Diabetologia (2004) 47:566–570
DOI 10.1007/s00125-004-1335-5

Diabetologia

Short Communication

The EGIR-RISC STUDY (The European group for the study of insulin resistance: relationship between insulin sensitivity and cardiovascular disease risk): I. Methodology and Objectives

S. A. Hills[1] · B. Balkau[2] · S. W. Coppack[3] · J. M. Dekker[4] · A. Mari[5] · A. Natali[1] · M. Walker[6] · E. Ferrannini[1] · Report prepared on behalf of the EGIR-RISC Study Group

[1] EGIR-RISC Coordinating Office, Department of Internal Medicine, University of Pisa School of Medicine, Pisa, Italy
[2] INSERM U 258, Villejuif, France
[3] Academic Medical Unit, The Royal London Hospital, London, UK
[4] EMGO Institute, Vrije University of Amsterdam, Amsterdam, The Netherlands
[5] CNR Institute of Biomedical Engineering, Padova, Italy
[6] Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

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

Received: 30 September 2003 / Revised: 16 December 2003
Published online: 14 February 2004

E. Ferrannini (✉)
EGIR-RISC Coordinating Office, Department of Internal Medicine, University of Pisa School of Medicine, Via Roma, 67, 56100 Pisa, Italy
E-mail: egirpisa@tin.it

Abbreviations: CVD, Cardiovascular disease · cIMT, carotid intima-medial thickness · CCA, common carotid arteries · CB, carotid bifurcation · ICA, internal carotid artery

See the Acknowledgements for the full list of members of the EGIR-RISC Study Group

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. Baseline examinations began in June 2002 and will continue through spring 2004.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
clinically healthy
aged between 30 and 60 years
available for follow-up in the next 10 years
Initial exclusion criteria
treatment for obesity, hypertension, lipid disorders, diabetes
pregnancy
cardiovascular disease
weight change 5 kg or more in last month
steroid treatment
chronic lung disease
cancer (in last 5 years)
kidney failure, kidney dialysis or transplant
recent major surgery
seizure disorder or epilepsy
inability to give informed consent
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 $pmol \cdot min^{-1} \cdot m^{-2}$. 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].

Ultrasound examination of extracranial carotid arteries. Carotid artery intima-media imaging follows a validated protocol [24]. Longitudinal B-mode image is taken of the right and left common carotid arteries (CCA), carotid bifurcation (CB), and internal carotid artery (ICA) from anterior, lateral and posterior angles. The image is recorded on videotape and sent to the reading centre for evaluation by a single reader blinded to the identity of the participant and centre. Diastolic frames of CCA, CB and ICA are selected to provide images of the near and far wall intima-media complex. Frames are digitised and analysed by an image analysis system (MIP: Institute of Clinical Physiology, CNR, Pisa, Italy [25]). Lines are drawn along the lumen-intimal and medial-adventitial interfaces, and the IMT is computed as an average of several measurements. A 30-s longitudinal B-mode image is made of the right CCA and concomitant blood pressure is measured using a sphygmomanometric cuff. An automatic contour detection algorithm determines the average minimum and maximum CCA diameter. Indices of arterial compliance, pressure-elastic modulus and Young's elastic index are calculated. Quality is controlled by performing repeated scans at regular intervals.

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.

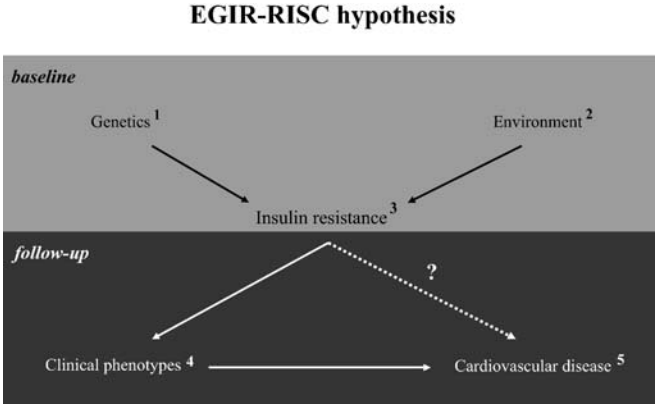

Fig. 1. Schema of the main hypothesis tested by the EGIR-RISC Study. At baseline (*lighter background*), information is obtained on genetic factors (*1* = DNA collection) and relevant environmental factors (*2* = obesity and physical activity) and insulin resistance is measured (*3* = insulin clamp). At follow-up (*darker background*), changes in carotid atherosclerosis (*4* = cIMT) are related to insulin resistance and/or clinical phenotypes (*5* = diabetes, dyslipidaemia, hypertension)

Acknowledgements. The RISC Study is partly supported by EU grant QLG1-CT-2001-01252. Additional support for the RISC Study has been provided by AstraZeneca (Sweden). Dr. R. Catalini (Ancona, Italy) is thanked for his advice and help in preparing the carotid ultrasound protocol. The EGIR Group is supported by Merck Santé (France).

EGIR-RISC Study Group

Project Management Board: B. Balkau, S.W. Coppack, J.M. Dekker, E. Ferrannini, A. Mari, M. Walker.

RISC Recruiting centres

Amsterdam, The Netherlands: R.J. Heine, J. Dekker, G. Nijpels, W. Boorsma. *Athens, Greece*: A. Mitrakou, S. Liatis, K. Kyriakopoulou. *Belgrade, Yugoslavia*: N. Lalic, K. Lalic, A. Jotic, L. Lukic, M. Civcic. *Dublin, Ireland*: J. Nolan, T.P. Yeow, M. Murphy, C. DeLong, G. Neary, M.P. Colgan. *Frankfurt, Germany*: T. Konrad, H. Böhles, S. Fuellert, F. Baer, H. Zuchhold. *Geneva, Switzerland*: A. Golay, V. Barthassat, V. Makoundou, T.N.O. Lehmann, E. Harsch Bobbioni, T. Merminod. *Glasgow, Scotland*: J. Petrie, C. Perry, F. Neary, C. MacDougall, K. Shields, L. Malcolm. *Kuopio, Finland*: M. Laakso, U. Salmenniemi, A. Aura, R. Raisanen, U. Ruotsalainen, T. Sistonen, M. Laitinen. *London, England*: S. Coppack, P. McIntosh, G. Hood, P. Khadobaksh. *Lyon, France*: M. Laville, A. Brac de la Perriere, C. Louche-Pelissier, C. Maitrepierre, J. Peyrat, A. Serusclat. *Madrid, Spain*: R. Gabriel, E.M. Sánchez, R. Carraro, A. Friera, B. Novella. *Malmö, Sweden (1)*: P. Nilsson, M. Persson, G. Östling, *(2)*: O. Melander, P. Burri. *Milan, Italy*: P.M. Piatti, L.D. Monti, E. Setola, F. Minicucci, A. Colleluori. *Newcastle-upon-Tyne, England*: M. Walker, I.M. Ibrahim, D. Carman, Y. McGrady, D. L. Richardson. *Odense, Denmark*: H. Beck-Nielsen, P. Staehr, K. Hojlund, V. Jensen, C. Olsen. *Perugia, Italy*: G.B. Bolli, F. Porcellati, C. Fanelli, M. Romolini, F. Calcinaro, A. Saturni. *Pisa, Italy*: E. Ferrannini, A. Natali, E. Muscelli, S. Pinnola, M. Kozakova. *Rome, Italy*: G. Mingrone, P. Di Rocco, A. Favuzzi. *Vienna, Austria*: W. Waldhäusl, M. Roden, C. Anderwald, A. Hofer.

Core laboratories and reading centres: *Lipids (Dublin, Ireland)*: J. Nolan, P. Gaffney. *Hormones (Odense, Denmark)*:

H. Beck-Nielsen, C. Olsen, L. Hansen. *Albumin:creatinine (Amsterdam, The Netherlands)*: J. Dekker, A. Kok; *Genetics (Newcastle-upon-Tyne, UK)*: M. Walker. *Stable glucose isotope (Pisa, Italy)*: A. Gastaldelli, D. Ciociaro. *Ultrasound reading centre (Pisa, Italy)*: E. Ferrannini, M. Kozakova. *ECG reading centre (Amsterdam, The Netherlands)*: J. Dekker, G. Nijpels.

Coordinating office: *Pisa, Italy*, E. Ferrannini, S.A. Hills, L. Landucci.

Data management: *Villejuif, France*, B. Balkau, L. Mhamdi.

Mathematical modelling and website management: *Padova, Italy*, A. Mari, G. Pacini, C. Cavaggion.

Further information on the RISC project and participating centres can be found on http://www.egir.org.

References

1. Reaven GM (1988) Banting Lecture. Role of insulin resistance in human disease. Diabetes 37:1595–1607
2. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP (1992) Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes 41: 715–722
3. Isomaa B, Almgren P, Tuomi T et al. (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 24:683–689
4. Bonora E, Formentini G, Calcaterra F et al. (2002) HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. Diabetes Care 25:1135–1141
5. Resnick HE, Jones K, Ruotolo G, Jain AK, Henderson J, Lu W, Howard BV, Strong Heart Study (2003) Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic American Indians: the Strong Heart Study. Diabetes Care 26:861–867
6. Laakso M (1993) How good a marker is insulin level for insulin resistance? Am J Epidemiol 137:959–965
7. Weyer C, Hanson RL, Tataranni PA, Bogardus C, Pratley RE (2000) A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: evidence for a pathogenic role of relative hyperinsulinemia. Diabetes 49:2094–2101
8. Ferrannini E, Balkau B (2002) Insulin: in search of a syndrome. Diabetic Med 19: 724–729
9. Howard G, O'Leary DH, Zaccaro D et al. (1996) Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Circulation 93: 1809–1817
10. Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U on behalf of EGIR (1996) Insulin action and age. Diabetes 45:947–953
11. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G on behalf of EGIR (1997) Insulin resistance and hypersecretion in obesity. J Clin Invest 100:1166–1173
12. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Jrvinen H on behalf of EGIR (1997) Insulin resistance, hyperinsulinemia, and blood pressure. Role of age and obesity. Hypertension 30:1144–1149
13. Baldeweg SE, Golay A, Natali A, Balkau B, Del Prato S, Coppack SW on behalf of EGIR (2000) Insulin resistance, lipid and fatty acid concentration in 867 healthy Europeans. Eur J Clin Invest 30:45–52
14. Vaag A, Lehtovirta M, Thye-Ronn P, Groop L (2001) Metabolic impact of a family history of type 2 diabetes. Results of a European multicentre study (EGIR). Diabetic Med 18:533–540
15. Natali A, Toschi E, Camastra S, Gastaldelli A, Groop L, Ferrannini E, on behalf of EGIR (2000) Determinants of post-absorptive endogenous glucose output in non-diabetic subjects. Diabetologia 43:1266–1272
16. Ferrannini E, Camastra S, Coppack SW, Fliser D, Golay A, Mitrakou A on behalf of EGIR (1997) Insulin action and non-esterified fatty acids. Proc Nutrition Soc 56:753–761
17. Camastra S, Bonora E, Del Prato S, Rett K, Weck M, Ferrannini E, on behalf of EGIR (1999) Effect of obesity and insulin resistance on resting and glucose-induced thermogenesis in man. Int J Obes 23:1307–1313
18. Rose GA, Blackburn H, Gillum RF, Prineas RJ (1982) Cardiovascular survey methods. World Health Organization, Geneva
19. Leng GC, Fowkes FG (1992) The Edinburgh Claudication Questionnaire: an improved version of the WHO/Rose Questionnaire for use in epidemiological surveys. J Clin Epidemiol 45:1101–1109
20. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ (2001) A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 24:539–548
21. Mari A, Tura A, Gastaldelli A, Ferrannini E (2002) Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. Diabetes 51 [Suppl 1]:S221–S226
22. Brage S, Brage N, Franks PW et al. (2004) Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure. J Appl Physiol 96:343–351
23. Craig CL, Marshall AL, Sjostrom M et al. (2003) International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35:1381–1395
24. Mercuri M, McPherson DD, Bassiouni H, Glagov S (1998) Non-invasive imaging of atherosclerosis. Kluwer, The Netherlands
25. Mazzone AM, Urbani MP, Picano E et al. (1995) In vivo ultrasound parametric imaging of carotid atherosclerotic plaque by videodensitometric technique. Angiology 46: 663–672