The European Association for the Study of Diabetes

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At the meeting of the European Association for the Study of Diabetes (EASD) in Munich, Germany, on 5–9 September 2004, a number of important topics were addressed related to the causes and complications of diabetes.

Renal replacement treatment

Norbert Lameire (Gent, Belgium) discussed the concept of "integrated renal care" of persons with end-stage renal disease (ESRD), noting the need for nephrology referral long before dialysis is required. At the time of referral to his unit, the mean creatinine clearance is 29 ml/min and few patients have good glucose or blood pressure control. He suggested calculation of creatinine clearance from serum creatinine and patient age and body size measurements {e.g., Cockcroft-Gault equation: (140 − age) × weight/([Cr] × 72) or Modification of Diet in Renal Disease Study Group equation: $170 \times [Cr]^{-0.999} \times age^{-0.176} \times sex \times race \times [BUN]^{-0.170} \times [albumin]^{0.318}$}, with referral when the glomerular filtration rate (GFR) is <40–50 ml/min. When renal replacement treatment is required, he suggested that peritoneal dialysis, hemodialysis, and transplantation should be seen as all being components of care to be utilized when appropriate for the individual patient (1). Thus, with the ultimate plan being to "transplant when you can," there is evidence of better survival during the initial 2 years of ESRD with peritoneal dialysis. Subsequent survival may improve with hemodialysis, so that after 10 years hemodialysis provides either better (2) or equivalent (3) outcome. A synthesis is the recommendation that peritoneal dialysis be the initial modality, with subsequent shift to hemodialysis, an approach that may be particularly useful for persons with diabetes, who may have greater peritoneal membrane vascular surface area than those without diabetes. Over several years of treatment with peritoneal dialysis, the ultrafiltration capacity of the peritoneal membrane decreases from peritoneal inflammation as well as from frank infection, leading to a decrease in solute absorption. The eventual better effect of hemodialysis, then, may be seen "not as a treatment failure" but as a rational use of both modalities. The creation of vascular access in the diabetic patient "is a nightmare even for the best vascular surgeon," Lameire noted, as the radial artery is often sclerosed forcing the "second choice" of an elbow fistula, so that graft patency rates are typically worse in diabetic patients (4), further making initial use of peritoneal dialysis advantageous for diabetic patients. When appropriate insulin can be administered intraperitoneally, often leading to improved glycemic control (5), and lifestyle issues may be less problematic with treatment at the patient's home. With hemodialysis, gradual ultrafiltration is important so that fewer abrupt falls occur in blood pressure.

In his dialysis population, Lameire showed evidence that peritoneal dialysis is associated with better preservation of residual renal function. Particularly during the initial phases, these patients had better blood pressure control and less anemia, with better calcium, phosphate, and vitamin D homeostasis even with GFR as low as 5 ml/min. Volume control, β2 microglobulin, "middle molecule," and total solute clearance were improved, and there was lower mortality, improved quality of life, and more liberal diet and fluid intake was possible, resulting in better nutritional status. Predictors of loss of residual renal function include female sex, non-Caucasian ethnicity, prior history of diabetes, and prior history of congestive heart failure (CHF), with peritoneal dialysis associated with a 65% lower rate of loss of residual renal function controlling for these factors (6). The prevalences of left ventricular hypertrophy (LVH) and of hypertension were lower with peritoneal dialysis. Peritoneal dialysis may remove factors promoting sclerosis of the glomerulus, and there may be nephrotoxic effects of hemodialysis membranes and dialysis fluid impurities. Hemodialysis is more expensive than peritoneal dialysis, another potential benefit to the health care system of the latter, although the lower physician reimbursement rates for patients receiving peritoneal dialysis may be an inducement for private hemodialysis facilities to utilize this modality. There is also evidence that renal transplantation graft survival is somewhat better in persons who had had peritoneal dialysis than in those who had been treated with hemodialysis, and there is evidence that delay of function of the renal transplant, an independent risk factor for the long-term survival of the renal graft, occurs more commonly in persons who had previously been maintained on hemodialysis than those treated with peritoneal dialysis (7). There is no increase in incidence of intra-abdominal infections with transplantation after peritoneal dialysis (8), although after simultaneous pancreas-kidney transplantation, there is a somewhat increased peritonitis rate, so that the catheter should be removed at the time of the surgical procedure. Lacking controlled studies comparing hemodialysis and peritoneal dialysis, however, Lameire acknowledged that persons with residual function may be more likely to be maintained on peritoneal dialysis, with the better survival a consequence of lesser

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Abbreviations: AGE, advanced glycation end product; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; CHF, congestive heart failure; CNS, central nervous system; CVD, cardiovascular disease; EASD, European Association for the Study of Diabetes; ESRD, end-stage renal disease; FFA, free fatty acid; GFR, glomerular filtration rate; IGT, impaired glucose tolerance; IRS, insulin receptor substrate; LVH, left ventricular hypertrophy; MIRKO, muscle insulin receptor knockout; NF-κB, nuclear factor-κB; NIRKO, neural insulin receptor knockout; PARP, poly(ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; SOCS, suppressors of cytokine signaling; TZD, thiazolidinedione.

degree of renal impairment rather than of the choice of dialysis method.

Ram Gokal (Manchester, U.K.) further discussed dialysis outcome and management for patients with diabetes. The U.S. Renal Data System 2003 report projects ESRD incidence to increase from current annual levels of ~100,000 to ~500,000 persons by 2030, with the majority of cases by that time likely to be caused by diabetes. At all age-groups, life expectancy is decreased on dialysis. Relative survival is particularly bad for diabetic patients >55 years of age, and this is even more the case among women. As the number of persons requiring dialysis increases, and as these patients become progressively older, they have more and more severe comorbidities and increasing mortality rates. Cardiovascular disease (CVD) is highly important for persons with diabetes and ESRD (9). There is increasing prevalence of foot ulcer among persons with diabetes and worsening degrees of nephropathy (10).

Another important consideration is the great dependence of persons with ESRD on medical personnel for care. Expenditures are increasing, and it appears likely that nephrology specialist resources will increasingly be overwhelmed, particularly given the importance of excellent treatment. Clinical outcome depends on blood pressure control and on adequacy of dialysis. An elevation in the calcium-phosphate product is associated with high coronary artery calcification score (11) and presumably with increased cardiac risk. Mortality is decreased with administration of both statins and ACE inhibitors (12). Furthermore, diabetic persons with ESRD often benefit from aggressive intervention, with evidence that coronary revascularization is associated with improvement in outcome (13). Such approaches may not, however, be appropriate for all, as one-third of those with diabetes and ESRD survive <2 years on dialysis, suggesting that a palliative care approach may be appropriate for selected severely disabled patients. Indeed, some patients "reach end of the road," with ~20% withdrawing from treatment because of poor quality of life and/or access issues related to peritonitis or to failure to "preserve [the] vessels" of the upper extremity. This may be more common, Gokal suggested, among patients with diabetes. Although hemodialysis is the major form of renal replacement treatment in the U.S., it is administered to between 2 and 90% of persons with ESRD in different countries. Gokal supported Lameire's assessment that "all groups [on peritoneal dialysis] do better," suggesting the need for controlled studies to assess the question. It may, he noted, be appropriate to start dialysis earlier, at GFRs ~10 ml/min, and with diabetic persons, who tolerate uremia particularly poorly, to initiate dialysis at a GFR of 15 ml/min. Such an approach may lessen development of complications and appears particularly useful for nutritional maintenance. Malnutrition is common among persons with diabetes regardless of the form of dialysis, in those with type 1 diabetes associated with uremia itself, and often in those with type 2 diabetes representing a syndrome caused by inflammation and atherosclerosis (14). Increases in inflammatory cytokines in ESRD are associated with particularly poor outcome (15), suggesting that consideration of these factors may allow improvement in outcome.

Harald Bergrem (Stavanger, Norway) discussed issues of quality of care for persons with diabetic nephropathy, pointing out that diabetic kidney failure will affect one-third of persons with diabetes, with prevalence expected to double over the next 20 years. It is possible that progression can be slowed by blood pressure control, ACE inhibitors, glycemic control, smoking cessation, and statins, with a reduction in the rate of fall in GFR from 10 to 4 ml · min^{-1} · $year^{-1}$ possible with such approaches. Bergrem noted, however, that "late referral" is unfortunately common (16), leading to higher mortality and morbidity, longer hospital stay, and higher cost. He reviewed a group of studies, showing that referral to the nephrologist <4 months before dialysis is needed is associated with 1.2- to 7.7-fold higher mortality. A National Institutes of Health consensus conference in 1994 suggested referral at a serum creatinine of 2 mg/dl, with most nephrologists suggesting a level of 2–3 mg/dl (17). Noting the International Diabetes Federation guideline of creatinine 2.5–3.5 mg/dl, Bergrem commented that "as a nephrologist I would say this is a late referral."

In addition to issues strictly related to dialysis, benefits of early referral include better blood pressure control, use of ACE inhibitors and angiotensin receptor blockers (ARBs), earlier anemia treatment, more attention to treatment of low calcium and high phosphate levels, and correction of metabolic acidosis (18). An analysis of 4,304 patients with type 2 diabetes, however, pointed out that nephrology referral for creatinine >1.5 mg/dl or frank proteinuria would require the service of many additional neprologists, although added survival of 3 years without dialysis could be anticipated (19). An interesting point is that erythropoietin treatment of renal anemia may "falsely decrease" HbA_{1c}. Furthermore, often ACE inhibitors are discontinued as creatinine rises, although the data suggest that this worsens patient prognosis. These considerations suggest that a particularly useful approach might be the combined diabetes-renal clinic, which can lead to improved blood pressure, lipids, and glycemia and may eliminate what Bergrem suggested was a financial disincentive to the diabetologist "because the patient disappears to another level of care."

CHF

Ines Thrainsdottir (Stockholm, Sweden) discussed the detection, classification, and epidemiology of CHF, noting the importance of hypertension, CVD, and LVH, as well as diabetes, as risk factors. There is a clear relationship between diabetes and a variety of forms of cardiac disease. The UKPDS (U.K. Prospective Diabetes Study) shows that for each 1% increase in HbA_{1c}, there is an 8–15% increase in the rate of development of CHF. Approximately one-third of persons with CHF have diabetes, Thrainsdottir noted, with the Reykjavik study of some 20,000 persons followed from 1967 to 2002 showing that prevalence of CHF increases with increasing glycemia, from ~3% of those with normal glycemic levels, to ~5% of persons with impaired glucose tolerance (IGT), and to ~15% of persons with established diabetes. CHF occurs at an earlier age in persons with diabetes, particularly among men. In a study of 8,231 persons with and 8,845 without diabetes, the former were 2.5-fold more likely to develop CHF (20). Conversely, in the Campania study, the incidence of diabetes was 29% in persons with CHF vs. 19% in control subjects, with CHF doubling the risk of diabetes in multivariate analysis (21). Thrainsdottir stated that diabetes also was a risk factor for mortality among persons with CHF in the Reykjavik study. The relationship between diabetes and CHF may be due to

diabetic cardiomyopathy, more extensive CVD, autonomic dysfunction, endothelial dysfunction, or to the hypercoagulable state, with repeated episodes of oxidative stress being a potential underlying explanatory factor.

Christian Schneider (Dusseldorf, Germany) discussed the pathophysiology of CHF in diabetes. In its initial stages, diabetes is seen without clinical CHF, although after Valsalva maneuver, diastolic dysfunction, characterized by impaired ventricular filling, can be demonstrated in 60% of normotensive persons with diabetes (22). Subsequently, systolic dysfunction is seen, with diminished ejection fraction. B-type natriuretic peptide (BNP) is a marker, with levels increased among persons with diastolic dysfunction (23). Levels of BNP are increased in patients with diabetes without overt CHF, but there is large overlap between persons with and without diastolic dysfunction (24), so it is uncertain whether this will prove a useful diagnostic test. Diastolic dysfunction is associated with a number of metabolic abnormalities, including increased free fatty acids (FFAs), carnitine deficiency, changes in calcium homeostasis, insulin resistance, and endothelial dysfunction. Cardiac autonomic neuropathy with sympathetic denervation may play an important role, as well as direct effects of hyperglycemia in increasing cardiomyocyte apoptosis and necrosis. Angiotensin II contributes to loss of viable cardiac cells and increasing fibrosis, as well as playing a central role in increasing left ventricular remodeling, giving rise to the transition from diastolic to systolic dysfunction. Aldosterone also has many deleterious effects, leading to changes in magnesium and potassium, causing arrhythmia, and increasing myocardial fibrosis.

From a clinical view point, there is evidence from the Framingham Study that left ventricular mass is increased in persons with diabetes, with intermediate increase among persons with IGT (25). Schneider commented that the heart of the person with diabetes is "stiff, thick, and vulnerable," with reduced reserve for ischemic events. CVD is, of course, quite common in diabetes, with autopsy studies showing high-grade stenosis in ~50% of young men with diabetes and in ~80% of men >65 years old (26). Severe silent ischemia is present in 20% of persons with diabetes, with silent myocardial infarction occurring at a rate of ~8%/year among persons with diabetes. These factors suggest that recurrent cycles of myocardial injury and remodeling occur, with reduced cardiac output activating the renin-angiotensin aldosterone system, leading to increased vascular resistance, with subsequent further myocardial injury and remodeling. Adrenergic tone, BNP, endothelin, and a variety of inflammatory cytokines contribute further to this process.

Insulin resistance at the vascular level and its relationship to endothelial dysfunction was discussed by Bart Staels (Lille, France). Atherosclerosis evolves over decades from foam cell to fatty streak to atheroma, initially consisting of fibrous plaque, with subsequent evolution into a complex lesion leading to rupture (27). With normal endothelial function, exposure to acetylcholine or to increased flow leads to vasodilation, mediated by factors such as nitric oxide (NO). With endothelial dysfunction this mechanism is inoperative. Endothelin is increased and NO decreased, leading to vasoconstriction rather than vasorelaxation. Clinical tools for assessment of endothelial function include biochemical measures such as of von Willebrand factor, thrombomodulin, and the adhesion molecules vascular cell adhesion molecule, intercellular cell adhesion molecule, and E- and P-selectin and functional assessment of flow-mediated vasodilation in brachial, leg, or coronary arteries. Plethysmographic measurement of postischemic vasodilation is a commonly employed method that can be used to demonstrate that endothelial dysfunction is present in persons with diabetes and those with pre-diabetic insulin-resistant states.

An important contributory process is the chronic inflammatory state of atherosclerosis. At the cellular level, endothelial cells express cell adhesion and attractant molecules leading to leukocyte entry into the vessel wall. A potent proinflammatory pathway involves nuclear factor-κB (NF-κB) signaling initiated by cytokines or oxidative stress. Potential therapeutic approaches that may modulate inflammation include reduction in cytokines, use of antioxidants, and use of agents to decrease the inflammatory signal transduction. Since the angiotensin II signaling pathway is of great importance, leading to NF-κB activation and prooxidant factors, Staels suggested the use of peroxisome proliferator–activated receptor agonists, which act at the level of NF-κB, and of sartans to block the angiotensin II receptor. A free fatty acid–induced increase in intracellular diacylglycerol levels leads to an increase in protein kinase C (PKC), another potential link between type 2 diabetes and CVD, with elevations in PKC worsening insulin resistance, inducing oxidative stress, and increasing NF-κB. Dyslipidemia plays a role, with small dense LDL particles highly susceptible to oxidation, inducing further vascular wall inflammation. A related concept is that of abnormal insulin signaling at the level of the vascular wall. Insulin stimulates NO-dependent vasodilation, which plays a role in increasing tissue glucose uptake, but insulin may also induce endothelin 1, leading to vasoconstriction. Under circumstances of vascular insulin resistance, insulin acts to increase phosphatidylinositol 3-kinase (PI3K), increasing both the Akt and mitogen-activated protein kinase pathways. In animal models of insulin resistance, macrophages also show decreased insulin response, causing abnormal responses to insulin such as the increase in expression of CD36, the scavenger receptor that plays a role in oxidized LDL uptake (28). It may be possible to modulate the abnormal vascular insulin response. Staels noted effects of olive oil and fish oil, of Coenzyme Q_{10} (29), and of physical exercise in improving endothelial function, as well as the actions of a number of therapeutic agents on endothelial function, including allopurinol, statins, sartans, fenofibrate, and the thiazolidinediones (TZDs). Interventions that increase adiponectin may be beneficial, while reduction in proinflammatory adipokines may also improve endothelial function.

Aldo Maggioni (Firenze, Italy) and Thomas F. Luscher (Zurich, Switzerland) (30,31) discussed prevention and treatment of heart failure in persons with diabetes. There is increasing frequency of diabetes, and it is assuming increasing importance as an independent risk factor for development of CHF, with >10% of persons with diabetes having CHF and >30% of those with CHF having diabetes. Persons with CVD who also have known diabetes have particularly high risk, but this is also seen in persons with CVD who have abnormal glucose tolerance. The prevention of heart failure in persons with diabetes includes meticulous glycemic control, blood pressure

control, lipid control, and the use of blockers of the renin-angiotensin system. Prevention of heart failure by antihypertensive treatment has been demonstrated, for example, with diuretics (32) and ARBs (33). The UKPDS showed that each 1% increase in HbA_{1c} is associated with a 16% increase in CHF (34) and that HDL, LDL, blood pressure, smoking, and HbA_{1c} are risk factors (35). Comprehensive prevention approaches are effective in the prevention of CVD (36). Treatment of persons with diabetes and additional CVD risk factors with ramipril decreases mortality, myocardial infarction, stroke, and CVD death (37). The use of losartan rather than atenolol in hypertensive diabetic persons is associated with decreased total and CVD mortality and trends to decreased myocardial infarction and stroke (38). Studies in progress will assess the roles of echocardiographic assessment and BNP measurement and of valsartan, nateglinide, insulin, and n-3 fatty acid treatment in high-risk persons with impaired fasting glucose, IGT, and early diabetes. Despite the current interest in glycemic treatment of persons with CVD and with critical illness, no trials have been reported addressing glycemic treatment of persons with diabetes and heart failure. Subgroup analysis of trials, including persons with diabetes, have shown benefit of β-blockers (39), ACE inhibitors (40), ARBs (41), and the aldosterone blockers spironolactone and eplerenone. Metoprolol, carvedilol, and bisoprolol have all been shown of benefit, with evidence of a significant difference in favor of carvedilol over metoprolol in persons with diabetes (42). Diuretics should be the next agent, and although no randomized trials exist showing benefit other than symptom relief, a torasemide study (43) suggested that improvement may be seen in outcome with these agents. In the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) study, however, chlorthalidone worsened fasting glucose, so that these agents must be used with caution as they may have adverse effect (44), with a further caution in the ALLHAT study being that heart failure was not well defined, leading to uncertainty as to whether chlorthalidone truly reduced heart failure. Drugs precipitating CHF should also be considered, with NSAIDS and Cox 2–specific inhibitors important causes and TZDs also requiring consideration. Heart failure risk factors in TZD-treated patients include a prior history of heart failure, symptomatic coronary disease, hypertension, and LVH; the use of insulin; and renal failure (45).

This symposium can be viewed at http://www.easd-lectures.org/.

EASD thiamine symposium

Maximo Porta (Torino, Italy) introduced a symposium on thiamine, pointing out that vitamin B_1, or thiamine, acts as a cofactor in three enzymatic pathways, transketolase, pyruvate dehydrogenase, and α-ketoglucarate dehydrogenase, all potentially shifting metabolism from glycolysis and increasing Krebs cycle activity. Thiamine administration, then, may decrease cycling through a variety of pathways of glucose-related damage (46), by decreasing production of lactate, triose phosphate, and pentose phosphates, with the potential to decrease advanced glycation end product (AGE) production. Hans-Peter Hammes (Heidelberg, Germany) discussed evidence from animal models of retinopathy suggesting benefit of vitamin B_1 in the treatment of diabetes complications. The retinal microcirculation is abnormal in the earliest lesions of diabetic nephropathy, with endothelial cells, basement membrane, and pericytes the three most important structures determining the effects of diabetes on the retina. Progressive vascular occlusion and increased vascular permeability are, Hammes suggested, the "two fundamental changes" of diabetic retinopathy. In experimental models, pericyte loss is seen initially, with subsequent endothelial cell loss, leading to the histological finding of acellular capillaries, which are, rather than microaneurisms, the most important lesions. Hyperglycemia-induced mitochondrial overproduction of reactive oxygen species activates all major pathways of diabetic cellular damage. The use of thiamine derivatives may ameliorate this abnormality by shifting hyperglycemia-induced disturbances via transketolase activation (47). Benfotiamine, a thiamine monophosphate derivative, inhibits hyperglycemia-induced activation of glycolysis, AGE production, PKC activation, and NF-kB activation. In rat diabetic models, which have somewhat decreased transketolase activity, benfotiamine markedly increases retinal transketolase, with normalization of retinal hexosamine, methylglyoxal-type AGE levels, and PKC and NF-kB activity. In retinal digest preparations, acellular capillary formation is markedly increased in diabetes, whereas this is almost completely normalized by benfotiamine. In vivo, in untreated diabetic rats, after a 26-week period of benfotiamine treatment acellular capillary formation was reduced, while there was no effect in reducing pericyte loss, lowering blood glucose or HbA_{1c}, or increasing body weight. As pericytes are survival factors for endothelial cells, however, their loss leads to endothelial cell death (48), a process that involves the angiopoeitin-tie system (49). Angiopoeitin-2 leads to capillary regression and is upregulated in the diabetic retina before pericyte loss. Angiopoeitin-2 overexpression or its direct injection into the vitreous induces pericyte drop out, while heterozygous inhibition prevents pericyte loss. In addition to capillary endothelium, many supporting glial cells in the ganglion cell and inner nuclear layers of the retina express angiopoeitin-2, with high glucose leading Müller glial cells to overexpress angiopoeitin-2. Benfotiamine inhibits endothelial cell overexpression of angiopoeitin-2 and reduces vascular endothelial growth factor and angiopoeitin-1 expression in the retina exposed to high glucose but does not correct glucose-induced angiopoeitin-2 upregulation in diabetic glia. Poly-(ADP-ribose) polymerase (PARP) is predominantly expressed by glial cells, with high glucose levels increasing this, whereas benfotiamine decreases PARP protein expression. Hammes concluded that diabetic retinopathy reflects an abnormal interaction of glial and vascular cells under conditions of high glucose, with benfotiamine reducing the major pathways of hyperglycemic damage, acting as a PARP inhibitor, and prevents retinopathy in animal models, suggesting a potential therapeutic role of this or related substances.

Paul Thornalley (Essex, U.K.) discussed prevention of diabetic nephropathy and correction of dyslipidemia by thiamine and benfotiamine, further suggesting that this approach may reverse multiple pathways of hyperglycemic damage. Vascular cells can accumulate high cytosolic glucose, activating polyol and hexosamine pathways, leading to the accumulation of triose phosphate intermediates, which can increase AGE formation and increase diacylglycerol, with consequent PKC activation and mito-

chondrial effects increasing reactive oxygen species. Thiamine enters cells via high-affinity binding transporters and is phosphorylated to thiamine pyrophosphate, the active coenzyme form, 90% mitochondrial, while 10% is cytosolic and susceptible to loss during hyperglycemia. Benfotiamine enters cells via a different transporter, with intracellular metabolism to thiamine pyrophosphate. Both thiamine supplements and, to a greater extent, benfotiamine increase circulating thiamine levels in animal models, while the plasma levels are decreased in diabetes models, perhaps in part due to increased renal thiamine clearance, which may be an early adverse effect of hyperglycemia. Albuminuria increased with diabetes, a phenomenon that can be prevented by high-dose thiamine and by benfotiamine (50). Furthermore, diabetes increases glomerular PKC activity, an effect prevented with both benfotiamine and thiamine. Accumulation of AGEs, including methylglyoxal and carboxymethyl lysine, is blocked by thiamine and benfotiamine (51). Renal glomerular transketolase activity is decreased ~30% by diabetes, again with reversal by benfotiamine and thiamine administration. Not all the benefits of thiamine can be seen with benfotiamine administration, with the former but not the latter appearing to be of benefit in a streptozotocin-induced diabetes model of dyslipidemia (52), perhaps because postprandial thiamine hepatic loading is more effective than that obtained with benfotiamine. Thornalley commented that an appreciable proportion of persons with diabetes have mild thiamine deficiency and noted that these studies suggest that this should be identified and treated, although acknowledging that it remains to be determined whether high-dose thiamine and benfotiamine can add further benefit. He suggested that up to 250–300 mg thiamine can be administered daily, although the typical supplementary dose is 50 mg and the recommended dietary allowance is 1–1.5 mg daily. Doses up to 600 mg of benfotiamine daily have been administered, he stated, with no evidence of toxicity.

Liver, β-cell, and brain: roles in glucose homeostasis

C. Ronald Kahn (Boston, MA) gave the 36th Claude Bernard Lecture (which can be viewed at http://www.easd-lectures.org/), discussing studies of the effect of insulin in a variety of tissues. He characterized the lecture as an exercise based on the goals of the Diabetes Genome Anatomy Project, an "initiative whose goal is to unravel the interface between insulin action, insulin resistance and the genetics of type 2 diabetes" (www.diabetesgenome.org). Honoring Claude Bernard, who as the founder of medical physiology developed "la médecine expérimentale," Kahn began by reviewing the dual abnormality of glucose homeostasis in type 2 diabetes, with decreasing ability of insulin to suppress hepatic glucose output and stimulate peripheral glucose uptake, as well as with deficiency in insulin secretion, together leading to hyperglycemia. The molecular aspects of insulin action involve the initiation of tyrosine phosphorylation of the insulin receptor, leading to subsequent phosphotyrosine modification of insulin receptor substrate (IRS)-1 to -4 and a variety of other peptides, with their phosphotyrosines acting as docking sites for subsequent phosphorylation of further enzymes, as for example the PI3K pathway stimulating glucose transport and glycogen, lipid, and protein synthesis and the Ras/mitogen-activated protein kinase pathway being involved in cell growth, differentiation, and gene expression. Kahn emphasized that this schema is oversimplified, as there are >1,000 enzymatic systems with which insulin may interact, and further noted the glucose transport effects of mitogen-activated protein kinase, exemplifying the tremendous cross-over between the pathways that we attempt to correlate with specific aspects of insulin actions.

Use of "knockout" models allows study of insulin signaling in the pathogenesis of diabetes. Insulin signaling knockout models have been studied in a number of specific tissues, including fibroblasts, brown fat, and β-cells, to assess biochemical signaling, physiological effects, and gene expression/proteonomics/genetics. Insulin receptor knockout mice develop severe growth retardation and ketoacidosis. IRS-1 knockout mice have insulin resistance in muscle and fat and growth retardation, as well as β-cell dysfunction, but do not develop diabetes. IRS-2 knockout mice have insulin resistance and a defect in β-cell and neural proliferation, with development of hyperglycemia. IRS-3 and -4 knockout mice have normal birth weight and growth and normal or only mildly abnormal glucose homeostasis. RNA interference studies can be used in studying insulin action, with short mRNA sequences delivered to specific organs using adenoviral constructs to decrease specific aspects of RNA signaling, in what have been termed "knockdown" models. Knockdown of IRS-1 or -2 alone produces mild hyperglycemia, with knockdown of both by >70% required to produce severe hyperglycemia and hepatic steatosis. There are slightly different effects of IRS-1 and -2, the former more tightly associated with inhibiting PEPCK and glucose-6-phosphatase, hence presumably acting to a greater extent on gluconeogenesis, while the latter has greater effects on fat metabolism, inhibiting sterol regulatory element–binding protein-1c and fatty acid synthase.

Physiology and pathophysiology, which Claude Bernard referred to as the "roots" of medicine, can be analyzed with tissue-specific tissue knockout models. Flanking a specific gene (such as the insulin receptor gene) with "lox" sites allows such studies in muscle, liver, adipose tissue, β-cells, brain, skin, and other tissues. The first to be developed was the muscle insulin receptor knockout (MIRKO) mouse. Surprisingly, MIRKO mice were found to have normal glucose tolerance, which was found to involve a shift of glucose uptake from muscle to fat, leading to the concept that insulin resistance in one tissue can have profound actions on other tissues, indicating communication by mechanisms not yet fully established between tissues. In a model with muscle-specific GLUT4 knockout, insulin resistance was seen in fat and liver, and fat cell-specific peroxisome proliferator–activated receptor γ knockout is associated with hepatic insulin resistance, further suggesting cross-talk between tissues. A candidate "MIRKO factor" has been isolated, a low–molecular weight protein secreted by muscle.

"Body fat is the ultimate tissue communicator in insulin resistance," Kahn stated, noting the tremendous differences between effects of central and peripheral obesity. Substances that are secreted by muscle and act to inhibit insulin signaling include FFAs, leptin, tumor necrosis factor-α, interleukin-6, and resistin, while adiponectin is a fat secretory product that appears to increase tissue insulin signaling. In the FIRKO (fat insulin receptor knockout mouse), there is decreased

body fat throughout the lifespan and resistance to the development of obesity even with increased caloric intake, leading paradoxically to improved glucose tolerance and insulin sensitivity. These mice actually have a longer lifespan as a consequence. Levels of leptin and tumor necrosis factor-α are decreased, while those of adiponectin are increased, potentially mediating this phenomenon. Kahn discussed the question of how cytokines produce insulin resistance in the liver, muscle, and other tissues, under circumstances of obesity, infection, inflammation, and trauma, a tissue-tissue communication that he termed "nonclassical endocrinology." A potential mechanism involves the suppressors of cytokine signaling (SOCS) proteins, which activate the SOCS gene leading to production of SOCS-1 and -3. These normally act to inhibit their own generation, but also lead to decreased tyrosine phosphorylation of IRS-1 and -2 by binding to the insulin receptor and inhibiting its action on IRS-1 and -2, although not decreasing phosphorylation of the insulin receptor itself. Kahn described studies using knockout technology to lower levels of SOCS-1 and 3 in the *db/db* mouse, reporting that this markedly improves hepatic steatosis.

In the pancreas, which Bernard thought of mainly as a digestive organ, insulin action is also important for β-cells, which express insulin and IGF-1 receptors, IRS-1 to -4, and PI3K. Glucose and insulin both induce phosphorylation of the β-cell insulin receptor and of IRS-1 and -2. Insulin increases intracellular calcium and stimulates insulin secretion in isolated β-cells, and this is reduced in IRS-1 knockout β-cells. Disruption of IRS-1 causes β-cell dysfunction, while disruption of IRS-2 leads to a reduction in β-cell mass. The βIRKO (β-cell insulin receptor knockout) mouse has loss of first-phase insulin release in response to glucose, with consequent IGT. Exploring the question of whether persons with type 2 diabetes have altered insulin signaling in β-cells, Kahn showed evidence that human islets from persons with type 2 diabetes have a 70% reduction in the insulin receptor, 85% reduction in IRS-1, and 70% reduction in Akt (protein kinase B), while levels of SHIP2, a phosphatase that normally turns off insulin signaling, are doubled.

Another aspect of insulin resistance and tissue communication is the β-cell response to insulin resistance. The IR/IRS-1 double heterozygous knockout has markedly increased β-cell growth, leading Kahn to address the question of what signal determines β-cell growth. He discussed whether it is a metabolic intermediate such as glucose or FFAs or a more specific factor produced by muscle and/or fat. Both the MIRKO and FIRKO mouse fail to show β-cell hyperplasia, while marked β-cell hyperplasia is seen in the LIRKO (liver-specific insulin receptor knockout) model. A double tissue-specific LIRKO/βIRKO mouse, however, has reduced islet mass, suggesting that insulin may either directly be a growth factor or may play a permissive role.

Finally, Kahn addressed potential roles of insulin action in the brain in glucose homeostasis and the question of brain-tissue communication in the pathogenesis of diabetes, a source of interest since the "picure diabetes" studies performed by Bernard in 1855. He noted that insulin receptors are expressed in central nervous system (CNS) neurons and that insulin has been suggested to play roles in food uptake and neural growth in the CNS. He described neural insulin receptor knockout (NIRKO) mice, which fail to express the insulin receptors that are present in leptin-responsive neurons. NIRKO mice show selective decrease in insulin signaling in paraventricular nuclei, although leptin response remains. These animals show increased food intake and obesity with evidence of insulin resistance, suggesting an important role of the CNS insulin receptor in the control of appetite as well as that of glucose homeostasis. Claude Bernard speculated in 1859 that decreased CNS sympathetic tone might play a role in picure diabetes. In the NIRKO mice, insulin fails to suppress hepatic glucose output, while stimulation of hypothalamic insulin receptors enhances hepatic insulin action (53). Thus, there is important communication between the brain and the liver. Insulin in the brain may have a role in the response to hypoglycemia, with NIRKO mice showing attenuated increase in Epi and Nep during hypoglycemia. Thus, appetite regulation, control of hepatic glucose production, and the response to hypoglycemia are all affected by insulin action in the brain.

Kahn concluded that the use of models with tissue-specific impairment in specific receptors related to insulin action has revealed that different tissues contribute uniquely to the pathogenesis of type 2 diabetes, although not always in the predicted fashion. Tissues possess mechanisms of communication, such that resistance in one tissue affects insulin signaling or metabolism in others. Insulin has effects in tissues such as neurons and β-cells not previously thought to be insulin responsive. Kahn noted that there is also a role of the insulin receptor in endothelial cell vasoconstriction as well as proliferation, promising further lessons from studies of this tissue, and reminded the audience of Claude Bernard's caution, "It is what we think we already know that prevents us from learning."

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