The Endocrine Society Meeting: Topics in Insulin Sensitivity and Hypertension

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Lectures at the Endocrine Society's 85th Annual Meeting in Philadelphia, Pennsylvania, 19–22 June 2003, showed the fascinating interplay of basic science and clinical medicine needed to appreciate our advancing knowledge of diabetes.

Signaling pathways mediating insulin sensitivity

A series of new intracellular signaling pathways related to insulin action are being identified. John C. Lawrence (Charlottesville, VA) discussed the mTOR pathway. The immunosuppressant rapamycin inhibits cell growth by interfering with the function of a kinase, termed mammalian target of rapamycin (mTOR). mTOR is defined by high affinity binding to rapamycin, regulating various steps in carbohydrate, lipid, and protein metabolism, particularly the protein kinases eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and the 40S ribosomal protein S6 kinase (p70s6k), which is involved in protein synthesis. mTOR is present in adipocytes and induced during adipogenesis. mTOR is regulated by an amino acid nutrient-sensing pathway that is particularly stimulated by branched chain amino acids such as leucine and growth factors such as insulin via protein kinase B (PKB), with a potential feedback loop whereby mTOR increases serine phosphorylation of insulin receptor substrate-1 and -2. Both insulin and PKB increase the phosphorylation of mTOR via another protein, mTOR kinase. 4E-BP1 is another protein with multiple phosphorylation sites affected by insulin; in particular, there are three threonine sites that are all affected by rapamycin. Lawrence concluded that mTOR, in combination with a series of other proteins, one with the intriguing name Raptor because of an effect of rapamycin that causes it to dissociate from the complex, binds and phosphorylates substrates such as 4E-BP1.

Gustav E Lienhard (Hanover, NH) addressed the question of the signal transduction pathway from the insulin receptor leading to GLUT4 translocation to the plasma membrane, mediating glucose transport. Insulin activates phosphoinositide 3-kinase, leading in turn to activation of a serine kinase originally identified as a proto-oncogene isolated from an AKR mouse T-cell lymphoma (Akt, also referred to as PKB). Akt in turn phosphorylates serine residues on specific proteins, including the 160-kDa protein Akt substrate (AS)160. AS160 has low intrinsic GTPase activity, but this is increased by a GTPase activating protein that converts GTP to GDP, leading to the dissociation of AS160 from the GLUT4 vesicle and to its movement to the plasma membrane. The Rab GTP binding proteins constitute a large family of GTP binding proteins involved in the recycling of proteins such as cell surface receptors from endosomes to the cell surface. Lienhard suggested that Rab, which is required for GLUT4 translocation, exists in GTP and GDP forms that can be interchanged. After insulin stimulates phosphorylation of AS160, its GTPase activating protein activity toward the Rab required for GLUT4 translocation is inactivated.

Steven Shoelson (Boston, MA) discussed the nerve factor (NF)-κB complex, containing this transcription factor present in an inactive cytoplasmic form, bound to a complex of inhibitory κB (IκB) proteins that acts by phosphorylating NF-κB. He suggested that these molecules are mediators of the association of inflammation with insulin resistance and pointed out three independent lines of evidence that suggest a role of inflammation. Epidemiologic studies show elevated levels of acute-phase reactants and inflammatory mediators in diabetes, cell biology shows that inflammatory cytokines attenuate insulin signaling through cross talk, and clinical data, which Shoelson reviewed, show a potential role of these molecules based on the hypoglycemic potential of salicylates (see summary in 1).

NF-κB is a site of integration of multiple proinflammatory inputs, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and lipopolysaccharide, leading to amplification with release of cytokines such as IL-6, IL-1β, and TNF-α, with activation of their respective receptors and subsequent production of further signaling proteins. IκB kinase (IKK)-β activity is increased in animal models of obesity, including the *fa/fa* and high-fat–fed rodent models, in association with increased levels of plasminogen activator inhibitor-1, signal transducer and activator of transcription (STAT)-3 and -6, suppressor of cytokine signaling 3 (SOCS3), and NF-κB itself. To explore its effects, animals with constitutive activation of IKK in fat (FIKK) and liver (LIKK) have been developed. IKKα protein levels are increased in fat from FIKK mice, which show somewhat greater weight gain, increased adipocyte size, and insulin resistance in glucose uptake compared with control subjects, although fat cells in these animals are histologically normal, with no evidence of inflammation. Increased levels of adipocyte products are seen in these animals, including IκB, resistin and resistin-like molecule-α, adiponectin (a possible compensatory effect), and peroxisome proliferator–

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Abbreviations: 4E-BP1, 4E binding protein 1; ARB, angiotensin II (type 1) receptor blocker; AS, Akt substrate; CHD, coronary heart disease; CHF, coronary heart failure; CVD, cardiovascular disease; ESRD, end-stage renal disease; FFA, free fatty acid; HSD1, hydroxysteroid dehydrogenase type 1; IκB, inhibitory κB; IKK, IκB kinase; IL, interleukin; LVH, left ventricular hypertrophy; MCH, melanin concentrating hormone; mTOR, mammalian target of rapamycin; NCEP, National Cholesterol Education Program; NF-κB, nerve factor-κB; PCOS, polycystic ovary syndrome; PKB, protein kinase B; PPAR, peroxisome proliferator–activated receptor; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor; WHO, World Health Organization; WHR, waist-to-hip ratio.

activated receptor (PPAR)-γ and -α. The animals become insulin resistant with hyperinsulinemia and hyperglycemia, suggesting effects on other tissues, and before development of insulin resistance, serum amyloid A, IL-6 receptor-α, and IL-1 receptor type 1 are increased. Oral salicylates, which block IKKβ, completely reverse the phenotype. In the LIKK mice, liver is histologically normal without evidence of inflammation. These animals also show evidence of systemic insulin resistance to a greater extent than that seen with FIKK. IL-6 levels are elevated, and anti–IL-6 antibody reverses the insulin resistance, suggesting that it is a mediator. Just as Shoelson's studies with salicylates suggest promising approaches to the abnormalities in this signaling pathway in diabetes, it is therefore likely that further understanding of the molecular biology of insulin action will lead to additional therapies to improve insulin sensitivity.

The metabolic syndrome

Jeffrey Flier (Boston, MA) gave the Edwin B. Astwood lecture discussing fat cells, hormones, and obesity-related disorders, as well as current understanding of the adipocyte as an endocrine tissue. The classical endocrine glands and hormones affect fat by both direct and indirect mechanisms. The core biology of fat is to store energy as triglyceride and then release it when needed. In addition to their role as fuels, fatty acids can also be signaling molecules as ligands for various PPARs and for membrane receptors, as well as by altering kinase cascades in a number of cells. Fat also produces signals acting on distant targets. Adipose tissue through aromatase p450 enzymes can turn weak androgens into weak estrogens, with a role in obesity-related malignancy and other effects. Adipose tissue produces complement factors such as adipsin (complement factor D; discovered in 1985), a secreted serine protease with its gene massively deficient in *ob/ob* and *db/db* rodents, although adipsin repletion in these rodents does not reverse the phenotype of these animals. Adipose tissue also produces inflammatory molecules such as TNF-α. Other factors overexpressed by obese fat cells include angiotensinogen and plasminogen activator inhibitor 1. Resistin and adiponectin are other molecules produced in fat, and adiponectin is an important circulating hormone-like molecule with complement-like features that are highly associated with insulin sensitivity and lipid oxidation, acting via two novel receptors that activate AMP kinase.

"The mother of all adipocyte hormones," Flier said, "is leptin." This cytokine-like product of fat cells has inactivating mutations causing the *ob* mouse and similar illness in humans, curable with leptin administration. Flier pointed out that the "simple view of leptin as an anti-obesity hormone," which increases with increasing fat cell size and signals the brain to decrease food intake, is incomplete. "More compellingly," he said, leptin acts as a starvation signal via reductions in circulating leptin levels. With starvation, adaptations include lowering leptin and insulin and increasing cortisol and other hormones. In women, caloric restriction decreases thyroxin and causes ovulatory delay, both of which are improved by administration of leptin. When subcutaneous leptin is given every 6 h to men under conditions of starvation, leptin increases to nonstarvation levels. In these individuals, there is suppression of testosterone, with coordinate changes in pulsatile leutinizing hormone secretion, and suppression of thyroid-stimulating hormone over the short term and of thyroxin over the long term; however, these effects are reversed by leptin administration.

These findings suggest a role of leptin in hypothalamic amenorrhea, in the oligomenorrhea associated with athletic activity, and in anorexia nervosa. Leptin may play a role in the timing of puberty, although GnRH appears not to be a direct target of leptin, and it will be important to determine the mechanism of these changes. A key site of leptin is in the arcuate nucleus of the hypothalamus, where a group of neurons produce Agouti-related peptide and neuropeptide Y (NPY) promote feeding and another group of neurons produce α-melanocyte–stimulating hormone, proopiomelanocortin, and cocaine- and amphetamine-regulated transcript, which suppress feeding and body weight. "A major part of the circuit" is made up of neurons expressing melanocortin 4 receptors, and abnormality at this receptor may account for 5% of severe human obesity. Melanin concentrating hormone (MCH) is expressed in the lateral hypothalamus, and its expression is upregulated in the *ob/ob* mouse, with intracerebroventricular administration of MCH increasing feeding. In the *ob/ob* mouse, the absence of MCH "limits the phenotype." Flier suggested that this locus, which is associated with activated hypothalamopituitary adrenal axis and increased fat mass, although not with hyperphagia, may be "an excellent obesity drug target" with antagonists appearing powerful in suppressing dietary-induced obesity.

Partial leptin resistance is seen in spontaneous obesity in rodents and in humans, although not interfering with leptin effects on the reproductive axis. Leptin signaling involves janus kinase 2, with downstream activation of other kinases in a cascade leading to expression of proopiomelanocortin, Agouti-related peptide, and neuropeptide Y. An antagonistic factor is SOCS3, which is induced by activation of the leptin receptor and then causes feedback inhibition of leptin receptor signaling. Compared to mice on a low-fat diet, the leptin activation of STAT-2 on a high-fat diet is decreased in obese animals. There are leptin transport proteins at the blood-brain barrier that are decreased in obesity, and antagonists at the leptin-responsive neurons. SOCS3 haplo-insufficiency causes resistance to high-fat diet–induced obesity, as well as preventing the increase in leptin, insulin, and glucose levels, suggesting that it is a mediator of leptin resistance.

Adipocytes also are a source of glucocorticoids mediated by the enzyme 11 β-hydroxysteroid dehydrogenase type 1 (HSD1), leading to production of cortisol from corticosterone, while other tissues, such as the kidneys, inactivate cortisol to corticosterone via HSD2 (vide infra). In a mouse overexpressing HSD1, there is obesity, particularly visceral, local cortisol excess in fat, hyperphagia, and hypertension, perhaps driven by induction of angiotensinogen. There is a positive correlation between BMI and HSD1 activity of subcutaneous fat, suggesting that inhibitors of adipocyte HSD1 may be therapeutically important.

Eleuterio Ferranini (Pisa, Italy) discussed the metabolic syndrome, which he characterized as being both topical and controversial. From an epidemiologic standpoint, the syndrome lends itself to measurement across populations. It is possible to carry out fascinating studies of its pathophysiology. What is needed clinically is the determination of approaches of high specificity and therapeutic benefit

in order to apply the concept in individual patients. There are several definitions, beginning with Reaven's work, in which he postulated that the cluster of a particular form of dyslipidemia with high triglyceride and low HDL cholesterol, insulin resistance, and abnormal glucose tolerance could constitute a syndrome (2). Subsequent work has extended the syndrome to include obesity with predominance of visceral fat, prothrombosis, antithrombolysis, microalbuminuria, hypertension, endothelial dysfunction, and low-grade inflammation.

The World Health Organization (WHO) definition requires insulin resistance and/or impaired glucose tolerance and two or more of the following: dyslipidemia (low HDL and/or high triglyceride), hypertension, increased waist-to-hip ratio (WHR), and microalbuminuria (3). In population-based studies in Europe and the U.S., using this definition there is a 20–30% prevalence that increases with age and is higher in men. In a multicenter study assessing insulin resistance in a variety of populations, the European Group for the Study of Insulin Resistance studied 1,466 subjects aged 18–85 years without diabetes or hypertension, approximately half of whom had a BMI <25 kg/m^2 using a 2-h euglycemic clamp with high physiologic insulin levels, as "a direct measure of the sensitivity of all the tissues in the body to insulin."

Ferranini compared insulin-resistant and -sensitive individuals in this population, using "a purely statistical criterion" comparing the lean subjects with the fourth quartile of insulin sensitivity. This gives the phenotype of 225 insulin-resistant and 1,125 insulin-sensitive subjects, the former with an *M* value (the amount of glucose required to maintain euglycemia during the insulin infusion) of approximately half that of the latter. The former are more likely to have a family history of diabetes, higher BMI and WHR, impaired glucose and increased lipid oxidation, increased endogenous glucose production, increased free fatty acids (FFAs), and higher systolic and diastolic blood pressure, although within the normal range. Addressing the question of the use of fasting insulin as a surrogate measure, individuals with insulin resistance have higher levels on average, but for a given insulin level, there is a wide range of insulin sensitivities. Indeed, defining insulin resistance by the top quartile of the insulin level of a lean population leads to a different population from that based on insulin sensitivity. Thus, although hyperinsulinemia is a compensatory response to insulin resistance, there is a degree of maladaptation, leading to varying degrees of chronic exposure to elevated insulin levels at tissues not showing the same resistance as that to glucose metabolism. There is also a strong association between fat mass and insulin resistance, but, again, obesity and insulin resistance define different populations and lean insulin-resistant individuals show a similar phenotype to those who are obese. Particularly among women, insulin resistance is associated with increased triglyceride levels, with an additional effect of the insulin level, confirming the concept of a maladaptive effect.

In another study comparing untreated hypertensive and normotensive men with similar age and BMI, there were similar HDL and LDL and slightly higher triglyceride levels; visceral fat, however, was ~60% higher in the hypertensive group and showed a linear relationship to blood pressure, correlating in turn with the degree of insulin resistance. Thus, Ferranini said, ectopic fat accumulation "carries a great impact," possibly being conducive to the development of hypertension.

Addressing the question of whether the metabolic syndrome is predictive of adverse outcome, Ferranini presented data from the Mexico City Diabetes Study of 2,268 subjects who had an OGTT at baseline and at 7-year follow-up. There were 173 subjects without diabetes initially who converted to diabetes at 7 years, of whom 98 had normal glucose tolerance and 75 impaired glucose tolerance at baseline. Compared with those who were and remained normal glucose tolerant, converting subjects had higher waist circumference, higher fasting and 2-h insulin, higher triglyceride, lower HDL, and higher systolic and diastolic blood pressure, regardless of the initial degree of glucose intolerance. Thus, these subjects had the metabolic syndrome. Of those with normal blood pressure at baseline, 215 developed hypertension at follow-up, with an excess of converters among those with diabetes. Glycemic abnormality predicts hypertension and increased blood pressure predicts glycemic abnormality, with hyperinsulinemia an important additional predictive factor, Ferranini noted, further suggesting the usefulness of the concept of metabolic syndrome. He concluded that "the syndrome itself is atherogenic," but suggested that insulin resistance causes atherosclerosis via the "intermediate phenotypes" of increased blood pressure, dyslipidemia, and abnormal glycemia, suggesting that therapy not be primarily directed at insulin resistance. The metabolic syndrome exists, and he asserted, "It predicts itself so it's not just an innocent cluster." Whether it is directly atherogenic is not clearly established, and whether it can be prevented is an important therapeutic question.

Gerald Shulman (New Haven, CT) discussed the role of FFAs in the pathophysiology of insulin resistance. "In order to progress to frank hyperglycemia," β-cell failure is preceded by defects in skeletal muscle glucose uptake for a number of years. Using nuclear magnetic resonance spectroscopy to measure intramuscular ^{13}C in glycogen and noninvasively measure glycogen synthesis, it is possible to quantitate normal and diabetic muscle glucose uptake into glycogen. "There is a profound defect" in the diabetic group, with approximately half the glucose uptake of that in normal subjects during a euglycemic-hyperinsulinemic clamp, almost all in normal and diabetic individuals leading to glycogen synthesis. He discussed evidence that, of the potential rate-limiting steps in glycogen synthase, hexose kinase, and GLUT4, the defect is shown to be at the GLUT4 glucose transport step, which then might be a useful pharmacologic target for diabetes treatment, while hexose kinase and glycogen synthase would presumably not be good targets for treatment (see summary in 4). The degree of insulin resistance is directly correlated with the FFA level, and intramuscular lipid is an even stronger predictor, suggesting that "the more fat you have inside muscle, the more insulin resistant you are." This is suggestive of the Randall hypothesis that increased FFAs inhibit glycolysis, although Shulman's findings suggest that the mechanism of adverse effect of FFAs involves direct interference with GLUT4 via abnormal trapping or intrinsic activity of GLUT4, with the intracellular fatty acid metabolite fatty acid-CoA and diacylglycerol activating a serine kinase cascade involving PKC-θ, leading to increased serine and decreased tyrosine phosphorylation of insulin receptor substrate-1, re-

sulting in the defect in GLUT4 translocation. In a related study, mice completely lacking adipose tissue, like patients with severe lipodystrophy, have high triglyceride and FFA levels and develop severe insulin resistance. These mice have both muscle and liver insulin resistance with increased fatty acid-CoA in both tissues, suggesting that fat accumulates in abnormal sites causing insulin resistance and that transplantation of adipose tissue normalizes the muscle and liver. "It's not so much how much fat we have. It's how it's distributed. . . Anything we can do to lower intramyocellular and hepatic fat is important." Shulman suggested that the PPAR-γ agonists act by increasing adipocyte triglyceride stores and decreasing hepatic and muscle fat. In patients with lipodystrophy, with fatty liver and severe insulin resistance, a 3- to 6-month period of leptin administration reverses fatty liver, normalizes hepatic insulin sensitivity, and improves that in muscle, consistent with these concepts. A new study addressing the association of insulin resistance with aging compared matched lean older with younger individuals, showing that there was mildly decreased glucose tolerance and hyperinsulinemia but severely decreased muscle insulin sensitivity, with increased muscle and liver fat. Intramyocellular mitochondrial oxidation and ATP synthase flux measurements with nuclear magnetic resonance technologies show 30–40% decreases in the older group, suggesting that accumulation of fatty acid metabolites due to decreased metabolism lead to impairment in insulin sensitivity. Shulman pointed out that regular exercise is an excellent approach to reversing this defect.

Steven Haffner (San Antonio, TX) discussed the definition of the metabolic syndrome, stating, "I don't know what the definition is. . . [and] no one else does." The idea of clustering cardiovascular risk factors was initially thought due to obesity, and subsequently was suggested by Reaven to be due to insulin resistance. The metabolic syndrome could be defined by clinical outcome, the approach taken by the National Cholesterol Education Program (NCEP) (5), or by underlying cause, as recommended by the WHO. Of clinical outcomes, Haffner suggested both the prediction of cardiovascular disease (CVD) and that of type 2 diabetes may be important. Pre-diabetes "somewhat imprecisely" has been thought to include both impaired glucose tolerance and impaired fasting glucose. The Nurses Health Study has shown that individuals not developing diabetes have the lowest CVD risk and that those who develop diabetes have a 3.75-fold increase in risk before the onset of diabetes (6). A critical question is whether prevention strategies decrease CVD, as suggested by the STOP-type 2 diabetes trial (7). The definition can require insulin resistance, markers of obesity and inactivity, or low-grade inflammation, each implying a different therapeutic approach.

The NCEP guidelines focus on abdominal obesity, hypertension, triglyceride, HDL, and impaired fasting glucose, with the therapeutic implication being a focus on obesity. The WHO definition is more complicated, requiring insulin resistance, based on diabetes, impaired fasting glucose, impaired glucose tolerance, or a hyperinsulinemic clamp or homeostasis model assessment calculation, the latter depending on the nonstandardized measurement of insulin concentrations, as well as blood pressure ≥140/90 mmHg, triglyceride 150 mg/dl and/or HDL <35/40 mg/dl (men/women), increased BMI or WHR, and microalbuminuria. An important difference between the definitions is that the WHO requires insulin resistance and the NCEP does not, although most individuals satisfying the metabolic syndrome requirements by the latter will in fact have insulin resistance. A third alternative is to regard the metabolic syndrome as an accumulation of different risk factors, with all the risks then residing in the individual risk markers. Haffner noted that both the NCEP and WHO definitions permit the inclusion of type 2 diabetes, although the more recent definition of the American College of Endocrinology has excluded this group (8). He further pointed out that some components of the various definitions may be better for predicting diabetes, whereas others may be better for prediction of coronary heart disease (CHD). Another important aspect of the definitions is the simplicity of their application in clinical use. Using the NCEP guidelines, the overall rate of metabolic syndrome in the U.S. is 23%, increasing from ~7% in people <30 years of age to 40% in those >60 (9). The apparently higher rate of metabolic syndrome in Hispanic women, Haffner pointed out, is due to a peculiarity of the NCEP guidelines, which allows low HDL and high triglyceride to be counted as separate risk factors. Use of cluster analysis suggests that hypertension is statistically separate from insulin resistance, suggesting different causal mechanisms.

There are very high CVD rates in individuals with metabolic syndrome (10), although Haffner suggested that this might be due to the inclusion of diabetic subjects. Using National Health and Nutrition Examination Survery data in individuals aged ≥50 years, 17% of the population has type 2 diabetes and 85% of those with diabetes have the metabolic syndrome (11). Haffner cited a recently presented analysis of the ADOPT (A Diabetes Outcome Progression Trial) study of ~4,500 recently diagnosed type 2 diabetic patients, of whom 78 and 82% had the metabolic syndrome using the WHO and NCEP criteria, respectively, with the prevalence of CHD much lower in type 2 diabetic subjects who did not have the syndrome. Analysis of two studies in which Haffner participated, the San Antonio 14-year follow-up and the Insulin Resistance Atherosclerosis Study, suggests that the WHO criteria may be more predictive of diabetes, whereas the NCEP criteria are more sensitive for CVD risk. Haffner implied that the use of markers of inflammation may allow additional sensitivity in the definition of the metabolic syndrome, with CRP related to insulin resistance and the development of type 2 diabetes in the Insulin Resistance Atherosclerosis Study. Intervention with lifestyle decreases CRP, with metformin showing weaker effect and thiazolidinediones and statins also decreasing this.

Willa Hsueh (Los Angeles, CA) discussed potential approaches to treatment of the metabolic syndrome, noting that a study of Mexican-American adults with a parent having diabetes compared with their age, BMI, WHR, blood pressure, lipid, and fasting glucose and insulin-matched spouses, showed significantly greater carotid intima-media thickness, "suggesting that genetics is very important, and may be one of the determinants." In the Diabetes Prevention Program, a lifestyle intervention reduced likelihood of diabetes 58% and metformin decreased risk 31% (12). Agents that improve endothelial function may also prevent CVD and diabetes. In the West of Scotland Coronary Prevention Study, pravastatin decreased diabetes incidence 30% (13). In the Heart Outcomes

Prevention Evaluation trial, among 5,720 patients >55 years of age without known diabetes but with vascular disease who were followed-up for a mean of 4.5 years, ramipril decreased diabetes 34% (14). The Losartan Intervention For End point reduction in hypertension study showed both improved endothelial function and a 25% decrease in diabetes among individuals treated with this agent (15). There is a suggestion that the PPAR-γ agonists prevent diabetes in trials with troglitazone and of CVD protection. In a mouse model not expressing the LDL receptor, aortic lesions develop with a high-fat diet and are accelerated with chronic angiotensin II infusion. Hsueh showed that treatment with rosiglitazone, pioglitazone, and a nonthiazolidinedione PPAR-γ ligand significantly attenuate this, as well as decreasing inflammatory cytokines levels. In response to a question as to whether the polycystic ovary syndrome (PCOS) should be considered to imply the presence of the metabolic syndrome, Hsueh noted that many of these women qualify because of existing additional factors, and Haffner commented, "As a clinician you are entitled to use your clinical judgment."

Richard S. Legro (Hershey, PA) reviewed aspects of the relationship between the PCOS, characterized clinically by hyperandrogenism, chronic anovulation, and polycystic ovaries, and the cardiovascular system, raising a cautionary note in recalling Mark Twain's adage, "What you know that isn't true will cause you more harm than what you don't know at all." Current thought, he stated, is that women with PCOS are insulin resistant, that insulin resistance is a CVD risk factor, and therefore that women with PCOS are at increased CVD risk. Clearly, glucose intolerance, obesity, dyslipidemia, and physical inactivity are increased. There are surrogate markers of endothelial dysfunction, cardiac dysfunction, oxidative stress, increased inflammatory markers, and altered hemostasis. Legro suggested that we be cautious, however, before concluding that these women definitely experience an increase in CVD.

He reviewed the existing literature on the topic. Risk factors clearly are worse in PCOS. After adjustment for BMI, hormone use, and insulin levels, women age <40 years with PCOS have higher LDL cholesterol levels (16). A study of 206 women with PCOS, compared with age-matched control subjects and followed for 10 years, showed increases in BMI, WHR, systolic blood pressure, LDL cholesterol, insulin, and triglyceride and decreases in total HDL and HDL2 levels (17). Interestingly, there is no evidence of association of PCOS with hypertension, with a study of 14 PCOS vs. 18 control subjects matched for age, race, and BMI showing comparable fasting glucose levels, higher 2-h postload glucose (144 vs. 101 mg/dl), 59% lower insulin sensitivity, but ambulatory systolic and diastolic blood pressures similar and no difference in degree of left ventricular hypertrophy (LVH) (18).

There is a paucity of evidence of increased risk of CVD per se. Some indirect data are available; one study showed that carotid intima-media thickness was increased in women over age 44, with 9 of 125 patients vs. 1 of 142 control subjects having a plaque index ≥3, which was significant after adjustment for age and BMI (19). Another study measuring coronary artery calcification (CAC) reported increased levels in 39% of 36 women with PCOS, in 21% of 71 age- and weight-matched control subjects, and in 9% of age-matched women from a CAC database (20). For definite CVD, the data are also scanty. In a study of 102 women undergoing cardiac catheterization, hirsutism and the WHR were significantly increased (21). A study of 45 women hospitalized with CHD (29 with myocardial infarction and 16 with angiographically confirmed coronary disease), who had participated in a breast cancer–risk study of 11,284 women aged 40–49 years, showed that urinary excretion of estrone-glucuronide, pregnanediol-glucuronide, and testosterone-glucuronide adjusted by creatinine were similar to levels in control subjects, although anovulatory cycles appeared more frequently in the women who developed CHD many years later (22). Among 786 women who had wedge resection for PCOS between 1930 and 1979, during a 30-year follow-up, there was no increase in CVD or cancer death, although there was a significant 3.6-fold increase in deaths from diabetes (23). Further study in this group, correcting for BMI, showed increased cerebrovascular disease risk and a significant increase in cholesterol adjusted for BMI (24). Finally, in the large Nurses' Health Study, menses were very regular during 715,293 person-years, regular during 264,924, irregular during 126,404 (11%), and very irregular during 49,292 (4%), with CHD risks 1.25- and 1.67-fold greater in the latter two groups, remaining significant after adjustment for BMI, with evidence of more frequent diabetes, hypercholesterolemia, and hypertension (25). Chronic anovulation represents, Legro stated, a heterogeneous group, with both hypo- and hyperestrogenism; therefore, the implications of this study may not be entirely clear.

He concluded, "There are an excess of studies documenting risk factors and a paucity of studies documenting events. There is a need for larger multicenter prospective studies of women with PCOS through the menopause and beyond. . . We really need to find this out before we start intervening as much as we are."

Issues in hypertension

Robert Carey (Charlottesville, VA) discussed the angiotensin II type 2 receptor (AT2), outlining his presentation by noting that the actions of angiotensin II are carried out by angiotensin II binding to either AT1 or AT2, which generally oppose each other. AT2 activates a vasodilator cascade of bradykinin, nitric oxide (NO), and cGMP and, when AT1 is blocked, simulation of AT2 lowers the blood pressure via vasodilation, stimulation of natriuresis, and, in the heart, protecting against fibrosis, reduced contractile function and LV remodeling in heart failure and following myocardial infarction, which may be particularly important as AT2 increases in expression in response to vascular and cardiac injury.

Angiotensin II acts by binding to AT1 or AT2, with most of our understanding of the peptide based on its action at AT1, causing vasoconstriction, sodium reabsorption, inhibition of renin, stimulation of aldosterone secretion, and cardiac inotropism. In contrast, we know little about the function of AT2, although this is clearly a counterregulatory vasodilatory receptor, downregulating AT1 expression, and inhibiting growth and cellular proliferation. AT2 is a GTP-binding protein (G-protein)-coupled receptor, binding to and hydrolyzing GTP to GDP, showing 34% homology with AT1. The AT2 signaling mechanism involves G-protein coupling activating phosphotyrosine phosphatases leading to inhibition of extracellular signal-regulated kinases (ERKs), forms of mitogen-activated pro-

tein kinases. AT2 activation increases ceramide synthesis and arachadonic acid metabolites, with kininogenase activation leading to bradykinin production, leading to NO synthase causing NO release from endothelial cells, causing vasodilation.

The AT2 receptor is expressed to highest extent in the fetus, and its expression is higher in primates and sheep than in rodents, which is important as rodents are used experimentally in a wide variety of biologic studies. AT2 is expressed in a wide variety of tissues, including the cardiac atrial and ventricular myocytes, coronary arteries, aorta, small resistance arteries, particularly in vascular smooth muscle, the adrenal medulla and glomerulosa, the renal tubules, particularly proximally but also in the distal tubules and collecting ducts, and in afferent and efferent vessels. AT2 protein is present in the proximal and distal tubule. AT2 stimulation leads to increased renal interstitial tubule bradykinin, a process potentiated by the AT1 blocker losartan and inhibited by the AT2 antagonist PD123319. AT2 inhibits AT1 receptor–mediated sodium and bicarbonate reabsorption, and AT2 null mice have decreased pressure natriuresis and show decreased renal nitric oxide and cGMP levels both at baseline and in response to angiotensin II infusion or sodium depletion. Renal vasodilation stimulated by AT1 blockade can be nullified by AT2 receptor blockade. Angiotensin II induces renal vasoconstriction, but vasodilation is seen when angiotensin II is administered with losartan, which can be prevented by the AT2 antagonist. Vascular AT2 overexpression eliminates the vasoconstrictive response to angiotensin II. AT1 is internalized leading to downregulation, but AT2 is not, leading to a prolonged vasodilatory response with AT1 blockade with valsartan, which again can be blocked with the AT2 antagonist. AT2 activation downregulates the transforming growth factor β receptor, as well as AT1. Studies with AT1 and AT2 antagonists, then, suggest that some of the beneficial actions of AT1 receptor blockade are mediated by the AT2 receptor. Carey pointed out that coronary artery AT2 expression is twice as great as that of AT1, explaining the biphasic response to angiotensin II administration, with the initial vasoconstrictor and subsequent vasodilatory responses blocked by AT1 and AT2 antagonists, respectively, the latter NO and bradykinin dependent. Myocardial remodeling, fibrosis, and LVH following myocardial infarction are lessened in animals overexpressing AT2.

Noting that ACE also affects the kinase system, it will be important to perform comparative studies to assess the relative effects of ACE inhibitors and AT1 blockers on increasing bradykinin levels. Asked how these agents may protect persons with diabetes, Carey suggested that there is systemic suppression of the RAS system in diabetes, with renal AT2 > AT1 downregulation, suggesting that disproportionate AT2 downregulation is related to diabetic renal disease. He concluded that stimulation of AT2 may mediate some of the beneficial effects of inhibition of AT1. The pancreas and particularly the islet cells have high AT2 expression, suggesting an important potential effect at this site, although studies to clarify these actions have not been carried out.

Elijah Saunders (University of Maryland School of Medicine, Baltimore, MD) discussed the medical management of hypertension in African-American men, pointing out that there is little difference between men and women in hypertension treatment other than that related to social and adherence factors, but that there is a need to focus on African Americans because of pathophysiologic differences, although he suggested that "it's not genetics," with greater genetic difference within than between races. The prevalence of hypertension increases with increasing age, and blacks have more hypertension than whites at all ages, but this is particularly significant at younger ages, particularly among men. This leads to tremendous premature CVD morbidity and mortality, with the average life expectancy of males in Harlem, New York, shorter than that for men in Bangaldesh (26). One-third of the adult black population has hypertension, with more advanced degrees of hypertension particularly common. The BMI is higher in the black population, diabetes is more common, cigarette smoking is more common, and dyslipidemia, although not occurring more frequently, is less commonly diagnosed among blacks. Blacks have 30% more nonfatal and 80% more fatal strokes, similar CHD prevalence but 50% more CHD mortality, and five times more end-stage renal disease (ESRD) than whites. Hospitalization for heart failure is more frequent among blacks.

In selecting antihypertensive treatment, coexisting diabetes and heart and renal disease should be the major determining factors. Saunders noted that blacks with uncomplicated hypertension, who are not at particularly increased risk, appear to respond well to diuretics, and perhaps best to calcium channel blockers. With diabetes, renal disease, or CHD, agents blocking the renin-angiotensin aldosterone system appear particularly useful. Blacks appear to require somewhat higher doses of ACE inhibitors than whites. There is, however, a paucity of blacks in many studies of ACE inhibitors, with the HOPE study, for example, only having 1.3% blacks. Given the frequency of diabetes and hypertension-related ESRD in the Black population, the African American Study of Kidney Disease and Hypertension enrolled hypertensive subjects with GFR <50% of normal, showing that the ACE inhibitor ramipril and, to lesser degree, the β-blocker metoprolol, were associated with less rapid deterioration in renal function than the calcium channel blocker amlodipine (27). In the ALLHAT study, the ACE inhibitor lisinopril was slightly less effective than the diuretic chlorthalidone and the calcium channel blocker amlodipine in lowering blood pressure, although with similar outcome in terms of the primary end point of fatal CHD or nonfatal myocardial infarction (28). This has led to the development of guidelines for blacks with hypertension, with the goal of 130/80 mmHg, and with the suggestion that treatment initiation be with two agents in individuals having initial blood pressure >155/100 mmHg or in those with proteinuria, the latter particularly requiring agents blocking the renin-angiotensin system.

When asked about the question of low renin hypertension in blacks, Saunders stated that there is some skewing of this population to this phenomenon, perhaps suggesting greater degrees of sodium retention, although "from a practical and clinical point of view we do not think it has [therapeutic] meaning anymore." He ended by noting, "I think the environment, the socioeconomics, the access to care, and psychosocial stress are [more important] factors," and suggested that "you must get help" from community support groups where necessary to con-

vince blacks with hypertension to comply with the complex treatment regimens required for adequate blood pressure control.

Domenic Sica (Richmond, VA) discussed the role of angiotensin II (type 1) receptor blockers (ARBs) in the treatment of diabetic nephropathy and heart disease, suggesting that this is "a contentious issue," particularly because of cost factors. Vascular dysfunction may have an amplifying effect on CVD, heart failure, and diabetic nephropathy, perhaps acting via excessive albumin excretion. A systolic blood pressure between 130 and 140 mmHg is associated with a 2.2-fold greater risk of CVD over that for lower blood pressure in individuals with microalbuminuria. With aggressive treatment of blood pressure and other risk factors, regression from micro- to normoalbuminuria may occur. However, once macroalbuminuria occurs, there is a largely irreversible increase in risk. A recent study of patients with type 2 diabetes randomized to conventional treatment versus treatment with ACE inhibitors, irrespective of blood pressure and aiming for blood pressure <140/85 mmHg, cholesterol <190 mg/dl, triglyceride <150 mg/dl, and HbA_{1c} <6.5%, and aspirin was associated with threefold greater frequency of reversion from micro- to normoalbuminuria, as well as with 53% reduction in CVD (29). There is evidence of similar benefit of ARBs, with seven of these agents now available, and "for all intents and purposes the drugs are quite comparable," although no head-to-head studies against one another or against an ACE inhibitor have been carried out. In a 6-month study of 332 type 2 diabetic patients with microalbuminuria treated with amlodipine versus valsartan, mean albumin excretion showed nil vs. 50% decrease, with 30 vs. 15% reverting to normoalbuminuria (30). Proteinuria may respond better to higher doses than those needed for maximal blood pressure response. In a 2-year trial, patients with diabetes and microalbuminuria randomized to conventional therapy, 150 mg irbesartan, or 300 mg irbesartan daily showed progression from micro- to macroalbuminuria in 15, 10, and 5% of patients, despite similar clinic blood pressure levels, although 24-h blood pressure profiles may differ (31). There are two "hard end point" trials in individuals with type 2 diabetes and established nephropathy with ARBs, with losartan up to 100 mg daily in 1,513 subjects for 3.4 years showing a 28% decrease in risk of ESRD (32), and the Irbesartan Diabetic Nephropathy Trial of 150–300 mg irbesartan, amlodipine, or conventional treatment in 1,715 subjects for 2.6 years similarly showing a 25% decrease in the composite end point of ESRD, doubling of serum creatinine, or death (33).

Aldosterone

Gordon Williams (Boston, MA) discussed new understanding of aldosterone, noting that 50 years ago the hormone was discovered and isolated by Sylvia Simpson (later Sylvia Tate) and James Tate. Within the next decade, much of the current understanding of the effect of aldosterone on increasing sodium reabsorption and potassium excretion and its control by angiotensin II and by potassium were established. A study appearing more than a decade ago, however, showed that unilateral nephrectomy followed by aldosterone administration led to increased fibrosis, leading to initiation of the Randomized Aldactone Evaluation Study (RALES), which showed that the aldosterone antagonist spironolactone reduced CVD mortality and hospitalization in individuals with coronary heart failure (CHF) treated with ACE inhibitors, diuretics, and digitalis (34), leading Williams to comment that "now we have to reexamine what it is that we think aldosterone is doing." Aldosterone and salt, in the setting of CHF, produce fibrosis leading to cardiovascular damage, although fibrosis is probably not the primary event, with animal studies showing that renal evidence of albuminuria, renal vascular injury, and glomerular damage were increased by aldosterone and blocked by administration of the mineralocorticoid receptor antagonist eplerenone. Similar findings can be seen in prevention of stroke in animal models. Necrosis and inflammatory infiltrate appear to be the primary event, with fibrosis occurring subsequently. Activation of cytokines, prostaglandins, plasminogen activator inhibitor 1, and other factors appear to be caused by aldosterone, but "there is a large blank spot" in discovering "the proximal event." Animal models studied to date have used high doses of aldosterone, but with L-nitroarginine methyl ester, a nonspecific inhibitor of NO synthase, to decrease NO production, and a subpressor dose of angiotensin II, there is substantial CV damage and proteinuria, and either blocking the mineralocorticoid receptor or adrenalectomy eliminate this response, suggesting that similar findings occur with low levels of aldosterone.

When the mineralocorticoid receptor is blocked, potassium levels increase, which could be the explanatory factor. Studying this by increasing dietary potassium shows that eplerenone has greater effect than increasing potassium levels in preventing vascular damage in animal models. Sodium restriction or diuretic treatment might be particularly damaging, by increasing aldosterone levels, but in the L-nitroarginine methyl ester/angiotensin II treatment model, a low-salt diet despite increasing aldosterone levels 10-fold actually has beneficial effects, suggesting that aldosterone only has adverse effects with sufficiency of salt. The caveoli are subcellular organelles acting as docking stations for many cellular functions, with the caveolin protein serving to link intracellular transduction mechanisms with extracellular ions. There are three forms of caveoli protein, with caveolin 1 in vascular and fat cells and caveolin 3 in myocytes. Both are involved in the binding to the mineralocorticoid receptor with initial events, while caveolin 2 plans a role in internalization of aldosterone with transport into the nucleus. Sodium restriction may decrease aldosterone-mediated vascular damage by decreasing caveolin 1 and 3, diminishing subsequent intracellular kinase cascades, a new understanding of this dietary intervention.

In individuals with hypertension, LVH and diabetic nephropathy are benefited by ACE inhibition, leading to the question as to whether this is an effect of blocking angiotensin II or blocking aldosterone. Comparing treatment of individuals with hypertension and LVH with enalapril, eplerenone, or both, LVH decreases with either treatment alone and to a greater extent with administration of both agents, suggesting that the ACE inhibitors act principally by decreasing angiotensin II, with additive benefit from antagonism of the effect of aldosterone. In a similar study of subjects with diabetes, hypertension, and albuminuria, ACE inhibitors decrease the albumin-to-creatinine ratio by half, blocking the mineralocorticoid receptor is even more effective, and combined treatment reduces albuminuria by 75–90%. In a final

and most impressive clinical study, 6,632 patients treated with eplerenone or placebo plus conventional treatment after myocardial infarction complicated by left ventricular dysfunction and heart failure showed a 15% reduction in mortality in the eplerenone group, with a 21% reduction in sudden death (35).

John Funder (Melbourne, Australia) further discussed the question of the effect of aldosterone on the heart, making the tantalizing suggestion that "in most of these circumstances aldosterone doesn't matter." In the RALES, an average dose of 26 mg spironolactone daily increased survival 30% and decreased hospitalization 35%. The actual question should be, Funder said, "is inappropriate mineralocorticoid activation bad for the heart?" When aldosterone levels are too high, with high-salt diet, myocardial and coronary artery disease occur, with prevention in animal models by eplerenone. Markers of inflammation, including COX2, osteopontin, and monocyte chemoattractant protein, levels increase. This phenomenon is not simply hypertension driven, as blood pressure levels remain high with eplerenone or adrenalectomy, although there is reduction in the inflammatory markers and the elevated levels can be reproduced in the adrenalectomized model with aldosterone infusion. Most cardiac effects of mineralocorticoid activation, Funder stated, are in fact driven by circulating cortisol. Mineralocorticoid receptor expression is particularly high in the hippocampus, heart, and kidney, with aldosterone and cortisol equally good in competing for the mineralocorticoid receptor, but circulating levels of glucocorticoids are many times higher than those of aldosterone, leading Funder to ask why all of us are not hypertensive. The answer appears to be the expression of HSD2 in epithelial target tissues, leading to conversion of cortisol to cortisone, which shows low binding, while aldosterone is not a substrate for HSD2. In evolutionary terms, the mineralocorticoid receptor is actually a high-affinity glucocorticoid receptor. The evolution of aldosterone is much more recent, with this "special steroid" secreted at low levels in response to potassium and angiotensin II involved in the adaptation from aquatic to terrestrial environments. With congenital HDS2 deficiency, the mineralocorticoids receptor is activated by cortisol leading to the syndrome of apparent mineralocorticoid excess with severe hypertension. HSD2 (as well as HSD1) is blocked by the licorice derivative carbenoxolone, explaining the association of excessive licorice ingestion with hypertension. In humans, 90% of renal mineralocorticoids are occupied by cortisol, but cortisol transformation by HSD2 into cortisone leads to NADH generation, which appears to prevent the activation of the mineralocorticoid receptor by cortisol despite its continuing to occupy the receptor. Administration of carbenoxolone prevents NADH generation and leads to receptor activation, which can be blocked by eplerenone. In an animal model, eplerenone lowers and aldosterone activates coronary artery inflammation and postinjury coronary narrowing. Funder suggested that a similar vascular inflammatory response occurs when mineralocorticoid receptors occupied by glucocorticoids become transcriptionally activated by a change in redox state. The therapeutic anti-inflammatory effect of mineralocorticoid antagonists, then, may actually represents an antiglucocorticoid effect.

Diabetic nephropathy

Ariel Zisman (Miami, FL) presented sobering information from the U.S. Renal Data System. Statistics from their website (www.usrds.org) show the increasing incidence of diabetic ESRD adjusted for age, sex, ethnicity, and race from ~10/million in 1980 to an estimated 130/million in 1999. Among adults, the incidence increased from ~450 to 700 per million per year from 1991 to 1999, with particularly high rates among blacks and Native Americans. In the U.S. Medicare population in 1998–1999, 406,800 of 1,095,480 individuals (37%) with ESDR had diabetes, comprising 10% of individuals with diabetes, as opposed to 3.5% of the Medicare population without diabetes having ESRD. Furthermore, ~12 (18% of adults), 8, and 6% of nondiabetic persons with ESRD develop diabetes during the first, second, and third year of dialysis. Of persons with diabetes and ESRD, 0.5–0.7% have a myocardial infarction annually, 2–2.5% require revascularization, and 8–10% have a stroke or transient ischemic attack, as opposed to ~0.4, 1.8, and 6%, respectively, of individuals with ESRD without diabetes. In diabetic subjects, CVD accounts for 51, 66, and 75% of deaths for individuals with no, micro-, and macroalbuminuria, with annual CHD mortality 0.7, 2, and 3.5%/year, and 12.1%/year in persons with creatinine ≥2 mg/dl or on renal replacement therapy.

Zisman discussed several practical points. There is good correlation between spot and 24-h urine collections, recognizing the high amount of day-to-day variability in the urine albumin-to-creatinine ratio; therefore, several specimens should be tested over a 6-month period for determining a subject's status. Urinary infection, postural changes, exercise, and CHF can contribute to variability. Once the urine albumin exceeds 300 mg/g creatinine, a 24-h urine collection gives somewhat less variability.

In terms of treatment, Zisman suggested that there is no evidence of superiority of either ARBs or ACE inhibitors, and no difference in frequency of hyperkalemia, although combination treatment does not appear to further increase potassium and appears more effective in blocking the renin-angiotensin pathway. He suggested further that eplerenone may be beneficial in individuals with diabetes, noting that up to 40% of individuals with diabetes treated with ACE inhibitor have "aldosterone escape," with lesser reduction in albuminuria. Nondihydropyridine calcium channel blockers may be more effective than dihydropyridines in reducing albuminuria, particularly in combination with an ACE inhibitor. Up to 30% increase in serum creatinine may occur with ACE inhibitor or ARB treatment, seen within 8 weeks, and actually appears to be a marker for long-term benefit of this treatment (36). In subjects with a greater increase in creatinine, or developing hyperkalemia, evaluation to exclude renal artery stenosis is appropriate. Zisman pointed out that typically three to four agents are required for adequate blood pressure control and suggested that for individuals with persistent proteinuria despite full dosage ACE inhibitor and/or ARB treatment, the addition of a nondihydropyridine calcium channel blockers, and possibly an aldosterone blocker, may be appropriate.

References

1. Bloomgarden ZT: Inflammation and insulin resistance. *Diabetes Care* 26:1619–1623, 2003
2. Reaven GM: Banting Lecture 1988: role of

insulin resistance in human disease. *Diabetes* 37:1595–607, 1988
3. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
4. Bloomgarden ZT: American Association of Clinical Endocrinologists (AACE) consensus conference on the insulin resistance syndrome: 25–26 August 2002, Washington, DC. *Diabetes Care* 26:933–939, 2003
5. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III): Third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 106:3143–421, 2002
6. Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC, Manson JE: Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care* 25:1129–1134, 2002
7. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, the STOP-NIDDM Trail Research Group: Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet* 359:2072–2077, 2002
8. American College of Endocrinology Position Statement: Insulin resistance syndrome [article online], 2002. Available from http: //www.aace.com/clin/guidelines/ACEIRSPositionStatement.pdf. Accessed 7 July 2003
9. Ford ES, Giles WH, Dietz WH: Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 287:356–359, 2002
10. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT: The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 288:2709–2716, 2002
11. Alexander CM, Landsman PB, Teutsch SM, Haffner SM: NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 52:1210–1214, 2003
12. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, the Diabetes Prevention Program Research Group: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002
13. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A: Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 103:357–362, 2001
14. Yusuf S, Gerstein H, Hoogwerf B, Pogue J, Bosch J, Wolffenbuttel BH, Zinman B, the HOPE Study Investigators: Ramipril and the development of diabetes. *JAMA* 286: 1882–1885, 2001
15. Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H, the LIFE Study Group: Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 359:995–1003, 2002
16. Talbott E, Clerici A, Berga SL, Kuller L, Guzick D, Detre K, Daniels T, Engberg RA: Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. *J Clin Epidemiol* 51: 415–422, 1998
17. Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, Kuller L: Coronary heart disease risk factors in women with polycystic ovary syndrome. *Arterioscler Thromb Vasc Biol* 15:821–826, 1995
18. Zimmermann S, Phillips RA, Dunaif A, Finegood DT, Wilkenfeld C, Ardeljan M, Gorlin R, Krakoff LR: Polycystic ovary syndrome: lack of hypertension despite profound insulin resistance. *J Clin Endocrinol Metab* 75:508–513, 1992
19. Talbott EO, Guzick DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH: Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol* 20:2414–2421, 2000
20. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF 2nd, Fitzpatrick LA: Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 88:2562–2568, 2003
21. Wild RA, Grubb B, Hartz A, Van Nort JJ, Bachman W, Bartholomew M: Clinical signs of androgen excess as risk factors for coronary artery disease. *Fertil Steril* 54:255–259, 1990
22. Gorgels WJ, v d Graaf Y, Blankenstein MA, Collette HJ, Erkelens DW, Banga JD: Urinary sex hormone excretions in premenopausal women and coronary heart disease risk: a nested case-referent study in the DOM-cohort. *J Clin Epidemiol* 50: 275–281, 1997
23. Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS: Mortality of women with polycystic ovary syndrome at long-term follow-up. *J Clin Epidemiol* 51:581–586, 1998
24. Wild S, Pierpoint T, McKeigue P, Jacobs H: Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. *Clin Endocrinol* 52:595–600, 2000
25. Solomon CG, Hu FB, Dunaif A, Rich-Edwards JE, Stampfer MJ, Willett WC, Speizer FE, Manson JE: Menstrual cycle irregularity and risk for future cardiovascular disease. *J Clin Endocrinol Metab* 87: 2013–2017, 2002
26. McCord C, Freeman HP: Excess mortality in Harlem. *N Engl J Med* 322:173–177, 1990
27. Wright JT Jr, Bakris G, Greene T, Agodoa LY, Appel LJ, Charleston J, Cheek D, Douglas-Baltimore JG, Gassman J, Glassock R, Hebert L, Jamerson K, Lewis J, Phillips RA, Toto RD, Middleton JP, Rostand SG, the African American Study of Kidney Disease and Hypertension Study Group: Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA* 288:2421–2431, 2002
28. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group: Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 288:2981–2997, 2002
29. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O: Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 348:383–393, 2003
30. Viberti G, Wheeldon NM, the MicroAlbuminuria Reduction With VALsartan (MARVAL) Study Investigators: Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus: a blood pressure-independent effect. *Circulation* 106:672–678, 2002
31. Parving HH, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, Arner P, the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345:870–878, 2001
32. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S, the RENAAL Study Investigators: Ef-

fects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345:861–869, 2001
33. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I, the Collaborative Study Group: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345:851–860, 2001
34. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J, the Randomized Aldactone Evaluation Study Investigators: The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N Engl J Med* 341:709–17, 1999
35. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M, the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study Investigators: Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 348:1309–1321, 2003
36. Bakris GL, Weir MR: Angiotensin-converting enzyme inhibitor-associated elevations in serum creatinine: is this a cause for concern? *Arch Intern Med* 160:685–693, 2000