Title: Lipid and inflammatory biomarker profiles in early insulin resistance

Itahisa Marcelino Rodríguez,^{1,2} José Gregorio Oliva García,³ José Juan Alemán Sánchez,^{1,2,4} Delia Almeida González,^{2,5} Santiago Domínguez Coello,^{2,4} Buenaventura Brito Díaz,^{1,4} Fadoua Gannar,⁶ María del Cristo Rodríguez Pérez,^{1,4} Roberto Elosua,^{7,2} and Antonio Cabrera de León,^{1,3,8}.

¹Research Unit, ³Endocrine Diseases Unit, and ⁵Immunology Unit, of the University Hospital Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, Spain

²Cardiovascular Research Network of the Carlos III Institute of Health, Spain

⁴Primary Care Authority, Santa Cruz de Tenerife, Spain

⁶Faculty of Sciences of Bizerte, Carthage University, Tunis, Tunisia

⁷Cardiovascular Epidemiology and Genetics, IMIM. Barcelona, Spain

⁸University of La Laguna, La Laguna, Spain

***Correspondence to:** Dr. Antonio Cabrera de León. Unidad de Investigación, Hospital de La Candelaria. Carretera de El Rosario 145, 38010 Santa Cruz de Tenerife, Canary Islands, Spain. Email: acableo@gobiernodecanarias.org; Tel: +34 922 600 602; Fax: +34 922 600 562

Word count: Abstract 160, text 2761.

Abstract

Aims: To analyze the serum lipid and inflammatory biomarker profile in the early insulin resistance (e-IR).

Methods: Cross-sectional study of 5,943 adults without diabetes, stratified into no IR group (C-peptide <third tertile and glucose <100 mg/dL), e-IR group (C-peptide ≥third tertile and glucose <100 mg/dL), and advanced IR group (glucose ≥100 mg/dL).

Results: E-IR showed significant differences with no IR in the serum concentration of triglycerides ($P < 0.001$), HDL-cholesterol ($P < 0.001$), LDL-cholesterol ($P < 0.001$), sCD40L ($P < 0.001$), C-reactive protein ($P < 0.004$), leptin ($P < 0.001$) and adiponectin ($P < 0.001$). Adjusting for age, gender and abdominal obesity, corroborated the association of e-IR with highest-quintile of triglycerides (OR=3.88 [3.07-4.89]), HDL-cholesterol (OR=0.35 [0.28-0.44]), sCD40L (OR=0.47 [0.24-0.94]), C-reactive protein (OR=2.31 [1.29-4.12]), adiponectin (OR=0.11 [0.04-0.32]), PAI-1 (OR=3.29 [1.29-8.40]) and resistin (OR=1.25 [1.01-1.54]); the same biomarkers were associated with advanced IR although resistin was a protective factor (OR=0.73 [0.58-0.93]).

Conclusions: Euglycemic patients with e-IR present an unfavorable serum lipid and inflammatory biomarker profile. Measuring C-peptide in euglycemic patients with elevated triglycerides identifies e-IR.

Key words: Insulin resistance. C-peptide. Biomarkers. Population-Based Studies. Epidemiology.

Introduction

Type 2 diabetes mellitus is currently believed to be the result of insulin resistance (IR) combined with the loss of pancreatic β cell mass and functioning [1]. Insulin resistance appears early, before glucose intolerance becomes detectable, and involves lipid and protein metabolism imbalances along with mineral and electrolyte alterations [2-4]. The IR state is seen together with visceral obesity [5], ectopic fat deposits in the muscle, liver and pancreatic islets [6, 7], hypertension, dyslipidemia, endothelial dysfunction, elevated levels of plasminogen activator inhibitor-1 (PAI-1), and ferritin [8,9]. In addition, the accumulation of adipose tissue increases the secretion of cytokines and adipokines such as tumor necrosis factor-alpha, interleukin-6, leptin and resistin, among others, which can be cytotoxic for β cells [10]. These alterations give rise to an inflammatory environment with overexposure to acute phase proteins such as C-reactive protein (CRP) [11]. As IR progresses, hyperglycemia can ensue, leading to the production of oxygen reactive species which further potentiate inflammation [12]. When diabetes develops, there are numerous inflammatory cytokines capable of detecting IR and predict the fasting plasma glucose [13].

Because of these alterations, patients who develop type 2 diabetes mellitus have an increased risk of developing cardiovascular disease, which in turn may involve severe complications including myocardial infarction and stroke [14]. However, the increased cardiovascular morbidity and mortality associated with IR is also present even before type 2 diabetes mellitus per se appears [15]. This makes IR itself a good predictor of the cardiovascular events noted above [16]. In fact, earlier work has found that by classifying the population according to serum C-peptide and glucose concentration, it is possible to identify the early stage of IR, when the risk of cardiovascular disease is already elevated in the absence of hyperglycemia [17].

The aim of this study was to determine the serum lipid and inflammatory biomarker profile in a sample of people in the general population who do not have type 2 diabetes mellitus and whose serum glucose concentration is within the normal range, but who do have early IR.

Methods

Participants and design

The participants in this cross-sectional study were drawn from the *CDC de Canarias* cohort study. (The CDC acronym stands from Cardiovascular, Diabetes and Cancer.) Members of this cohort were randomly chosen during the years 2000 to 2005 from the general population of the Canary Islands (Spain), and ranged in age from 18 to 75 years. The methodology has been reported previously [18]. All participants recruited for the present analysis (n=6,729) were interviewed with the aid of a questionnaire designed to record information on their health-related antecedents (available at <http://www.cdcdecanarias.org>). In addition, all underwent physical examination to record anthropometric data and vital signs, and all provided a sample of venous blood after abstaining from food or drink for at least 10 hours. Blood samples were centrifuged *in situ* at room temperature and 2,000 rpm for 10 min, and transported daily to the laboratory. This study was approved by the Bioethics Committee of Nuestra Señora de la Candelaria University Hospital, and all participants provided their informed consent in writing.

Variables

Serum glucose and lipid concentrations (triglycerides, total and high-density lipoprotein [HDL] cholesterol) were determined within 24 hours after the blood sample was obtained, and are reported here in mg/dL. Low-density lipoprotein (LDL) cholesterol was calculated with Friedewald's formula (excluding cases with triglycerides >300 mg/dL), and non-HDL cholesterol was recorded by subtracting HDL cholesterol from total cholesterol. Serum samples were stored at -80 °C, and an aliquot was thawed and used to measure C-peptide (Biosource®, ng/mL) and resistin (Bio-Vendor®, ng/mL) with enzyme immunoanalysis techniques for the entire study population. A subset of randomly selected samples from a smaller number of individuals for the DARIOS Inflammatory Study [19] was used to determine insulin, leptin, PAI-1 and adiponectin concentrations with Luminex xMAP assays, CRP with a highly sensitive immunoturbidimetric assay (HORIBA-ABX Diagnostics) and soluble CD40 ligand (sCD40L) with an enzyme-linked immunosorbent assay.

Abdominal obesity was defined as a waist/height ratio of ≥ 0.55 [20]. Individuals with type 2 diabetes mellitus were excluded if they stated having the disease and were taking anti-diabetes medication, or if they were unaware of having the disease but had a fasting serum glucose concentration of ≥ 126 mg/dL. In these cases a second glucose measurement was obtained to verify the result.

To identify participants with IR, serum C-peptide and glucose concentrations were considered [17], and C-peptide values in the upper tertile (T_3) were assumed to indicate exposure to elevated levels [21-23]. The population was classified into three IR groups: Group 1 (individuals with no IR: blood glucose < 100 mg/dL and C-peptide $< T_3$), Group 2 (individuals with early IR: blood glucose < 100 mg/dL and C-peptide $\geq T_3$) and Group 3 (individuals with advanced IR: hyperglycemia defined as serum glucose concentration > 100 mg/dL after a 10-hour fast).

Statistical analysis

Normally distributed variables are reported as summary statistics (mean $\pm$ standard deviation); log natural transformation (LN) was used when needed to approximate the variable to the normal distribution. To compare mean values for the three IR groups, analysis of variance was used, with the Bonferroni test for post-hoc comparisons. For variables that failed to approximate a normal distribution, the nonparametric Kruskal–Wallis test was used, and we show the median values together with 25th and 75th percentiles.

Categorical variables are reported as relative frequencies and absolute frequencies for each category. Chi square test were used to compare proportions between groups.

The associations identified with bivariate analysis between IR categories and biomarker results were adjusted for age, gender and abdominal obesity with multivariate models for ordinal regression and multinomial logistic regression. For ordinal regression models each biomarker (LN) was used as an independent continuous variable, and the three IR categories were used as a dependent ordinal variable. Goodness of fit was determined and regression coefficients were calculated with 95% confidence intervals (95% CI) and statistical significance. For multinomial models the independent variable

(biomarker) was categorized in quintiles to estimate the risk (odds ratio, OR), in each quintile, of early or advanced IR compared to the first quintile, used here as the reference category. Goodness of fit was determined, and we show the OR (95% CI) and statistical significance for the fourth and fifth quintiles of each biomarker. The differences in risk of early and advanced IR across quintiles was plotted for each biomarker, and the significance of the trends in risk was calculated. All analyses were done with SPSS® version 21 software in Spanish.

Results

After persons with diabetes were excluded, the final study sample was 5,943 individuals, of whom 3,524 were included in the no IR group, 1,321 in the early IR group, and 1098 in the advanced IR group.

The distribution of values for the biomarkers and anthropometric indexes in each group are shown in Table 1. Differences between groups were evident for serum glucose and C-peptide concentrations, but these variables were excluded from further analysis because the values were conditioned by how we defined our three IR groups. In the early IR group the values for all biomarkers were significantly different compared to the no IR group except for resistin, PAI-1 and leptin in males. In the advanced IR group the values for all biomarkers except PAI-1 were significantly different compared to the no IR group. In comparisons between the early IR and advanced IR groups, the only markers that were significantly different were serum lipids and resistin. For the anthropometric indexes, significant differences between groups were seen for mean age, proportion of males, and abdominal obesity; the values in the early IR group were intermediate between those in the no IR and advanced IR groups.

In Table 2, the ordinal models fitted to estimate the association between each biomarker and IR progression yielded significant regression coefficients ($p < 0.01$) for insulin, triglycerides, HDL cholesterol, triglycerides/HDL cholesterol ratio, non-HDL cholesterol, CRP, adiponectin, and sCD40L. The coefficients for leptin and resistin approached statistical significance, whereas the coefficients for LDL cholesterol and PAI-1 were not significant.

Table 3 summarizes the estimated risks according to different multinomial logistic regression models for the association between each biomarker and IR category. For early IR, only LDL cholesterol and leptin showed no significant association, and for advanced IR, only leptin failed to show a significant association. Figure 1 shows the largest risks of early IR for each quintile of biomarker values according to the regression models. A significant trend toward increased risk was seen for values in the upper quintiles for triglycerides ($P=0.008$), triglycerides/HDL cholesterol ratio ($P=0.009$), non-HDL cholesterol ($P=0.011$), LDL cholesterol ($P=0.003$) and resistin ($P=0.009$). A trend toward decreased risk was seen with increasing quintile values for HDL cholesterol ($P=0.012$) and adiponectin ($P=0.047$). Figure 2 shows the risks of advanced IR for each quintile of biomarker values according to the same regression models. A significant trend toward increased risk was seen in upper quintile values for triglycerides ($P=0.043$), triglycerides/HDL cholesterol ratio ($P=0.022$), non-HDL cholesterol ($P=0.001$), LDL cholesterol ($P=0.011$) and PAI-1 ($P=0.046$), whereas the trend toward decreased risk approached significance for upper quintile values of HDL ($P=0.067$), adiponectin ($P=0.077$) and resistin ($P=0.077$).

Discussion

Approximately 20% of the general population without diabetes has early IR. These individuals are euglycemic but despite their normal blood sugar levels, they have high concentrations of C-peptide and insulin, and a profile indicative of dyslipidemia (elevated values of triglycerides, LDL and non-HDL cholesterol, and low concentrations of HDL cholesterol) and inflammation (elevated resistin, CRP and PAI-1, together with decreased adiponectin and sCD40L).

The population with early IR is on average 3 years older than the population without IR, and is further characterized by a higher male sex ratio and a higher prevalence of abdominal obesity and elevated serum leptin levels. However, we found that the relationship of IR with lipids and cytokines is independent of obesity. The atherogenic lipid profile is a consequence of the diminished production of glucose by the liver during the early, euglycemic stage of IR, because when insulin production increases to limit the action of glucose-

6-phosphatase and maintain normal levels of hepatic glucose production, an inevitable result is that glucokinase is stimulated, leading to the accumulation of fat in the liver and to triacylglycerol secretion [1]. When IR debuts, the pancreas has not yet been severely damaged. However, the elevated insulin levels along with equimolar C-peptide secretion have inflammatory effects in the vascular wall by favoring the accumulation of monocytes that stimulate increases in a number of cytokines [24,25]. This may explain the increase we observed in PAI-1, CRP and resistin levels in the early stage of IR. The cross-sectional design of our study means that we are not able to establish what happens before IR becomes detectable, but we speculate that factors such as a diet rich in sugars and saturated fats along with a sedentary lifestyle may be responsible for the dyslipidemic profile and low adiponectin concentration that can be seen before obesity appears [26]. Given that persons with early IR have normal blood glucose levels, IR goes undetected in usual clinical practice, and there are no indications for treatment that might prevent it from progressing to advanced IR. However, the lipid and cytokine profile we report here can be considered atherogenic and inflammatory, and in fact this population group is at elevated risk of coronary artery disease [17].

We use the term “advanced IR” to designate the presence of hyperglycemia regardless of C-peptide concentration. This population group makes up almost 20% of the general population, and their clinical status is currently identified as prediabetes or altered basal glucose level. On average, this group is 10 years older than the population without IR, and is characterized by a much higher male sex ratio, prevalence of abdominal obesity and a more markedly dyslipidemic profile (with significantly increased triglycerides, LDL and non-HDL cholesterol) compared to early IR. In contrast, insulin and C-peptide concentrations are lower in the advanced IR group, probably as a result of the progressive deterioration in pancreatic function after years of IR, and possibly related with the concomitant decrease in resistin. To our knowledge this possible evolution of resistin, independently of obesity, with elevated levels in early IR followed by decreased levels in advanced IR, has not been described previously. This pattern of changes in resistin levels may account for the contradictory findings published regarding the relationship between this cytokine and IR [27-29].

In the ordinal models we found that the relationship between IR and different biomarkers approached a direct linear association for triglycerides, non-HDL cholesterol and CRP, and an inverse association for HDL cholesterol, adiponectin and sCD40L. Although this was a cross-sectional study, these findings suggest that as IR progresses from the early stage toward the advanced stage, atherogenic and inflammatory biomarkers show a steady increase, while the concentrations of markers of a protective effect such as HDL cholesterol and adiponectin decrease. The linear associations for leptin and LDL cholesterol were not significant: adjustment for abdominal obesity showed that both were dependent on this latter variable. The association between IR and resistin did not approach linearity: resistin concentrations increased during early IR but decreased during advanced IR, as noted above.

In contrast to the ordinal models, the multinomial models showed the extent of the increase or decrease in risk of early or advanced IR according to biomarker values. Persons with values for triglycerides, triglyceride/HDL cholesterol ratio or PAI-1 in the upper two quintiles had a threefold greater risk of IR, and those with elevated CRP levels had a more than twofold greater risk, compared to the middle and lower two quintiles. In contrast, persons with HDL cholesterol or sCD40L values in the upper two quintiles had less than half the risk of IR, and those with adiponectin concentrations in the upper quintile had a risk of IR less than 20% that of the middle or lower two quintiles. Of particular interest is our finding that the upper two quintiles of resistin values were associated with a 25% increase in the risk of early IR, but with an almost 30% decrease in the risk of advanced IR.

In terms of clinical practice, our results indicate that no single biomarker for IR performed better than values of triglycerides in the upper quintile (>136 mg/dL in women and >181 mg/dL in men). The estimated risk of IR of 3.88 estimated with triglyceride values was not surpassed by any of the lipids or cytokines analyzed here. Triglycerides and blood glucose values are habitually measured as part of screening tests used in primary care in developed countries, and it thus seems advisable to measure C-peptide concentration in middle-aged people with elevated triglycerides and normal blood glucose values. It has been proven that measurement of fasting plasma insulin (and therefore C-peptide) does provide a surrogate estimate of insulin action as good

as calculation of HOMA-IR [30]. C-peptide is currently inexpensive to measure, and its values are much more stable than insulin given its 2-fold to 5-fold longer half-life. This laboratory result may identify persons with early IR, whom we found to have an atherogenic and inflammatory biochemical profile and to be at greater risk of coronary artery disease [17]. To this population group belong women who after suffering a gestational diabetes present normal blood glucose levels, as they show a high IR prevalence [31]. The interventions found in the Diabetes Prevention Program [32] to be effective in improving advanced IR should be investigated to evaluate their usefulness in early IR. Our epidemiological study is consistent with the results of experimental studies in pointing to the potential benefits of preventing cardiovascular disease associated with type 2 diabetes mellitus before hyperglycemia appears [1].

The main limitation of this study is its cross-sectional design, which precluded investigating changes in IR and serum values with time, although a provisionally useful approximation was possible thanks to our categorization of IR as absent, early or advanced. Another important limitation was the low number of measures available for some biomarkers, which we were unable to study in greater detail due to budgetary restrictions. This may have diminished the power of some of our analyses. In this regard, because of the low number of participants in whom values were available for all biomarkers, we were unable to analyze the possible correlations among all markers in a single model. Among the strengths of our study are the large sample drawn from the general population without diabetes, and the fact that we were able to measure most variables in our entire sample.

We conclude that the population with early IR has an unfavorable lipid and inflammatory profile regardless of the presence or not of obesity, and independently of gender or age. Given that this population subgroup goes unidentified in the course of usual clinical practice, determining C-peptide concentration in patients with elevated triglyceride values may aid in detecting IR.

Acknowledgments

The authors thank Karen Shashok for translation of the manuscript into English.

Declaration of conflicting interests. None. The authors declare that they have no conflict of interest.

Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

Funding

Canary Islands Health Service. Spanish Government (Instituto de Salud Carlos III, projects 0901314, PI12/01855, and RD12/0042/0031 and 0013. FEDER).

References

1. Pajvani UB, Accili D. The new biology of diabetes. *Diabetologia*. 2015; 58: 2459-2468.
2. Muniyappa R, Madan R, Quon MJ. Assessing Insulin Sensitivity and Resistance in Humans. De Groot LJ, Beck-Peccoz P, Chrousos G, et al., editors. NCBI Bookshelf. Endotext [Internet], 2000. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK278954>. Last Update: February 2, 2015
3. Weyer C, Tataranni PA, Bogardus C, Pratley RE. Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. *Diabetes Care*. 2001; 24: 89-94
4. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet*. 2009; 373: 2215-2221
5. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med*. 2014; 371:1131-1141
6. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest*. 2016; 126: 12-22
7. Khodabandehloo H, Gorgani-Firuzjaee S, Panahi G, Meshkani R. Molecular and cellular mechanisms linking inflammation to insulin resistance and β -cell dysfunction. *Transl Res*. 2016; 167: 228-256
8. Cersosimo E and DeFronzo RA. Insulin Resistance and Endothelial Dysfunction: The Road Map for Cardiovascular Diseases. *Diabetes Metab Res Rev*. 2006; 22: 423-436

9. Zhan Y, Tang Z, Yu J. Serum ferritin, diabetes, diabetes control, and insulin resistance. *Acta Diabetol.* 2014; 51: 991-998
10. Kharroubi I, Ladriere L, Cardozo AK, Cnop M, Eizirik DL. Free fatty acids and cytokines induce pancreatic beta cell apoptosis by different mechanisms: role of NF-kappa B and endoplasmic reticulum stress. *Diabetologia.* 2004; 47: A176– Endocrinology. 2004; 145: 5087-96.
11. Moran A, Steffen LM, Jacobs DR Jr, Steinberger J, Pankow JS, Hong CP, et al. Relation of C-reactive protein to insulin resistance and cardiovascular risk factors in youth. *Diabetes Care.* 2005; 28: 1763-1768
12. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, et al. Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells. *Diabetologia.* 1999; 42: 1417-1424
13. Daniele G, Guardado Mendoza R, Winnier D, Fiorentino TV, Pengou Z, Cornell J, et al. The inflammatory status score including IL-6, TNF- α , osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin resistance and hyperglycemia in type 2 diabetes mellitus. *Acta Diabetol.* 2014; 51: 123-131
14. Nwaneri C, Cooper H, Bowen-Jones D. Screening for type 2 diabetes and population mortality over 10 years. *Lancet.* 2013 ; 381: 901-912
15. Salazar MR, Carbajal HA, Espeche WG, Aizpurúa M, Leiva Sisniegues CE, Leiva Sisniegues BC, et al. Insulin resistance: The linchpin between prediabetes and cardiovascular disease. *Diab Vasc Dis Res.* 2016; 13: 157-163
16. Ariel D, Reaven G. Modulation of coronary heart disease risk by insulin resistance in subjects with normal glucose tolerance or prediabetes. *Acta Diabetol.* 2014; 51: 1033-1039
17. Cabrera de León A, Oliva García JG, Marcelino Rodríguez I, Almeida González D, Alemán Sánchez JJ, Brito Díaz B, et al. C-peptide as a risk factor of coronary artery disease in the general population. *Diab Vasc Dis Res.* 2015;12:199-207
18. Cabrera de León A, Rodríguez Perez MC, Almeida González D, Domínguez Coello S, Aguirre Jaime A, Brito Díaz B, et al. Presentation of the "CDC de Canarias" cohort: objectives, design and preliminary results. *Rev Esp Salud Publica.* 2008; 82: 519-534
19. Fernández-Bergés D, Consuegra-Sánchez L, Peñafiel J. Cabrera de León A, Vila J, Félix-Redondo FJ, et al. Perfil inflamatorio y metabólico. Estudio DARIOS Inflamatorio. *Rev Esp Cardiol (Engl Ed).* 2014; 67: 624-631
20. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev.* 2012; 13: 275-286
21. Bo S, Gentile L, Castiglione A, Prandi V, Canil S, Ghigo E, et al. C-peptide and the risk for incident complications and mortality in type 2 diabetic patients: a retrospective cohort study after a 14-year follow-up. *Eur J Endocrinol.* 2012;167:173-180
22. Kim ST, Kim BJ, Lim DM, Song IG, Jung JH, Lee KW, et al. Basal C-peptide level as a surrogate marker of subclinical atherosclerosis in type 2 diabetic patients. *Diabetes Metab J.* 2011; 35: 41–49
23. Marx N, Silbernagel G, Brandenburg V, Burgmaier M, Kleber ME, Grammer TB, et al. C-Peptide levels are associated with mortality and cardiovascular mortality in patients undergoing angiography: The LURIC Study. *Diabetes Care.* 2013; 36: 708-714
24. Marx N, Walcher D, Raichle C, Aleksic M, Bach H, Grüb M, et al. C-peptide colocalizes with macrophages in early arteriosclerotic lesions of diabetic

- subjects and induces monocyte chemotaxis in vitro. *Arterioscler Thromb Vasc Biol* 2004; 24: 540–545
25. Kappert K, Meyborg H, Clemenz M, Graf K, Fleck E, Kintscher U, et al. Insulin facilitates monocyte migration: a possible link to tissue inflammation in insulin-resistance. *Biochem Biophys Res Commun*. 2008; 365: 503-508
 26. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest*. 2006; 116: 1784-1792
 27. Schwartz DR, Lazar MA. Human Resistin: Found in Translation From Mouse to Man. *Trends Endocrinol Metab*. 2011; 22: 259-265
 28. Iqbal N, Seshadri P, Stern L, Loh J, Kundu S, Jafar T, et al. Serum resistin is not associated with obesity or insulin resistance in humans. *Eur Rev Med Pharmacol Sci*. 2005; 9: 161-165
 29. Domínguez Coello S, Cabrera de León A, Almeida González D, González Hernández A, Rodríguez Pérez MC, Fernández Ramos N, et al. Inverse association between serum resistin and insulin resistance in humans. *Diab Res Clin Pract*. 2008; 82: 256-261
 30. Abbasi F, Okeke Q, Reaven GM. Evaluation of fasting plasma insulin concentration as an estimate of insulin action in nondiabetic individuals: comparison with the homeostasis model assessment of insulin resistance (HOMA-IR). *Acta Diabetol*. 2014; 51: 193-197
 31. Noctor E, Crowe C, Carmody LA, Kirwan B, O'Dea A, Glynn LG, et al. ATLANTIC-DIP: prevalence of metabolic syndrome and insulin resistance in women with previous gestational diabetes mellitus by International Association of Diabetes in Pregnancy Study Groups criteria. *Acta Diabetol*. 2015; 52: 153-160
 32. Hivert MF, Christophi CA, Franks PW, Jablonski KA, Ehrmann DA, Kahn SE, et al. Lifestyle and Metformin Ameliorate Insulin Sensitivity Independently of the Genetic Burden of Established Insulin Resistance Variants in Diabetes Prevention Program Participants. *Diabetes*. 2016; 65: 520-526

Table 1—Distribution of biomarker values (mean $\pm$ SD or median [25th to 75th percentile]) and anthropometric measures (mean $\pm$ SD or percentage and 95% CI) in three IR groups from the general population without diabetes

Biomarkers				P
	No IR [n]	Early IR [n]	Advanced IR [n]	
Blood glucose	86.71 $\pm$ 6.78 [3524]	89.17 $\pm$ 6.63 [1321]	106.78 $\pm$ 6.20 [1098]	<0.001 ^{†,‡,§}
C-Peptide *	1.54 $\pm$ 0.40 [3524]	3.25 $\pm$ 1.31 [1321]	2.57 $\pm$ 1.31 [1095]	<0.001 ^{†,‡,§}
Insulin *	135.93 $\pm$ 187.75 [124]	366.93 $\pm$ 225.12 [73]	310.18 $\pm$ 280.69 [93]	<0.001 ^{†,‡}
Triglycerides *	100.94 $\pm$ 78.87 [3524]	138.20 $\pm$ 87.83 [1321]	146.91 $\pm$ 97.26 [1098]	<0.001 ^{†,‡,§}
HDL chol Women	57.18 $\pm$ 12.84 [2180]	51.77 $\pm$ 12.46 [773]	51.38 $\pm$ 13.89 [449]	<0.001 ^{†,‡}
HDL chol Men	48.26 $\pm$ 11.90 [1344]	43.77 $\pm$ 10.51 [547]	45.94 $\pm$ 14.14 [649]	<0.001 ^{†,‡,§}
Tg/HDL chol Women*	1.77 $\pm$ 2.39 [2180]	2.66 $\pm$ 1.96 [773]	2.95 $\pm$ 2.68 [449]	<0.001 ^{†,‡,§}
Tg/HDLchol Men*	2.71 $\pm$ 2.68 [1344]	4.09 $\pm$ 3.63 [547]	3.88 $\pm$ 3.21 [649]	<0.001 ^{†,‡}
LDLcholesterol	122.29 $\pm$ 35.26 [3509]	129.26 $\pm$ 35.13 [1300]	140.01 $\pm$ 35.79 [1076]	<0.001 ^{†,‡,§}
Non-HDL cholest.	142.22 $\pm$ 40.52 [3511]	156.82 $\pm$ 41.13 [1312]	168.85 $\pm$ 40.80 [1091]	<0.001 ^{†,‡,§}
C-Reactive prot.	1.60 (0.70-3.23) [494]	2.10 (1.05-3.90) [193]	1.80 (0.90-4.00) [296]	0.004 ^{†,‡}
Resistin Women*	6.12 $\pm$ 2.34 [2179]	6.14 $\pm$ 2.36 [773]	5.81 $\pm$ 2.56 [445]	<0.001 ^{†,§}
Resistin Men*	5.74 $\pm$ 2.18 [1344]	5.81 $\pm$ 2.21 [548]	5.34 $\pm$ 2.14 [649]	<0.001 ^{†,§}
Leptin Women	8.02 (4.7-15.1) [337]	11.13 (7.1-18.5) [122]	12.54 (6.3-21.2) [140]	<0.001 ^{†,‡}
Leptin Men	1.49 (0.9-2.58) [216]	1.81 (0.9-4.3) [77]	2.28 (1.1-4.1) [165]	<0.001 [†]
Adiponectin	3.61 (2.53-5.25) [125]	2.69 (1.7-3.87) [72]	2.38 (1.7-3.6) [93]	<0.001 ^{†,‡}
sCD40L *	10.18 $\pm$ 9.15 [415]	8.40 $\pm$ 7.36 [120]	8.76 $\pm$ 8.16 [209]	<0.001 ^{†,‡}
PAI-1 ng/mL	44.08 (31.99-51.25) [114]	47.25 (35.09-56.68) [63]	49.00(34.60-54.64) [68]	0.164
Anthropometric variables				
	No IR [3524]	Early IR [1321]	Advanced IR [1098]	
Age	39.1 $\pm$ 11.9	42.4 $\pm$ 12.6	49.3 $\pm$ 10.9	<0.001 ^{†,‡,§}
Male gender %	38.1 [n=1344]	41.5 [n=548]	59.1 [n=649]	<0.001 ^{†,‡,§}
Abdominal obesity %	30.3 [n=1063]	63.5 [n=837]	73.8 [n=806]	<0.001 ^{†,‡,§}

*P for LN. Significant difference between † groups 1 and 2, ‡ groups 1 and 3, and § groups 2 and 3.

Table 2—Multivariate analysis. Each row is for an ordinal regression model in which the estimated association of each biomarker (independent continuous variable) with IR (ordinal dependent variable) is adjusted for age, gender and abdominal obesity. Regression coefficients with 95% CI and *P* values for each independent variable (biomarker).

INDEPENDENT VARIABLE	Coefficient (CI 95%)	p
Insulin * (n=290)	1.90 (1.28- 2.53)	<0.001
Triglycerides * (n=5943)	0.91 (0.77- 1.06)	<0.001
HDL cholesterol * (n=5942)	-1.45 (-1.75- -1.15)	<0.001
Tg/ HDL cholesterol* (n=5942)	0.77 (0.65- 0.88)	<0.001
LDL cholesterol * (n=5885)	0.13 (-0.12- 0.38)	0.298
Non HDL cholesterol (n=5914)	0.70 (0.44- 0.96)	<0.001
CRP * (n=983)	0.26 (0.10- 0.43)	0.001
Resistin * (n=5938)	0.19 (-0.10- 0.39)	0.063
Leptin * (n=1057)	0.19 -(0.01- 0.38)	0.057
Adiponectin * (n=290)	-1.38 (-2.05- -0.70)	<0.001
sCD40L * (n=744)	-0.35 (-0.60- -0.09)	0.007
PAI-1 * (n=245)	0.06 (-0.03- 0.15)	0.165

* LN.

Table 3—Risk of early and advanced IR associated with elevated levels of different biomarkers. Each row is for a multinomial model with logistic regression in which the estimated association of each independent variable (in quintiles) with IR (categorical dependent variable) is adjusted for age, gender and abdominal obesity. Odds ratios with 95% CI and *P* values for the fourth and fifth quintiles of each independent variable (biomarker) compared to its first quintile, for the dependent variable categories early IR and advanced IR.

Independent variable	Early IR		Advanced IR	
	OR (95% CI)*	<i>P</i>	OR (95% CI)*	<i>P</i>
Insulin (n=290)	133.00 (22.17-798.04)	<0.001	18.17 (5.03-65.66)	<0.001
	45.93 (9.20-229.19)	<0.001	4.34 (1.53-12.29)	0.006
Triglycerides (n=5943)	3.88 (3.07-4.89)	<0.001	3.10 (2.39-4.03)	<0.001
	2.53 (2.01-3.18)	<0.001	2.03 (1.56-2.64)	<0.001
HDL cholesterol (n=5942)	0.35 (0.28-0.44)	<0.001	0.38 (0.30-0.49)	<0.001
	0.49 (0.40-0.60)	<0.001	0.45 (0.36-0.57)	<0.001
Tg/ HDL cholesterol (n=5942)	3.86 (3.06-4.87)	<0.001	3.08 (2.37-3.99)	<0.001
	2.55 (2.02-3.21)	<0.001	2.02 (1.56-2.63)	<0.001
LDL cholesterol (n=5885)	1.14 (0.91-1.43)	0.254	1.66 (1.27-2.16)	<0.001
	1.08 (0.87-1.35)	0.479	1.49 (1.14-1.94)	0.004
Non-HDL cholesterol (n=5914)	1.74 (1.38-2.19)	<0.001	2.34 (1.78-3.08)	<0.001
	1.68 (1.34-2.10)	<0.001	1.89 (1.42-2.46)	<0.001
CRP (n=983)	2.31 (1.29-4.12)	0.005	1.89 (1.15-3.08)	0.012
	2.34 (1.35-4.20)	0.003	1.30 (0.78-2.15)	0.313
Resistin (n=5938)	1.24 (1.00-1.53)	0.050	0.73 (0.58-0.93)	0.010
	1.25 (1.01-1.54)	0.040	0.74 (0.59-0.94)	0.013
Leptin (n=1057)	1.55 (0.86-2.81)	0.148	1.31 (0.77-2.24)	0.324
	1.15 (0.64-2.05)	0.646	1.04 (0.72-1.75)	0.874
Adiponectin (n=290)	0.11 (0.04-0.32)	<0.001	0.16 (0.06-0.45)	<0.001
	0.21 (0.08-0.56)	0.002	0.16 (0.06-0.44)	<0.001
sCD40L (n=744)	0.63 (0.32-1.23)	0.175	0.59 (0.33-1.07)	0.082
	0.47 (0.24-0.94)	0.033	0.46 (0.25-0.83)	0.010
PAI-1 (n=245)	3.29 (1.29-8.40)	0.013	3.08 (1.29-7.34)	0.011
	1.61 (0.54-4.83)	0.398	2.07 (0.77-5.55)	0.148

* The first of each pair of lines is the OR for fifth versus first quintile, and the second line is the OR for the fourth versus first quintile.

Figure 1—Risk of early IR (OR compared to the no IR group) according to the quintile distribution of biomarker values. Maximum and minimum significant risks are shown for each quintile.

Figure 2—Risk of advanced IR (OR compared to the no IR group) according to the quintile distribution of biomarker values. Maximum and minimum significant risks are shown for each quintile.