American College of Endocrinology Pre-Diabetes Consensus Conference: Part Three

ZACHARY T. BLOOMGARDEN, MD

The American College of Endocrinology held a Consensus Conference in Washington, DC, on 21–22 July 2008 on the topic of pre-diabetes, organized around a series of interrelated questions. This is the third of a three-part series summarizing presentations at the conference.

What are the appropriate measures to monitor pre-diabetes and its treatment?

Peter Wilson (Boston, MA) reviewed Framingham data, with 30-year follow-up now available, showing greater evidence of adverse cardiovascular disease (CVD) outcome with diabetes. It has been recognized for some time that there are clustered abnormalities within the metabolic syndrome spectrum. BMI, triglycerides, waist circumference, HDL cholesterol, and fasting and 2-h insulin form one group; fasting and 2-h insulin and glucose another; and BMI and diastolic and systolic blood pressure levels a third (1), with hyperinsulinemia a crucial factor in many of these associations (2). Homeostasis model assessment of insulin resistance is associated with left ventricular mass in women, though not in men (3). Meigs and colleagues (4) showed evidence that the coronary artery calcium score is associated with insulin resistance and with pre-diabetes. Metabolic syndrome is certainly related to outcome, with CVD two to three times and diabetes seven times more likely in those with 3–5 vs. 0–2 components of the syndrome. In this analysis, the population-attributable risk of metabolic syndrome is approximately one-third that of CVD and two-thirds that of diabetes for men, with glucose (as expected) the major determinant of diabetes risk among metabolic syndrome variables. Population-attributable risks of metabolic syndrome were somewhat lower for women. An alternative analysis divides metabolic syndrome factors into none vs. 1–2 vs. 3–5, with a suggestion of increased risk even in the intermediate group and of markedly increased risk in comparison with 3–5 vs. no metabolic syndrome factors. If a measure of insulin resistance is added to metabolic syndrome, both diabetes and CVD risks are markedly augmented (5). There are arguments against the use of metabolic syndrome, as its components are not all equally powerful in Framingham predictive models (6). Wilson also pointed out that waist circumference and BMI appear equivalent in the analysis of this population.

In the Framingham population, 20% had impaired fasting glucose (IFG) only, 5% impaired glucose tolerance (IGT) only, and 6% both, with likelihood of developing diabetes, based on fasting glucose, 1.3% for those with neither, 4.3% for those with IGT only, 9.2% for those with IFG only, and 25.5% for those with both. Wilson stated that diabetes prediction based on age, sex, family history, BMI, blood pressure, and lipids is as good as that based on the presence of IFG and IGT, although using the actual glucose levels improves prediction, and speculated that it might be reasonable to define pre-diabetes and diabetes based on composite risk score, which he termed "a weighted metabolic syndrome," rather than using glucose levels alone.

Steven Haffner (San Antonio, TX) discussed "how and when pre-diabetes progresses to diabetes." Isolated IFG and isolated IGT differ, with the latter more associated with insulin resistance and inflammation and the former with insulin deficiency. In a population of individuals with normal glucose tolerance, multivariate analysis shows that triglyceride, HDL cholesterol, systolic blood pressure, fasting glucose, and fasting insulin are significant markers of diabetes risk (7)—exactly the components of the metabolic syndrome as subsequently defined. Haffner reviewed analyses from San Antonio and Insulin Resistance Atherosclerosis Study datasets showing IGT somewhat more strongly associated with diabetes development than IFG. Both insulin resistance and decreased insulin secretion increase diabetes risk (8). Inflammatory measures are strongly correlated with insulin resistance, including C-reactive protein (CRP) and the leukocyte count (9), with both CRP and plasminogen activator inhibitor (PAI)-1 quartile associated with diabetes risk (10). Haffner analyzed the additional effect of metabolic syndrome in predicting diabetes. Individuals with metabolic syndrome without IGT had a 12% 7-year risk of diabetes, those with IGT without metabolic syndrome had a 25% risk, and those with both had a 55% risk, suggesting that they might be appropriate candidates for pharmacologic intervention (11). Another set of markers of diabetes risk pertains to its association with hepatic steatosis and to a progressive increase in the likelihood of diabetes associated with increasing alanine transaminase (ALT) levels (12), both of which are additive to the effect of CRP. This might offer another parameter useful in determining whether pharmacologic treatment would be indicated, with Haffner suggesting that those individuals whose annual likelihood of diabetes exceeded 8% might be candidates for such an intervention. For such a group, Haffner recommended use of IFG with a glucose cutoff of 110 mg/dl, plus IGT and metabolic syndrome, present in 7% of the population with a 10% annual diabetes risk. Adding ALT somewhat improved this. There is currently no information as to whether serial measurements of glucose levels would allow greater specificity. Another reason for performing a

Zachary T. Bloomgarden, MD, is a practicing endocrinologist in New York, New York, and is affiliated with the Division of Endocrinology, Mount Sinai School of Medicine, New York, New York.
DOI: 10.2337/dc08-zb12
© 2008 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

glucose tolerance test (GTT) is to ascertain cases of diabetes before initiating preventative treatment. A further approach would be to develop models to determine which individuals with pre-diabetes are at greatest risk of diabetes complications and, hence, would be better treatment candidates.

Should we measure parameters other than glucose and, if so, which ones?

Larry Blonde (New Orleans, LA) discussed glycemic and nonglycemic parameters to be monitored in pre-diabetic patients and whether there is evidence of an effect of glycemic variability on complications of pre-diabetes. There were 17.9 million diagnosed and 5.7 million undiagnosed people with diabetes in the U.S. in 2007. IFG is present in 26% and IGT in 15% of the population, and their prevalence increases with age (13).

A1C is associated with fasting and 2-h postload glucose levels (14) and could be considered for diabetes diagnosis, particularly given its relationship with progression of microvascular disease and given the somewhat poor reproducibility of the GTT, with fasting glucose having 6.4% and 2-h glucose 16.7% variability (15). A1C does not require fasting and has good standardization, although nonglycemic factors affecting A1C should be taken into account. In a study mainly of type 1 diabetic patients, the correlation between average glucose on continuous glucose monitoring and A1C had an r^2 of 0.84 (16), although Blonde noted that it appears likely that ethnic-specific values need to be developed for such an analysis. Individuals with A1C >6% could then be said to have pre-diabetes, while A1C ≥7% on two occasions might be considered acceptable in confirming the diagnosis of diabetes (17). Home glucose monitoring might also play a role in the diagnosis of diabetes (18). Another potential measure is the serum level of 1,5-anhydroglucitol, a monosaccharide similar to glucose ingested in the diet. Its tubular reabsorption is prevented by glycosuria, so higher levels equate to lower glycemia.

Blonde suggested several other interesting parameters that might be monitored. 25-hydroxy-vitamin D may be associated with risk of developing diabetes (19–21). Oxidative stress has been the subject of much investigation (22). Intermittent hyperglycemia may cause this by activating protein kinase C (PKC) (23), and there is evidence that postprandial glycemia is associated with elevations in prostaglandin F2α (24,25), a potential measure of this process. Advanced glycation end products may be involved in complications of pre-diabetes, and there is evidence that skin advanced glycation end product levels can be measured from small-punch biopsy sites (26). In the future, Blonde speculated, genetic markers such as *TCF7L2* will also be assessed in determining diabetes risk.

Can society afford the costs of treating or not treating the pre-diabetes state?

William Herman (Ann Arbor, MI) discussed costs of type 2 diabetes and approaches to assessing cost-effectiveness of diabetes prevention, suggesting it to be a highly useful endeavor. Taking into account diabetes, its complications, and general medical care, the annual direct cost of diabetes is 116 billion USD, with an additional indirect cost of illness, disability, and premature mortality totaling 58 billion USD (27). Of these amounts, 56% is incurred by individuals aged ≥65 years and 35% by those aged 45–64 years. Fifty percent of direct costs are in hospital, 24% for pharmaceutical products and supplies, 20% for outpatient care, and 6% for nursing home care. "We're spending a lot of the money on older people and a lot of it is driven by late, chronic complications," Herman summarized. Macrovascular disease contributes 52% of costs, but as diabetes duration increases, the costs of microvascular complications, particularly of diabetic nephropathy, become more important (28). Cost-effectiveness may be calculated as the ratio between the difference in cost of intervention versus usual care to the difference in health outcomes associated with intervention versus usual care. Cost-utility analysis includes costs of the interventions and outcomes, expressing outcomes in quality-adjusted life years (QALYs). Thus, perfect health might be assigned a utility of 1.0, pre-diabetes 0.8, diabetes 0.6, diabetes with complications 0.4, and death 0.0. Assuming the usual cost of pre-diabetes to be 800 USD and that of pre-diabetes with intervention 1,600 USD annually, and annual costs of uncomplicated and complicated diabetes of 1,800 and 3,000 USD, respectively, Herman presented an analysis of a Diabetes Prevention Program–type intervention that, on average, delayed diabetes onset by 5 years (3.4 years with metformin and 11.1 years with lifestyle modification). If complications began an average of 10 years after diagnosis, Herman calculated that, viewed over a 30-year period, standard and intervention approaches beginning with pre-diabetes cost 56,000 and 57,000 USD, respectively; however, with outcomes of 18 vs. 20 QALYs, the intervention approach thus costs 500 USD per QALY (29), representing a very inexpensive approach compared with typical intervention costs of well over 10,000–20,000 USD per QALY. "In essence," Herman said, "the costs of pre-diabetes will be paid later if we don't do anything today."

What future research is needed to further clarify the diagnosis and management of the pre-diabetes state?

Gerald Shulman (New Haven, CT) discussed the role of skeletal muscle in the pathogenesis of type 2 diabetes and the metabolic syndrome. Using nuclear magnetic resonance (NMR) spectroscopy, it is possible to noninvasively follow intracellular metabolism. This approach allows measurement of incremental change in muscle glycogen in normal versus type 2 diabetic individuals (30), showing a profound defect in muscle glycogen synthesis. Assessment of the rate-controlling step comparing uptake via GLUT4, hexokinase activity causing glucose-6-phosphate (G6P) formation, and glycogen synthase for glycogen formation (31) shows the defect to be in the glucose transport step. Hexokinase and glycogen synthase might then, Shulman commented, be poor pharmacologic targets. Use of calf-muscle proton NMR to measure intramyocellular fat shows this to be the best predictor of insulin resistance (32). The Randle hypothesis predicts that fat-induced insulin resistance involves competition between fatty acids and glucose in uptake by the inhibition by phosphofructokinase to increase G6P levels (33), but phosphate NMR measurement of G6P (34) and carbon NMR to measure glucose shows that fatty acids directly inhibit skeletal muscle GLUT4 (35). Fatty acids abolish insulin activation of phosphatidyl inositol-3-kinase (34). Elevated plasma free fatty acids increase intracellular diacyl glycerol, leading to kinase Cφ activation, which causes insulin receptor substrate-1 serine phosphorylation to reduce phosphatidyl inositol-3-kinase and thereby decrease GLUT4 activity. Targets to block this include

PKCφ, acetyl-CoA carboxylase, and uncoupling protein-3. In the liver, a similar pathway of fatty acid–induced insulin resistance via increased diacyl glycerol causes PKC-ε activation (36). There are, then, multiple potential sites that rationally could be exploited in developing interventions. In mice lacking adipose tissue, there is severe muscle and liver insulin resistance associated with doubling of both liver and muscle fatty acyl CoA, all normalized by adipose tissue transplantation (37). "It's not a question of how much fat we have," Shulman commented. "It's really how the fat is distributed." The important common mechanism of treatments, then, involves reduction of intracellular fat in muscle and liver, a potential mechanism of effect of the thiazolidinediones (TZDs).

Shulman reviewed studies of individuals with lipodystrophy in which leptin administration for 6 months normalized fasting glucose, with improvement in insulin-stimulated muscle glucose uptake (38). Proton NMR in these patients demonstrated that liver and muscle fat were markedly reduced by treatment. In a less extreme example, weight loss with a 1,200-calorie diet in obese type 2 diabetic patients decreased fasting glucose and hepatic triglyceride concentration (from 10 to 2%), associated with reduction of hepatic glucose production and near-normalization of hepatic insulin sensitivity, though without changing circulating cytokines (39). Intracellular fat and diacyl glycerol appear to underlie these abnormalities; fat cells that "hold onto fat" are the answer, as shown with the "fit fat" phenotype. Mitochondrial abnormalities, either acquired or inherited, may underlie some of these conditions.

Shulman asked whether the metabolic syndrome is derived from such states. In a study of insulin sensitivity among 400 lean, healthy, 20-year-old individuals, ^{13}carbon NMR was used to measure glycogen, proton NMR to measure intracellular fat, and magnetic resonance imaging to quantitate visceral fat (40). There were no differences in intra-abdominal fat between insulin-sensitive and insulin-resistant individuals, but insulin levels after a carbohydrate load markedly differed, with the insulin-resistant group forming less muscle glycogen and markedly more hepatic triglyceride, increased de novo lipogenesis, and an association with increased circulating triglyceride and reduced HDL cholesterol levels. These abnormalities normalize with exercise (41). Shulman pointed out that IFG and IGT may have different determinants, with fasting hyperglycemia caused by increased hepatic gluconeogenesis while postload hyperglycemia reflects a reduction in glucose uptake by liver and muscle.

Jack Leahy (Burlington, VT) discussed the β-cell. Each person has ~1 million pancreatic islets, which he described as multicellular organelles with complex interactions of nutrients, growth factors, neurotransmitters, and incretins regulated by numerous cellular receptors and nuclear receptor transcription factors. The curvilinear relationship between insulin action and insulin secretion suggests that declining β-cell function from an already decreased baseline underlies worsening glucose tolerance among initially insulin-resistant individuals. He cited a just-published study of healthy individuals with normal glucose tolerance who underwent hemipancreatectomy to become pancreas donors between 1997–2003, 43% of whom had pre-diabetes or diabetes at follow-up (42).

A large number of type 2 diabetes susceptibility genes that regulate the β-cell have been discovered (*TCF2*, *IGFBP2*, *WFS1*, *CDKAL1*, *SLC30A8*, *CDKN2A/B*, *HHEX/IDE*, *TCF7L2*, *KCNJ11*, *CDC123-CAMK1D*, *THADA*, and *NOTCH2*) while fewer susceptibility genes have been identified affecting insulin sensitivity or with as-yet unidentified effects (43–45). "We're really still in the stage of just cataloging," Leahy commented, pointing out that there are "many years of hard work" to better understand these factors.

When the β-cell begins to decompensate, entering a stage of β-cell failure, β-cell mass decreases up to 40% in pre-diabetes and ≥60% in diabetes. The acute insulin response decreases in pre-diabetes and to an even greater extent in diabetes (46). Autopsy studies show that both pre-diabetes and diabetes are associated with reductions in β-cell mass (47). There is, however, evidence that a number of treatments allow the β-cell to recover function in type 2 diabetes: insulin administration (48), β-cell rest using diazoxide (49), somatostatin (50), TZDs (which also potentially reduce fatty acid-induced β-cell toxicity, as has also been shown with acipimox [51]), administration of GLP-1, and anti-inflammatory treatment using an interleukin-1 receptor antagonist (52). β-Cell dysfunction, then, may be a reversible phenomenon involving increased apoptosis, amyloid deposition, lipotoxicity, oxidative stress, inflammation, or impaired incretin effect (53). Leahy amplified Shulman's comment on the difference between IGT and IFG, noting that IFG particularly reflects hepatic insulin resistance such that β-cell dysfunction may be less of a factor, whereas IGT primarily represents a mismatch between insulin response and need, with failure of postprandial glucose clearance in part due to insulin resistance but also involving β-cell dysfunction and potentially being caused by excess glucagon action.

The precise pathogenesis of the β-cell defects is, however, unknown, and the concept that there is a pathophysiological difference between IFG and IGT should be recognized as speculative. Furthermore, β-cell function represents a number of independent functional and mass-related contributions, so there may be no simple test that can be said to measure β-cell function, with the integrated insulin response to an oral or intravenous glucose challenge not likely to represent the same function as the individual components of this response. Whether β-cell mass is the major determinant of insulin response is actually unknown, and there is certainly no evidence that durability of response to a given treatment will be dependent on its effect on β-cell mass or that treatment of pre-diabetes should be focused primarily on the β-cell. Approaches to measurement of β-cell function include homeostasis model assessment of β-cell function, the proinsulin-to-insulin ratio, measurement of insulin and C-peptide responses to oral or intravenous glucose, the frequently sampled intravenous GTT, and application of the related "minimal model" to meals, static or graded hyperglycemic clamps, the disposition index, and measurement of pulsatile insulin secretion or of the entrainment of pulsatile secretion. Many of these measures are abnormal in relatives of type 2 diabetes individuals, but it is not clear that any of these tests are adequately specific or sensitive to understand abnormalities of type 2 diabetes and pre-diabetes. Positron emission tomography–based imaging of ^{11}C-dihydrotetrabenazine bound to type 2 vesicular monoamine transporters in a baboon model has been demonstrated (54), suggesting that it may be possible to develop approaches for noninvasive β-cell mass assessment. Gene expression studies of islets obtained at autopsy from individuals with and without type 2 diabetes (55) may allow further information about

mechanisms of β-cell dysfunction. Promising directions of research include studies of incretin action, with GLP-1 having effects on β-cell proliferation, apoptosis, and mass (56), and of the TZDs, although one need not suggest that these agents are directly beneficial for the β-cell, as lowering the insulin requirement can allow deficient insulin secretory function to become adequate.

George Alberti (London, UK) discussed future research needed to further clarify the diagnosis and management of the pre-diabetic state. He noted that there were 309 million individuals with IGT worldwide in 2007, and there is a projection that there will be 419 million by 2025. Notions of pre-diabetes suggested in the 1950s were replaced in 1980 by the functionally similar recommendation of the World Health Organization that statistical risk classes be used, but the concept was reintroduced in 2002 by Department of Health and Human Services Secretary Tommy Thompson as an approach describing the conditions of IGT and IFG in a fashion that would communicate their high risk to the public. As presently defined, pre-diabetes may, however, omit other individuals at equally high risk, such as those with strongly positive family history, obesity, hypertension, dyslipidemia, CVD, metabolic syndrome, or a history of gestational diabetes mellitus who do not have abnormalities of glucose tolerance by current definitions. Furthermore, Alberti pointed out that 30% of individuals with pre-diabetes as defined by IGT or IFG will revert to normal glucose tolerance, and over their lifetimes only half will develop diabetes. This led him to question whether this group alone is deserving of the term. Indeed, the relative weights of the different risk factors for lifetime risk of diabetes need to be better clarified.

The recommended diagnostic criteria have changed over time. In 1979 and 1985, the National Diabetes Data Group and the World Health Organization suggested that IGT be defined by fasting plasma glucose between 140 and 144 mg/dl and 2-h postload glucose of 140–198 and 144–196 mg/dl, respectively. In 1985, fasting glucose <140 mg/dl and 2-h glucose of 140–198 mg/dl were suggested, and in 1997 the fasting glucose criterion was changed to a glucose of 110–125 mg/dl, introducing the concept of IFG. In 2003, the ADA suggested the lower limit of IFG be 100 mg/dl.

The purpose of labeling a person as pre-diabetic, Alberti suggested, should be to identify those at high risk of developing diabetes and CVD. He reviewed studies in Mauritius in 1987, 1992, and 1998. The population in the island comprises three ethnic groups—Asian Indians, blacks (Creoles), and Chinese—that together constitute two-thirds of the world's population, making this an excellent model. Those with IGT alone or IFG alone had 3.1–3.2 times increased risk of diabetes over 10 years, while those having both had nearly a fivefold increase in diabetes risk, leading Alberti to emphasize the usefulness of the GTT. Over a decade, one-third of those with IFG or IGT became normal glucose tolerant, one-third progressed to diabetes, and one-third continued to have pre-diabetes, although the frequency of progression appears to be worsening in recent studies (57). The long-term outcome and, crucially, the use of glucose tolerance information to recommend treatments are being explored.

The actual cut points used may not, Alberti noted, be correct. The upper limit is the diabetes criterion, while the lower is an arbitrary figure. Those with IGT at levels exceeding 170 mg/dl fasting glucose probably have considerably greater risk than those with 2-h glucose levels closer to 140 mg/dl. The IFG criteria based on equalizing the numbers of individuals with IFG and IGT do not appear to be an evidence-based recommendation, and it is more accurate to understand that there is a continuum of risk within the glucose levels currently considered to represent pre-diabetes. First-phase insulin secretion becomes abnormal beginning at a fasting glucose of 95 mg/dl, Alberti stated, and he suggested that there is no real glucose cut point for CVD risk and that it is not clear why the risk of elevated 2-h glucose exceeds that of elevated fasting glucose.

One might, Alberti suggested, simply change the terminology and state that individuals with IFG or IGT in fact have diabetes, as has been suggested by the finding of retinopathy within populations of individuals with pre-diabetes. However, it would be rather complicated to abruptly double the number of individuals with diagnosed diabetes. What, he asked, is the pathophysiological basis for pre-diabetes? Islet abnormalities, insulin resistance, and genetic polymorphisms have been found, and the apparent distinction that IGT represents decreased glucose disposal while IFG is a state of glucose overproduction may be of importance. How, he asked, should we endeavor to detect individuals with pre-diabetes? This becomes an issue particularly if the GTT is not routinely performed, he stated, suggesting that it may be useful to develop screening approaches to determine appropriate candidates for the GTT, perhaps screening individuals based on obesity, family history or ethnic group, hypertension, dyslipidemia, or CVD. A possible stepwise approach is to start with a questionnaire such as that described by Tuomilehto (as summarized in ref. 58), perhaps supplemented by waist circumference measurement, followed first by fasting glucose measurement and then the GTT. Although A1C might be measured rather than performing a GTT, Alberti preferred the latter, suggesting that development of a home kit with oral glucose and test strips might be useful in population screening. An interesting study would compare the natural history of pre-diabetic individuals found by screening, pre-diabetic individuals found by history, and individuals having diabetes at initial presentation.

Finally, Alberti asked whether pre-diabetes should be treated, and if so, how? Recalling that one-third revert to normal glucose tolerance, he asked whether a single abnormal GTT should be repeated. We do not know whether the aim of treatment should be restoration of normal glucose tolerance or prevention of diabetes. Prevention of CVD will probably, he suggested, be impossible to ascertain and may not be an appropriate goal for a state defined by glycemia, and he noted that statins would be more likely than metformin to have a benefit in this regard. At present, he suggested that use of acarbose and metformin might be considered, noting that "we don't have the data" to recommend treatment with other agents.

References

1. Meigs JB, D'Agostino RB Sr, Wilson PW, Cupples LA, Nathan DM, Singer DE: Risk variable clustering in the insulin resistance syndrome: the Framingham Offspring Study. *Diabetes* 46:1594–1600, 1997
2. Meigs JB, Nathan DM, Wilson PW, Cupples LA, Singer DE: Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance: the Framingham Offspring Study. *Ann Intern Med* 128:524–533, 1998
3. Rutter MK, Parise H, Benjamin EJ, Levy D, Larson MG, Meigs JB, Nesto RW, Wilson

PW, Vasan RS: Impact of glucose intolerance and insulin resistance on cardiac structure and function: sex-related differences in the Framingham Heart Study. *Circulation* 107:448–454, 2003
4. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB: Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation* 112: 3066–3072, 2005
5. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr, Wilson PW: Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. *Diabetes Care* 30:1219–1225, 2007
6. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr: Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch Intern Med* 167:1068–1074, 2007
7. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK: Cardiovascular risk factors in confirmed prediabetic individuals: does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA* 263:2893–2898, 1990
8. Haffner SM, Mykkänen L, Festa A, Burke JP, Stern MP: Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. *Circulation* 101:975–980, 2000
9. Festa A, D'Agostino R Jr, Howard G, Mykkänen L, Tracy RP, Haffner SM: Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (insulin resistanceAS). *Circulation* 102:42–47, 2000
10. Festa A, D'Agostino R Jr, Tracy RP, Haffner SM: Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study. *Diabetes* 51:1131–1137, 2002
11. Lorenzo C, Okoloise M, Williams K, Stern MP, Haffner SM: The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. *Diabetes Care* 26:3153–3159, 2003
12. Hanley AJ, Williams K, Festa A, Wagenknecht LE, D'Agostino RB Jr, Haffner SM: Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes* 54: 3140–3147, 2005
13. Nichols GA, Hillier TA, Brown JB: Progression from newly acquired impaired fasting glusose to type 2 diabetes. *Diabetes Care* 30:228–233, 2007
14. Woerle HJ, Pimenta WP, Meyer C, Gosmanov NR, Szoke E, Szombathy T, Mitrakou A, Gerich JE: Diagnostic and therapeutic implications of relationships between fasting, 2-hour postchallenge plasma glucose and hemoglobin a1c values. *Arch Intern Med* 164:1627–1632, 2004
15. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, Heine RJ: Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. *Diabetologia* 39:298–305, 1996
16. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, the A1c-Derived Average Glucose (ADAG) Study Group: Translating the A1C assay into estimated average glucose values. *Diabetes Care* 31: 1473–1478, 2008
17. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB: A new look at screening and diagnosing diabetes mellitus. *J Clin Endocrinol Metab* 93:2447–2453, 2008
18. Schwedes U, Siebolds M, Mertes G, the SMBG Study Group: Meal-related structured self-monitoring of blood glucose: effect on diabetes control in non–insulin-treated type 2 diabetic patients. *Diabetes Care* 25:1928–1932, 2002
19. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ: Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely prospective study 1990–2000. *Diabetes* 57:2619–2625, 2008
20. Knekt P, Laaksonen M, Mattila C, Härkänen T, Marniemi J, Heliövaara M, Rissanen H, Montonen J, Reunanen A: Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* 20 May 2008 [Epub ahead of print]
21. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K: Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 167:1159–1165, 2007
22. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A: Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. *Diabetes* 52:2795–2804, 2003
23. Ceriello A, Bortolotti N, Motz E, Pieri C, Marra M, Tonutti L, Lizzio S, Feletto F, Catone B, Taboga C: Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: the possible role of hyperglycemia. *Metabolism* 48:1503–1508, 1999
24. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C: Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 295:1681–1687, 2006
25. Brownlee M, Hirsch IB: Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. *JAMA* 295:1707–1708, 2006
26. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, Cleary PA, Lachin J, Genuth S, the DCCT Skin Collagen Ancillary Study Group: Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA_{1c} as markers of diabetic complications. *Diabetes* 48:870–880, 1999
27. American Diabetes Association: Economic costs of diabetes in the U.S. in 2007. *Diabetes Care* 31:596–615, 2008
28. Caro JJ, Ward AJ, O'Brien JA: Lifetime costs of complications resulting from type 2 diabetes in the U.S. *Diabetes Care* 25: 476–481, 2002
29. Herman WH, Hoerger TJ, Brandle M, Hicks K, Sorensen S, Zhang P, Hamman RF, Ackermann RT, Engelgau MM, Ratner RE; Diabetes Prevention Program Research Group: The cost-effectiveness of lifestyle modification or metformin in preventing type 2 diabetes in adults with impaired glucose tolerance. *Ann Intern Med* 142:323–332, 2005
30. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG: Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 322:223–228, 1990
31. Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL, Shulman GI: Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 341:240–246, 1999
32. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42:113–116, 1999
33. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1:785–789, 1963
34. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103:253–259, 1999

35. Shulman GI: Cellular mechanisms of insulin resistance. *J Clin Invest* 106:171–176, 2000
36. Neschen S, Morino K, Hammond LE, Zhang D, Liu ZX, Romanelli AJ, Cline GW, Pongratz RL, Zhang XM, Choi CS, Coleman RA, Shulman GI: Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. *Cell Metab* 2:55–65, 2005
37. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI: Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem* 275:8456–8460, 2000
38. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI: Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 109:1345–1350, 2002
39. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI: Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 54:603–608, 2005
40. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, Cline GW, Befroy D, Zemany L, Kahn BB, Papademetris X, Rothman DL, Shulman GI: The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci U S A* 104:12587–12594, 2007
41. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, Rothman DL, Shulman GI: Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N Engl J Med* 335:1357–1362, 1996
42. Kumar AF, Gruessner RW, Seaquist ER: Risk of glucose intolerance and diabetes in hemipancreatectomized donors selected for normal preoperative glucose metabolism. *Diabetes Care* 31:1639–1643, 2008
43. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881–885, 2007
44. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Boström K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Råstam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjögren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–1336, 2007
45. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345, 2007
46. Brunzell JD, Robertson RP, Lerner RL, Hazzard WR, Ensinck JW, Bierman EL, Porte D Jr: Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *J Clin Endocrinol Metab* 42:222–229, 1976
47. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
48. Garvey WT, Olefsky JM, Griffin J, Hamman RF, Kolterman OG: The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. *Diabetes* 34:222–234, 1985
49. Greenwood RH, Mahler RF, Hales CN: Improvement in insulin secretion in diabetes after diazoxide. *Lancet* 1:444–447, 1976
50. Laedtke T, Kjems L, Pørksen N, Schmitz O, Veldhuis J, Kao PC, Butler PC: Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. *Am J Physiol Endocrinol Metab* 279:E520–E528, 2000
51. Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E: Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. *Am J Physiol Endocrinol Metab* 292:E1775–E1781, 2007
52. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY: Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 356:1517–26, 2007
53. Nauck MA, Wollschläger D, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Willms B: Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7–36 amide]) in patients with NIDDM. *Diabetologia* 39:1546–1553, 1996
54. Souza F, Simpson N, Raffo A, Saxena C, Maffei A, Hardy M, Kilbourn M, Goland R, Leibel R, Mann JJ, Van Heertum R, Harris PE: Longitudinal noninvasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. *J Clin Invest* 116:1506–1513, 2006
55. Marselli L, Thorne J, Ahn YB, Omer A, Sgroi DC, Libermann T, Otu HH, Sharma A, Bonner-Weir S, Weir GC: Gene expression of purified beta-cell tissue obtained from human pancreas with laser capture microdissection. *J Clin Endocrinol Metab* 93:1046–1053, 2008
56. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R: Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 144:5149–5158, 2003
57. Söderberg S, Zimmet P, Tuomilehto J, de Courten M, Dowse GK, Chitson P, Stenlund H, Gareeboo H, Alberti KG, Shaw J: High incidence of type 2 diabetes and increasing conversion rates from impaired fasting glucose and impaired glucose tolerance to diabetes in Mauritius. *J Intern Med* 256:37–47, 2004
58. Bloomgarden ZT: American College of Endocrinology Pre-Diabetes Consensus Conference: part one. *Diabetes Care* 31:2062–2069, 2008