taken with a random-zero sphygmomanometer, and the mean of the last 2 of 3 measurements was used. In all enrolled subjects systolic and diastolic arterial pressure values were recorded and a venous blood sample was drawn after an overnight fast for the determination of plasma glucose, serum insulin and lipid parameters (total, LDL and HDL cholesterol, triglycerides). Plasma glucose, total cholesterol (mg/dl) and triglyceride levels (mg/dl) were measured by enzymatic methods and LDL cholesterol (mg/dl) was calculated indirectly using the Friedewald's equation. HDL cholesterol was measured after precipitation of the other lipoprotein fractions by dextran sulfate.

For insulin determination we used a two-site immunoenzymometric assay (ST AIA-PACK IRI, Tosoh Bioscience Inc., 6000 Shoreline Ct., Suite 101, South san Francisco, CA, USA) with a cross-reactivity of 2% for human proinsulin and no cross-reactivity for human C-peptide. Reference