

2009 World Congress on the Insulin Resistance Syndrome: Cardiovascular Disease Concepts

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This is the first of four articles summarizing presentations at the seventh World Congress on the Insulin Resistance Syndrome, held in San Francisco, California, on 5–7 November 2009. This article pertains to cardiovascular disease (CVD) concepts.

Yehuda Handelsman (Tarzana, CA), chair of the International Committee for Insulin Resistance, “revisited” the treatment of insulin resistance, discussing aspects of the metabolic findings of BARI-2D’s insulin-sensitizing versus insulin-providing strategy comparison (1). “When it came to the control of glucose,” he said, “the group that was on sensitizers had a 0.5% improvement in A1C,” similar to that seen in PROactive (2). “We’ve seen [in RECORD (3) and ADOPT (4)],” he continued, “that if you are on sulfonylurea or metformin you lose control” whether in combination or single-agent treatment in comparison to the stability of glycemia with rosiglitazone. Furthermore, Handelsman pointed out that in BARI-2D, those undergoing CABG had better outcome with the insulin-sensitizing strategy.

Reviewing the concept of metabolic syndrome, which has been defined and redefined many times since first proposed as Syndrome X by Reaven (5), Handelsman emphasized the potential benefit of thiazolidinediones, but suggested that the flawed Nissen metaanalysis of rosiglitazone (6) has led to the misperception that insulin resistance treatment is not of benefit. He asked, “Should we treat insulin resistance pharmacologically?” If there is little evidence that treatment of the syndrome is effective beyond the benefit of treatment of the components, then we may need more data to justify insulin sensitizer administration outside of diabetes treatment. It may, however, be the case

that insulin levels per se can be used in guiding pharmacologic treatment, and Handelsman reviewed the evidence that pioglitazone did reduce CV event rates in subset analyses of PROactive. Insulin resistance treatment appears of benefit in the polycystic ovary syndrome (PCOS), and in nonalcoholic fatty liver disease (NAFLD), and may play a role in prevention (or even in treatment) of the many malignancies associated with obesity, with Handelsman noting that analysis of the RECORD trial showed significant reduction in pancreatic carcinoma with rosiglitazone.

Inflammation and insulin resistance

Steven Shoelson (Boston MA) discussed inflammation and the adaptive immune system in the pathogenesis of type 2 diabetes. The adaptive immune system has been thought to play roles in autoimmunity and in the pathogenesis of type 1 diabetes, but there is growing evidence of its role in type 2 diabetes and in atherosclerosis. Epidemiologic studies show that diabetes is associated with higher levels of circulating markers of inflammation—not only C-reactive peptide (CRP), but also the leukocyte count—and with levels of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-6. A recently rediscovered literature from clinical experience beginning in 1875 describes therapeutic benefit of salicylates in what is now considered to be type 2 diabetes, giving impetus to Shoelson’s study of the effect on diabetes of salicylates. The effect of salicylates was confirmed in animal models (7), with evidence that the molecular target of high-dose salicylates is not the COX-1 inhibition of platelet aggregation, as seen with 81-mg doses of aspirin, or COX-1/2

inhibition seen with 650-mg doses, but rather an effect on nuclear factor (NF)- κ B seen at high (3–5 g daily) doses. NF- κ B is activated by proinflammatory factors including IL-1, the toll-like receptor (TLR), TNF- α , and intracellular factors including oxidative and endothelial reticular stress, ceramides, and various protein kinase (PK)-Cs, with NF- κ B in turn having multiple cytokine and receptor effects. Obesity induces inflammation and hence insulin resistance (IR), and salicylates reduce this in animal models. Shoelson asked whether this might be used in not only glycemic treatment, but also the prevention of atherosclerotic complications, showing a study of mice not expressing the LDL receptor, in which early atherosclerotic events seen with a high-fat diet decreased with salicylate treatment.

Salsalate is insoluble in acid pH, hydrolyzed and absorbed in the duodenum, generic, and inexpensive, with an excellent safety profile. The first stage of the Targeting INflammation using SALSalate in type 2 Diabetes (TINSAL-type 2 diabetes) trial included 120 people randomized to 0, 3, 3.5, and 4 g daily for 14 weeks, with baseline age 55, BMI 34 kg/m², diabetes duration 6 years, and A1C ~7.6% (8). A1C decreased ~0.4% at 8 and 14 weeks, and fasting blood glucose (FBG) decreased 10–20 mg/dl, while there were 0.1% and 10 mg/dl elevations in the placebo group. Triglycerides fell and adiponectin increased. There was no change in weight, liver function, or electrolytes, and no adverse gastrointestinal effect. Compliance was high, with tinnitus occurring infrequently. A 240-person 1-year trial is now ongoing, with planned coronary calcium as well as metabolic measurements.

Lipids and IR

Ronald Krauss (Berkley, CA) discussed lipoprotein abnormalities in the IRS, reminding the audience of the heterogeneity of LDL particle size and density, with smaller particles having greater atherogenicity. In the Quebec Cardiovascular study, the cholesterol circulating in small LDL particles added to coronary heart disease (CHD) risk independently

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DOI: 10.2337/dc10-zb07

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of nonlipid risk factors, HDL and LDL cholesterol, triglyceride, lipoprotein (a), and apoB levels (9). Levels of small, but not of large, LDL were independently associated with CHD risk in the first 7 years of follow-up, but interestingly not during the subsequent 6 years of the study (10). Analysis of lipoprotein subfractions with nuclear magnetic resonance spectroscopy over 11 years of follow-up of 27,673 participants in the Women's Health Study showed that VLDL, IDL, small LDL, and large HDL were associated with CV risk (11). A systematic review of CV associations of LDL subfractions showed that higher LDL particle number is associated with CVD but failed to show evidence that measures of LDL subfractions add incremental benefit (12). Krauss suggested, however, that this reflects the high degree of intercorrelation of LDL particle measures with traditional risk, which obscures the extent to which these particles are risk mediators (13).

How then, Krauss asked, can we improve clinical risk prediction? He hypothesized that there are distinct clusters of lipoprotein subfractions representing specific mechanisms which independently confer risk. Using a new method of ion mobility to determine lipoprotein particle sizes and concentrations (14), he showed an analysis of the prospective Malmö cohort study of 4,594 persons who also had traditional lipid profiles with mean follow-up 12.2 years (15). HDL was most strongly associated with risk, then triglyceride, and non-HDL cholesterol, and, in this analysis, LDL itself was not a significant risk factor. Using principal component analysis to make weighted linear combinations of correlated variables to maximize the variation explained, the first component, including 41% of risk, was made up of LDL, IDL, and VLDL and gave apoB-related risk; the second component, with 24% of risk, comprised small and medium LDL and lower levels of large HDL; and the third component, giving 11% of the risk, was principally composed of HDL. The second component, Krauss explained, "captures the phenotype of IR," both in men and women, and was independent of LDL but not HDL. He asked whether these risk components represent biological pathways, using genetics to analyze their relationships. In genome-wide association studies, selecting single nucleotide polymorphisms (SNPs) associated with lipid changes, he analyzed changes in the three components with SNP genotype, suggest-

ing that if a SNP aligns strongly with one component, it would suggest this gene to be related. Indeed, the first component was related to a set of ApoB, apoE, and LDL-receptor SNPs; the second component with hepatic lipase, LPL, apoA5, and cholesterol ester transfer protein (CETP) SNPs; and the third component with a CETP SNP, suggesting that the components are indeed biologically meaningful, and that the pathways involved in the second component appear to be distinct (16). "Ultimately," he said, "these will be part of our diagnostic testing." The second component was strongly predictive of CV risk in both sexes, independent of LDL cholesterol. HDL was related to risk by two of the components, suggesting its actions in taking up cholesterol from macrophages, and, independently, its role as a marker of the IR syndrome.

Thomas Bersot (San Francisco, CA) discussed approaches to recognizing and managing CV risk in insulin resistant (IR) patients with low HDL cholesterol levels. "It's clear," he said, "that the way we eat and we live in this country is killing us." In a metaanalysis of multiple studies, mortality was increased by approximately one third during the first year after acute coronary syndrome in patients with diabetes (17). The "Get With The Guidelines" group studied 136,905 persons admitted after a CHD event in 2000–2006. 45% had prior vascular disease or diabetes, but only 21% were taking a lipid-lowering drug, with the LDL 94 in those who were and 108 in those not receiving treatment, while HDL was 39.6 in both groups (18), leading Bersot to observe that "the defining risk factor is low HDL cholesterol concentration." "Patients are not being identified and appropriately treated," he said, while "use of lipid-lowering drugs by only 21% ... is a tragedy." Bersot reviewed data from the National Health and Nutrition Examination Survey showing average levels of LDL and of HDL cholesterol 123 and 49.6 mg/dl, respectively, in the general population. Over a 6 year period, LDL decreased from 108 to 103, but HDL fell as well, from 43 to 39, which may reflect the increasing prevalence of obesity.

An important question concerns appropriate lipid targets for persons with diabetes or IR. In the Treating to New Targets (TNT) study, the lesser benefit with atorvastatin 10 mg than with 80 mg daily suggests LDL <70 mg/dl as the target for secondary prevention. Comparing diabetic versus nondiabetic persons in

TNT, RR reductions were 20% in both, but absolute risk reduction was 3% versus 1.5%. As low HDL is one of the mediators of risk in diabetic patients, non-HDL cholesterol and the ratio of total-to-HDL cholesterol (goal <3.0) may be the best parameters to follow in reducing CV risk. The ApoB-to-ApoA1 ratio, the absolute value of ApoB (goal <80 mg/dl), and LDL particle number may also be useful in risk assessment.

Bersot recommended use of the most effective statins at maximum doses to reduce LDL and triglyceride-rich lipoproteins in the endeavor to attain LDL and non-HDL targets, and suggested consideration of additional LDL reduction with ezetimibe, niacin, or bile acid sequestrants, and additional triglyceride reduction to lower non-HDL cholesterol with niacin, fibrates, or omega 3 fatty acids. The lipid arm of ACCORD in which simvastatin was administered with either fenofibrate or placebo has been completed, and the possible benefit in the high triglyceride/low HDL cholesterol subgroup somewhat confirms these recommendations (19). Results of the ongoing Atherosclerosis Intervention in Metabolic syndrome with low HDL/High triglyceride and Impact on Global Health Outcomes (AIM-HIGH) and other studies of addition of niacin to a statin will not be available for years. Of course, a crucial aspect of care is change in lifestyle, and it is infrequent for us to successfully encourage a patient to walk 40 min and eat three to four servings of fruits and vegetables daily.

The endothelium and IR

Jorge Plutzky (Boston, MA) discussed endothelial regulation of metabolic as well as vascular function, and hence that there is the potential that endothelial dysfunction itself may cause the abnormalities of IR. Energy balance, particularly of glucose and of lipids/fatty acids, has major consequences in obesity and in the control of glucose and lipid metabolism, and it is noteworthy that atherosclerosis may occur consequence of both. The endothelium has the largest surface area of any organ in the body, and is involved in both systemic and local responses and in a variety of disease processes. The endothelium may then, Plutzky suggested, act as transducer of circulating factors and tissue response—including mechanical forces such as blood pressure, shear stress, pulsatile flow, the cellular blood components, erythrocytes, leukocytes,

and platelets—and circulating nutrients, particularly lipids, and toxins. A particularly important mediator released by the endothelium is nitric oxide, having paracrine effect on vasodilation, inflammation, and effects of adhesion molecules. People at risk of developing type 2 diabetes show reduced microvascular hyperaemia (20) and decreased brachial artery reactivity (21), manifestations of abnormal endothelial function with decreased vasodilation (22). Both PPAR- α and - γ are present in the endothelium. Activation of either receptor improves flow-mediated vasodilation as well as inflammatory markers (23). Plutzky described a complex experimental model with specific deletion of PPAR- γ in the endothelium, which had no effect on body weight (or on adipocyte PPAR- γ) but decreased adipocyte size and adipocyte PPAR- γ response, with decreased skeletal muscle but increased liver triglyceride, the latter presumably related to the liver's fenestrated epithelium allowing passive uptake of fatty acids with the animals having high circulating fatty acid and triglyceride levels and abnormal vasomotor function on a high-fat diet (24).

IR and thrombosis

Peter Grant (Leeds, U.K.) discussed the association between diabetes and thrombotic risk, suggesting that diabetes and CVD represent aspects of the same condition underpinned by an “inflammatory atherothrombotic insulin resistance syndrome” related to obesity, with inflammation and thrombosis further enhanced by hyperglycemia. Inflammation and thrombosis should, Grant observed, be considered protective and related processes, with both diabetes and heart disease occurring as results of normal responses in abnormal settings. IR presumably evolved primarily as a defense for energy conservation, and it is interesting that IR causes a hypercoagulable state.

Grant described interesting biologic characteristics of the horseshoe crab, which has existed on earth for 250 million years, with a single vascular cell, the amebocyte, functioning both as a platelet and phagocyte, with bacterial endotoxin releasing amebocyte clotting factors leading to simultaneous clearance of bacteria and rapid clot formation, suggesting a primal linkage of these two processes. The mammalian coagulation cascade, platelet activation process, and immune cells have much more complex characteristics. Fibrinolysis is inhibited by plasminogen ac-

tivator inhibitor (PAI)-1, while C3 is the central regulatory protein of the complement cascade. Inflammation and thrombosis strongly interact to protect against injury and infection, with the IR state associated with a complex of endothelial dysfunction, macrophage abnormalities, and formation of thrombus involving complement, clotting factors, and platelets. Atherectomy specimens from diabetic patients have more lipid-rich material, with greater degrees of macrophage infiltration and thrombus formation (25). In IR, thrombotic risk is related to adipocyte factors, including local inflammation caused by release of free fatty acids. IR occurs not only in the liver, fat, and skeletal muscle, but there is increasing recognition of its effect also in the endothelium, in macrophages, in platelets, and in cardiac myocytes, all contributing to CVD. Endothelial dysfunction leads to reduction in generation of nitric oxide (NO), which acts as a vasodilator but also decreases platelet stabilization. The IR macrophage develops into a foam cell, and although thrombosis is a late manifestation, increased levels of PAI-1, the primary inhibitor of plasminogen activator, are seen earlier. Adipocyte production of PAI-1 is reduced by weight loss and can be inhibited pharmacologically with pentoxifylline, which inhibits TNF- α . Normoglycemic first-degree relatives of persons with diabetes have doubling of PAI-1 and increased fibrinogen levels (26). Clot structure-function relationships in these persons show progressively longer clot lysis time due to a denser, more tightly branched clot structure, as IR progresses to type 2 diabetes, with clot abnormalities also demonstrated in studies of metabolic syndrome (27). Such denser fibrin clots are associated with worse survival after acute events. Other effects of diabetes include increased antiplasmin binding and decreased plasmin generation, further reducing clot lysis. Grant pointed out that in part these abnormalities are related to glycemia, perhaps related to nonenzymatic glycation of clotting factors, and can be reversed with improved control. Platelet activation is increased by inflammation, potentially leading to a positive feedback cycle with enhanced clot formation. C3, which alters fibrin structure and prolongs the time to clot lysis, increasing thrombotic potential, is associated with IR and CVD, as is C-reactive protein (CRP), which alters endothelial cell function and increases thrombus formation.

Grant discussed approaches to thrombotic risk treatment in diabetes. Improving glycemic control is important, with evidence of pleiotrophic effect of some glucose-lowering medications. Thiazolidinediones reduce thrombin-induced expression of the platelet surface antigen CD40L, reduce PAI-1 levels, reduce platelet activation, reduce CRP, and reduce macrophage CD36 expression, all of which would be expected to improve clinical outcome, although Grant observed that the evidence that this occurs is limited. The role of anticoagulant treatment in diabetes is uncertain. The cyclooxygenase (COX)-1 inhibitor aspirin inhibits the thromboxane A2 platelet aggregation pathway. A metaanalysis of six primary prevention trials of 95,000 persons did not show an effect on mortality, but in 16 secondary prevention trials of 17,000 persons, there was a 23% reduction in events, from 8.2 to 6.7% (28). The effect of aspirin among diabetic patients is unclear. A study of 2,499 diabetic persons with acute coronary syndrome failed to show evidence of benefit of aspirin administration (29). Among 58,000 Swedish diabetic persons treated with aspirin, there was evidence of increased mortality due to serious bleeding, causing a net of 107 excess deaths in persons without CVD, although preventing 164 deaths among those with CVD (30), leading Grant to state, “We should be very cautious in patients with diabetes for primary prevention.” He reviewed the effect of clopidogrel in the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) substudy of 4,000 diabetic persons, showing evidence of greater effect than aspirin, although the agent should only be used in very high-risk primary prevention. The use of clopidogrel is complicated by evidence that there is a rebound increase in mortality on its withdrawal, which is particularly seen in diabetic patients. Prasugrel, a similar agent, showed evidence of benefit in the subgroup of persons with diabetes (31). Future approaches with novel anticoagulants and anti-inflammatory agents appear promising, with Grant mentioning antiplatelet agents, such as the thromboxane inhibitor picotamide; inhibitors of p2y₁₂ (32); and the thrombin receptor protease-activated receptor-1 (PAR-1) (33).

Glycemia and CVD

Darren McGuire (Dallas, TX) discussed “fallacy, faith, and fact” pertaining to the relationship of glucose control to CVD.

In the UK Prospective Diabetes Study (UKPDS), among some 4,000 type 2 diabetic persons followed for a decade, nearly one in four had an atherosclerotic event (34). Inversely, 30–50% of persons with CVD have diabetes, in many cases not diagnosed. CHD risk begins to increase at low glucose levels, well below those used for diagnosis of diabetes. Does glucose-lowering then improve CV risk? In the University Group Diabetes Program (UGDP), cumulative mortality was greatest in persons receiving tolbutamide, leading to a black box warning in the product information for all sulfonylureas.

McGuire noted that earlier studies showed no evidence of macrovascular benefit of glycemic treatment of type 2 diabetes, and that although there was nearly significant reduction in myocardial infarction in the UKPDS, there was a comparable degree of increase in stroke. The UKPDS did suggest significant CV benefit of metformin. Furthermore, McGuire considered PROactive to suggest CV benefit of pioglitazone treatment. Combining PROactive, ACCORD, ADVANCE, VADT, and the follow-up studies of the UKPDS, there is a trend to CV benefit of glycemic control started late in the natural history of diabetes, with a suggestion that greater benefit can be obtained with treatment from the time of diabetes diagnosis. Observational data shows improved outcome among persons receiving insulin sensitizers after acute myocardial infarction (35), but McGuire observed discordance between rosiglitazone versus pioglitazone in being associated with risk versus benefit, respectively. “Beware the product label” he concluded, those of sulfonylureas and rosiglitazone implying risk, while metformin seems sturdily associated with benefit (he did not discuss the sulfonylurea plus metformin UKPDS-subset study, in which CV risk was greater than that of sulfonylureas alone [36]). He also questioned whether glycemia as a surrogate marker is truly associated with reduction in CVD, noting that in the UKPDS, the drug associated with greater CV benefit, metformin, was associated with lesser A1C reduction.

McGuire also presented mixed evidence pertaining to in-hospital glycemic control. Certainly, diabetes and high glucose are associated with adverse outcome, both in general (37) and with increasing blood glucose at the time of hospitalization for acute coronary syndrome (38). The DIGAMI study should not be used as evidence favoring glycemic control, be-

cause it aimed for blood glucose levels between 126 and 199 mg/dl (39). Van den Berghe’s study showed benefit of intensive glycemic control for patients in a surgical intensive care unit (ICU) (40), but McGuire interpreted the medical ICU study as being negative (41), and the multicenter NICE-SUGAR ICU study showed a significant increase in mortality (42), which, McGuire said, “has led us to make a hasty retreat.” Hypoglycemia is associated with increased mortality (43) and certainly is “a marker of adverse outcome” (if not a mediator). McGuire interpreted the studies to suggest an in-hospital target glucose <180 mg/dl. More intensive glycemic treatment may be reasonable after CABG surgery, although he termed “the data [...] not as strong as the cardiovascular surgeons would like us to believe.”

References

1. BARI 2D Study Group, Frye RL, August P, Brooks MM, Hardison RM, Kelsey SF, MacGregor JM, Orchard TJ, Chaitman BR, Genuth SM, Goldberg SH, Hlatky MA, Jones TL, Molitch ME, Nesto RW, Sako EY, Sobel BE. A randomized trial of therapies for type 2 diabetes and coronary artery disease. *N Engl J Med* 2009;360:2503–2515
2. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefebvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schemthaler G, Schmitz O, Skrha J, Smith U, Taton J, PROactive investigators. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet*. 2005;366:1279–1289
3. Home PD, Pocock SJ, Beck-Nielsen H, Curtis PS, Gomis R, Hanefeld M, Jones NP, Komajda M, McMurray JJ, RECORD Study Team. Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial. *Lancet*. 2009;373:2125–2135
4. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O’Neill MC, Zinman B, Viberti G, ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006;355:2427–2443
5. Reaven GM. Syndrome X. *Blood Press Suppl*. 1992;4:13–16
6. Nissen SE, Wolski K. Effect of rosiglita-

- zone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007;356:2457–2471
7. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 2001;293:1673–1677
8. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE, TINSALT2D (Targeting Inflammation Using Salsalate in Type 2 Diabetes) Study Team. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. *Ann Intern Med* 2010;152:346–357
9. St-Pierre AC, Ruel IL, Cantin B, Dagenais GR, Bernard PM, Després JP, Lamarche B. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. *Circulation* 2001;104:2295–2299
10. St-Pierre AC, Cantin B, Dagenais GR, Mauriège P, Bernard PM, Després JP, Lamarche B. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Québec Cardiovascular Study. *Arterioscler Thromb Vasc Biol* 2005;25:553–559
11. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009;119:931–939
12. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. *Ann Intern Med* 2009;150:474–484
13. Krauss RM, Siri PW. Metabolic abnormalities: triglyceride and low-density lipoprotein. *Endocrinol Metab Clin North Am* 2004;33:405–415
14. Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, Reitz RE, Krauss RM. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. *Clin Chem* 2008;54:1307–1316
15. Musunuru K, Orho-Melander M, Caulfield MP, Li S, Salameh WA, Reitz RE, Berglund G, Hedblad B, Engström G, Williams PT, Kathiresan S, Melander O, Krauss RM. Ion mobility analysis of lipoprotein subfractions identifies three independent axes of cardiovascular risk. *Arterioscler Thromb Vasc Biol* 2009;29:1975–1980
16. Berneis K, La Belle M, Blanche PJ, Krauss RM. Analysis and quantitation of biotinylated apoβ-containing lipoproteins with streptavidin-Cy3. *J Lipid Res* 2002;43:1155–1159
17. Donahoe SM, Stewart GC, McCabe CH,

- Mohanavelu S, Murphy SA, Cannon CP, Antman EM. Diabetes and mortality following acute coronary syndromes. *JAMA* 2007;298:765–775
18. Sachdeva A, Cannon CP, Deedwania PC, Labresh KA, Smith SC Jr, Dai D, Hernandez A, Fonarow GC. Lipid levels in patients hospitalized with coronary artery disease: an analysis of 136,905 hospitalizations in Get With The Guidelines. *Am Heart J* 2009;157:111–117
 19. The ACCORD Study Group. Effects of Combination Lipid Therapy in Type 2 Diabetes Mellitus. *N Engl J Med* 2010 Mar 18. [Epub ahead of print]
 20. Jaap AJ, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1994;37:214–216
 21. Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, Matthaei S, Rett K, Häring HU. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 2000;101:1780–1784
 22. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 1999;48:1856–1862
 23. Brown JD, Plutzky J. Peroxisome proliferator-activated receptors as transcriptional nodal points and therapeutic targets. *Circulation* 2007;115:518–533
 24. Kanda T, Brown JD, Orasanu G, Vogel S, Gonzalez FJ, Sartoretto J, Michel T, Plutzky J. Rgamma in the endothelium regulates metabolic responses to high-fat diet in mice. *J Clin Invest* 2009;119:110–124
 25. Moreno PR, Murcia AM, Palacios IF, Leon MN, Bernardi VH, Fuster V, Fallon JT. Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus. *Circulation* 2000;102:2180–2184
 26. Mansfield MW, Heywood DM, Grant PJ. Circulating levels of factor VII, fibrinogen, and von Willebrand factor and features of insulin resistance in first-degree relatives of patients with NIDDM. *Circulation* 1996;94:2171–2176
 27. Carter AM, Cymbalista CM, Spector TD, Grant PJ, EuroCLOT Investigators. Heritability of clot formation, morphology, and lysis: the EuroCLOT study. *Arterioscler Thromb Vasc Biol.* 2007;27:2783–2789
 28. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC, Zanchetti A. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet* 2009;373:1849–1860
 29. Cubbon RM, Gale CP, Rajwani A, Abbas A, Morrell C, Das R, Barth JH, Grant PJ, Murphy SA, Riesmeyer J, Weerakkody G. Aspirin and mortality in patients with diabetes sustaining acute coronary syndrome. *Diabetes Care* 2008;31:363–365
 30. Welin L, Wilhelmsen L, Björnberg A, Odén A. Aspirin increases mortality in diabetic patients without cardiovascular disease: a Swedish record linkage study. *Pharmacoepidemiol Drug Saf* 2009;18:1143–1149
 31. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM, TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007;357:2001–2015
 32. Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest* 2004;113:340–345
 33. Derian CK, Damiano BP, Addo MF, Darrow AL, D'Andrea MR, Nedelman M, Zhang HC, Maryanoff BE, Andrade-Gordon P. Blockade of the thrombin receptor protease-activated receptor-1 with a small-molecule antagonist prevents thrombus formation and vascular occlusion in nonhuman primates. *J Pharmacol Exp Ther* 2003;304:855–861
 34. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet.* 1998;352:837–853
 35. Inzucchi SE, Masoudi FA, Wang Y, Kosiborod M, Foody JM, Setaro JF, Havranek EP, Krumholz HM. Insulin-sensitizing antihyperglycemic drugs and mortality after acute myocardial infarction: insights from the National Heart Care Project. *Diabetes Care* 2005;28:1680–1689
 36. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet.* 1998;352:854–865
 37. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, Goodman SG, Corbalan R, Purdy DA, Murphy SA, McCabe CH, Antman EM, TRITON-TIMI 38 Investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. *Circulation.* 2008;118:1626–1636
 38. Kosiborod M, Rathore SS, Inzucchi SE, Masoudi FA, Wang Y, Havranek EP, Krumholz HM. Admission glucose and mortality in elderly patients hospitalized with acute myocardial infarction: implications for patients with and without recognized diabetes. *Circulation* 2005;111:3078–3086
 39. Malmberg K, Rydén L, Efendic S, Herlitz J, Nicol P, Waldenström A, Wedel H, Welin L. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. *J Am Coll Cardiol* 1995;26:57–65
 40. van den Bergh G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in the critically ill patients. *N Engl J Med* 2001;345:1359–1367
 41. Van den Bergh G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006;354:449–461
 42. NICE-SUGAR Study Investigators, Finfer S, Chittock DR, Su SY, Blair D, Dodek D, Dhingra V, Bellomo R, Cook D, Dodek P, Henderson WR, Hébert PC, Heritier S, Heyland DK, McArthur C, McDonald E, Mitchell I, Myburgh JA, Norton R, Potter J, Robinson BG, Ronco JJ. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med* 2009;360:1283–1297
 43. Svensson AM, McGuire DK, Abrahamson P, Dellborg M. Association between hyper- and hypoglycaemia and 2 year all-cause mortality risk in diabetic patients with acute coronary events. *Eur Heart J* 2005;26:1255–1261