Abstract

Aims/hypotheses. Insulin resistance is thought to be a key predictor for the development of Type 2 diabetes mellitus and cardiovascular disease (CVD), a leading cause of morbidity and premature mortality in Europe. Insulin resistance is influenced by both genetic and lifestyle factors (e.g. obesity and physical inactivity). The RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) Study is using the infrastructure of an extended European collaborative research group to study insulin resistance and CVD risk in 1500 healthy people aged 30 to 60 years from 20 centres in 13 countries.

Methods. Baseline measurements of glucose tolerance and insulin sensitivity are made by the oral glucose tolerance test and the euglycaemic insulin clamp, respectively; carotid artery intima-medial thickness (by ultrasound), ankle/brachial pressure index and electrocardiography will enable evaluation of subclinical CVD at baseline and at follow-up. Classic CVD risk factors, as well as socioeconomic and lifestyle factors will be recorded at baseline; samples for measurement of biochemical and genetic markers will be collected and stored for future analyses. Investigations will be repeated after 3 and 10 years to evaluate the relationship between insulin resistance and the development of atherosclerosis as measured by carotid artery intima-media thickness. Development of Type 2 diabetes, dyslipidaemia, obesity, hypertension and cardiovascular events are additional endpoints.

Conclusions. This study will evaluate the importance of insulin resistance in the development of CVD and diabetes, and has implications for the development of prevention and treatment strategies. [Diabetologia (2004) 47:566–570]

Keywords Atherosclerosis · Cardiovascular disease · Diabetes · Epidemiology · Insulin resistance syndrome · Metabolic syndrome · Methodology

Background

It has been hypothesised [1] that insulin resistance per se may be atherogenic. Conclusive evidence for the direct role of insulin resistance as a cardiovascular (CVD) risk factor is, however, lacking. The San Antonio Heart Study first linked hyperinsulinaemia with the incidence of all three of diabetes, hypertension and dyslipidaemia [2]. Subsequent prospective studies [3, 4, 5] have reported an association between insulin resistance and incident CVD, but none of these studies included direct measures of insulin resistance and, in one of them [5], the association was lost when adjusted for established CVD risk factors. Hyperinsuli-
naemia is the compound physiologic output of insulin resistance, enhanced insulin secretion, and reduced insulin clearance [6]. Furthermore, hyperinsulinaemia may have pathophysiologic effects distinct from those of insulin resistance proper [7, 8]. In the only published study that used a direct measure of insulin resistance (the intravenous glucose tolerance test [IVGTT]-minimal model) in 1397 subjects [9], higher levels of insulin sensitivity were associated with less atherosclerosis in Hispanic and non-Hispanic white subjects but not in black subjects. This difference was partially mediated by lower levels of traditional cardiovascular risk factors.

The European Group for the Study of Insulin Resistance (EGIR) originally collaborated to pool data from euglycaemic insulin clamp studies performed at 21 centres across Europe in non-diabetic subjects of any age and BMI. This dataset made it possible to analyse the relation of insulin resistance to the principal components of the metabolic syndrome, namely age [10], BMI and fat distribution [11], arterial blood pressure [12] and serum lipid concentrations [13]. Additional information concerned the influence of family history of diabetes on insulin sensitivity [14], the relationship between peripheral insulin action and hepatic glucose production [15] and the impact of insulin resistance on lipolysis [16] and thermogenesis [17]. The main limitations of the EGIR database were its cross-sectional nature, and lack of standardised protocols and centralised laboratory analyses. Thus, there is a need for a prospective evaluation of insulin resistance as an independent CVD risk factor.

**Objectives**

The RISC Study is the logical expansion of the EGIR studies: pan-European, prospective, with standardised protocols and centralised evaluation of measures. In addition it features measurements of carotid artery intima-medial thickness (cIMT) and genetic and lifestyle factors (body composition and physical activity). The three principal objectives are: (i) to establish whether insulin resistance predicts deterioration of CVD risk markers, diabetes, obesity, atherosclerosis and CVD; (ii) to determine genetic and environmental contributions to insulin resistance and CVD; (iii) to develop a method based on mathematical modelling to identify insulin resistant subjects in clinical practice.

**Protocol and methodology**

RISC is a prospective (3- and 10-year follow-up), observational, cohort study. Participants are recruited from the local population, according to specific inclusion criteria (Table 1). The population is stratified by sex and by age according to 10-year age groups. Base-

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**Table 1. Inclusion and exclusion criteria**

<table>
<thead>
<tr>
<th><strong>Inclusion criteria</strong></th>
<th><strong>Initial exclusion criteria</strong></th>
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<tbody>
<tr>
<td>clinically healthy</td>
<td>treatment for obesity, hypertension, lipid disorders, diabetes pregnancy</td>
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<tr>
<td>aged between 30 and 60 years</td>
<td>chronic treatment</td>
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<tr>
<td>available for follow-up in the next 10 years</td>
<td>chronic lung disease</td>
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<td></td>
<td>cancer (in last 5 years)</td>
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<td>kidney failure, kidney dialysis or transplant</td>
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<td></td>
<td>recent major surgery</td>
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<td></td>
<td>seizure disorder or epilepsy</td>
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<td></td>
<td>inability to give informed consent</td>
</tr>
</tbody>
</table>

**Exclusion criteria after clinical examinations**

- systolic/diastolic blood pressure ≥140/90 mmHg or treatment
- fasting plasma glucose ≥7.0 mmol/l (126 mg/dl) or treatment
- 2h plasma glucose ≥11.1 mmol/l (200 mg/dl) or treatment
- total cholesterol ≥7.8 mmol/l (300 mg/dl) or treatment
- triglycerides ≥4.6 mmol/l (400 mg/dl) or treatment
- ECG abnormalities
- acute myocardial ischaemia injury or pericarditis
- poor ultrasound imaging of carotid artery

**Ethical considerations.** Before starting the study local ethics committee approval is obtained by each recruiting centre. Volunteers are given detailed written information on the study as well as an oral explanation. Written consent is required for the RISC Study itself and separate consent is obtained for the genetic analyses.

**Lifestyle and medical history questionnaire.** Information is collected on socioeconomic status of the participant and their partner, personal medical history and family history of CVD, stroke, hypertension and diabetes in first-degree relatives, as well as information on body shape of family members, smoking and alcohol habits, and physical activity. All medication is recorded, prescribed (including hormone replacement therapy and oral contraceptive), as well as self medication such as vitamin supplements and homeopathy. A modified version of the Rose questionnaire [18] and the Edinburgh claudication questionnaire [19] are used for exclusion. The lifestyle questionnaire was prepared in English, translated into the 11 languages of the study and back-translated into English to ensure homogeneity.

**Physical examinations.** Height is measured on a clinic stadiometer. Body weight, BMI, percent body fat and fat-free mass are evaluated by the TANITA bioimpe-
dance balance (Tanita International Division, UK); waist, hip and thigh circumferences are measured by tape measure according to a standardised written protocol. Sitting blood pressure and heart rate are measured three times (OMRON 705 cp, OMRON Healthcare Europe, The Netherlands). A resting 12-lead ECG is recorded for central coding.

**Ankle:brachial pressure index.** Two measures are taken at the brachial artery, and pedal and tibial arteries for calculation of the systolic pressure ratio.

**Biological samples.** Fasting blood samples are taken for exclusion criteria (Table 1) before and during a 75 g OGTT, together with samples for central analysis of glucose, insulin and C-peptide. Urine is tested with Multistix 10 (Bayer, Leverkusen, Germany) and samples are frozen for central measurement of albumin:creatinine ratio.

Blood collected during the studies is separated into plasma and serum, aliquoted and stored at −20°C for glucose and glucagon, and −80°C for lipids and NEFA. Serum aliquots are stored at −80°C for insulin, C-peptide and insulin split products; urine samples are stored at −20°C. Samples are transported on dry ice at pre-arranged intervals to central laboratories.

Extra aliquots of serum and plasma from the OGTT and clamp are stored for future analyses of inflammatory markers, haemostatic factors and other research questions.

**Insulin sensitivity and beta-cell function.** Insulin sensitivity and beta-cell function will be estimated from the OGTT and the euglycaemic insulin clamp data according to validated mathematical models [20, 21].

**Euglycaemic insulin clamp.** The target plasma glucose concentration is between 4.5 and 5.5 mmol/l; insulin is infused at a rate of 240 pmol·min$^{-1}$·m$^{-2}$. Bedside plasma (or blood) glucose is measured at 5- to 10-min intervals to ensure it remains within 0.8 mmol/l (±15%) of the target glucose concentration. The steady-state period (for calculation of insulin sensitivity) is between 80 to 120 min.

**Measures of physical activity.** Each participant is fitted with a CSA Actigraph (MTI: Manufacturing Technology, Fort Walton Beach, Fla., USA) attached to a waist belt for 1 week. The Actigraph is a small (43 g) single-channel recording accelerometer capable of continuous data collection for up to 22 days. Data are summed over 1-min periods and processed to evaluate energy expenditure over the recording period, and periods of moderate and intense activity [22].

Qualitative information on physical activity is collected with the 7-day International Physical Activity Questionnaire (IPAQ), a previously validated assessment tool for international studies [23].

**Genetic analyses.** Two 4-ml EDTA whole blood samples are collected for centralised leukocyte DNA extraction by a kit-based method. Genotyping is done by PCR amplification and variant detection by using a variety of standard techniques (e.g. restriction digest, direct sequencing, etc). Once the DNA is extracted it is subject to quality control, catalogued and stored.

Candidate genes implicated with development of insulin sensitivity (e.g. **TNF-alpha**, **IRS-1**, and **PPAR-gamma**) and those implicated in the development of cardiovascular disease (e.g. **Lp(a)**, **ACE**, and **PAI-1**) are genotyped. As new candidate genes emerge they will be genotyped. The importance of specific genotypes is being assessed in relation to lifestyle determinants of insulin resistance and cardiovascular disease.

**Follow-up.** Annual telephone follow-up will correct addresses, schedule examinations and ask about medical treatment since the last visit as well as changes and additions to medication.

After 3 years selected examinations will be repeated: cIMT will be recorded to examine development of atherosclerosis; progression to diabetes will be assessed by OGTT; obesity will be measured by standard criteria. Ankle:brachial pressure index will measure progression of peripheral artery disease and biological variables such as lipids will be examined. The lifestyle questionnaire will be repeated to record changes in medical status, smoking and alcohol habits and prescribed and non-prescribed medication. Hospital records with a diagnosis of CVD will be reviewed.
Death certificates will be abstracted for the underlying causes of death.

Data analysis and management. Study documentation is accessed from a website, where recruitment information is updated weekly (password protected). Data are entered into the program Epi Info 2002 (Centres for Disease Control, Atlanta, Ga., USA) at the recruiting centres and sent by e-mail as an Excel file to the coordinating office or via the website data transfer system. Data are maintained centrally on a computer with restricted access and with back-up.

The participant’s name and address is kept only at the recruiting centre, documents identifying the participant are stored separately from the study data. Data are identifiable only by a 9-digit code and the coordinating centre only receives the coded information. Identification codes are used only as long as necessary to maintain confidentiality.

Power calculations. The primary endpoint is the change in cIMT. From currently published studies [24], this change is at least 0.01 mm over 1 year, with a standard deviation of 0.06 mm; over 3 years a mean change of at least 0.03 mm and standard deviation below 0.11 mm is estimated. To detect a difference of 0.03 mm in the mean cIMT a change between the insulin resistant subjects (in the lower 20% of the insulin sensitivity distribution) and the remainder of the population, with an alpha error=0.05 and power=0.80 and a two-sided test, 1500 subjects will be adequate (or a one-sided test in 1200 subjects, which is adequate in the case of drop outs). The secondary endpoint of deterioration in CVD risk factors (e.g., blood pressure, lipids, glucose metabolism, body composition) requires fewer subjects, so this endpoint will also be tested.

Comments

The main advantages of the RISC Study are: (i) a large sample of Europeans, (ii) gold-standard methodology for measurement of insulin sensitivity and cIMT, (iii) state-of-the-art measurement of physical activity, (iv) availability of DNA samples, (v) centralised laboratory assays, (vi) centralised continuous quality control of data.

Results will include testing whether insulin resistance is an independent risk factor for CVD and Type 2 diabetes, confirming that insulin resistance is a predecessor of the intermediate phenotypes (obesity, dyslipidaemia, hypertension) and assessing the influence of key genetic and environmental factors (Fig. 1). Additionally, an estimate will be provided of insulin resistance and secretion from parameters measured by clinic based methods.
Further information on the RISC project and participating centres can be found on http://www.egir.org.

References