Gut and Adipocyte Peptides

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This is the third in a series of articles on presentations at the American Diabetes Association Annual Meeting, San Diego, California, 10–14 June 2005.

Glucagon-like peptide-1 (and related hormones)

Daniel Drucker (Toronto, Canada) reviewed evidence that the incretin effect, the phenomenon of enteral glucose loading increasing the insulin secretory response to an increase in blood glucose, is reduced in type 2 diabetes, and that the incretin glucagon-like peptide (GLP)-1 may ameliorate this defect. The major limitation to use of GLP-1 in clinical treatment is its rapid clearance by the enzyme dipeptidyl peptidase (DPP)-IV. GLP-1 stimulates insulin secretion, inhibits glucagon secretion, and delays gastric emptying. Loss-of-function studies with a mouse model not expressing the GLP-1 receptor (GLP-1R$^{-/-}$), and with the GLP-1R antagonist exendin-(9-39), show effects of GLP-1 on the hypothalamic-pituitary-adrenal (HPA) axis, the reproductive system, the portal glucose receptor, gastric motility, and a number of other potential targets. GLP-1R$^{-/-}$ mice have normal insulin sensitivity, arguing against an effect of the system on insulin action, but have glucose intolerance, and administration of exendin-(9-39) similarly is associated with glucose intolerance in mice, in primates, and in humans. Drucker noted that there is controversy as to whether the product of DPP-IV degradation may act at a second GLP-1R, perhaps having additional effects on metabolism, but that GLP-1 does not lower glucose levels in GLP-1R$^{-/-}$ mice, arguing against such a receptor. In the model, pancreatic insulin mRNA levels are reduced and somatostatin mRNA levels are increased. The perfused pancreas isolated from GLP-1R$^{-/-}$ animals shows normal insulin response to glucose, although without response to GLP-1. GLP-1R$^{-/-}$ β-cells appear to have increased sensitivity to the incretin glucose-dependent insulinotropic polypeptide (GIP), perhaps representing an adaptive upregulation of GIP and/or of GIP response, and mice neither expressing the GLP-1 nor the GIP receptor show glucose intolerance with decreased insulin response to glucose, and fail to show a glucose-lowering response with administration of DPP-IV inhibitors, suggesting that the mechanism of DPP-IV inhibition involves both peptides. GLP-1R$^{-/-}$ mice have increased β-cell susceptibility to apoptotic streptozotocin injury. In the *ob/ob* (leptin-deficient) GLP-1R$^{-/-}$ mouse, insulin gene levels are upregulated and there is islet hyperplasia to the same extent as seen in the *ob/ob* mouse with normal GLP-1R function. With partial pancreatectomy, however, although exendin-(9-39) does not block β-cell hyperplasia, the GLP-1R$^{-/-}$ mouse has reduced β-cell regeneration and is hyperglycemic. Taken together, these studies suggest that trophic effects of GLP-1 on the β-cell occur physiologically. There are areas with high density of GLP-1R binding sites in the hypothalamus, but their role is uncertain. The GLP-1R$^{-/-}$ mouse has normal body weight and minimal perturbation in food ingestion on a standard diet, although showing lesser degrees of obesity on a high-fat diet. Gastric emptying is not changed in the GLP-1R$^{-/-}$ mouse, which shows a response to cholecystokinin although not to exendin. Studies in these mice also suggest that there is a neural portal vein and/or liver glucose sensor, which may be in part activated by GLP-1 to enhance the response to ingested food.

Robert Rizza (Rochester, MN) presented information pertaining to effects of GLP-1 on insulin action, noting that a major confounding factor is the increase in portal insulin levels and the decrease in glycemia with GLP-1, both indirectly improving the glucose-lowering effect of insulin. GLP-1 concentrations are somewhat lower in persons with diabetes and, to a lesser extent, with impaired glucose tolerance, and GLP-1R activation is increased well beyond the physiologic range both with agonists and with administration of DPP-IV inhibitors. In vitro, GLP-1 increases adipocyte 2-deoxy-glucose uptake, rat soleus muscle glycogen synthesis, and heptaocyte glycogen synthase A levels. In mouse models, the effect of insulin in increasing glucose uptake is enhanced without change in glucose effectiveness by administration of GLP-1. In obese hyperglycemic Zucker rats, administration of exenatide increases the glucose infusion rate required to maintain euglycemia, suggesting increased insulin action. In nondiabetic humans, however, the glucose disappearance rate with peripheral infusion of GLP-1 is equal to that with insulin infusion to produce similar insulin levels. A measure of "glucose effectiveness" appears to be increased in persons without diabetes administered GLP-1, but Rizza showed another study of persons with type 2 diabetes, in which GLP-1 increased insulin and reduced glucose levels modestly, without change in glucose effectiveness with insulin clamped. In a 6-week study of persons with type 2 diabetes undergoing continuous GLP-1 infusion, the glucose infusion requirement was increased during a hyperinsulinemic-euglycemic clamp, although with somewhat lower glucose levels, potentially explaining improved insulin sensitivity (1). Rizza showed an interesting study in persons with type 1 diabetes, with glucose infusion rates during a euglycemic clamp modestly increased by GLP-1, again suggesting possible effect on insulin action.

David D'Alessio (Cincinnati, OH) discussed the effect of GLP-1 on body weight. Central administration of GLP-1 decreases, whereas that of exendin-(9-39) increases food intake (2). There is a taste-aversive effect of central GLP-1, not seen with leptin in doses similarly decreasing food intake (3). Furthermore, central administration of GLP-1 induces neuronal growth in central emetic areas. Thus, GLP-1 may suppress food intake by acting

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Abbreviations: AGRP, agouti-related peptide; ARIC, Atherosclerosis Risk in Communities; BBB, blood-brain barrier; CART, cocaine-amphetamine–regulated transcript; CNTF, ciliary neurotrophic factor; DPP, dipeptidyl peptidase; FFA, free fatty acid; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; GLP-1R, GLP-1 receptor; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; POMC, proopiomelanocortin; PPAR, peroxisome proliferator–activated receptor; REE, resting energy expenditure; SOCS, suppressor of cytokine signaling; STAT, signal transducers and activators of transcription; TLR, toll-like receptor; TNF, tumor necrosis factor.

as a satiety factor, as a regulator of energy balance like leptin or insulin, or intriguingly, as a mediator of a "visceral illness" response. Examining the brain GLP-1 system, the nucleus of the solitary tract (NTS) receives signals from gut visceral afferents and contains a small number of GLP-1–staining neurons. Central GLP-1 synthesis occurs almost exclusively in the NTS, but GLP-1Rs are widespread, found in the amygdala, the hypothalamic paraventricular nucleus, the NTS itself, and circumventricular areas where the blood-brain barrier (BBB) is attenuated with greater potential effect of circulating GLP-1. A number of stimuli increase central GLP-1, including leptin and balloon distension of the stomach, although ingestion of a large meal does not increase central GLP-1 levels. Acting in a fashion similar to that of lithium chloride, GLP-1 decreases food intake, causes taste aversion, decreases water intake, decreases locomotor activity, decreases body temperature, reduces gastric emptying, and activates sympathetic outflow and the hypothalamic-pituitary-adrenal axis. The GLP-1 antagonist exendin-9, when infused in the fourth ventricle, decreases the anorexic response to other stimuli, suggesting that central GLP-1 may mediate a number of food aversion responses. In neonatal rats treated with monosodium gluconate, which induces lesions in the arcuate nucleus, GLP-1–mediated anorexia is prevented under a variety of circumstances.

Thus, central food intake, aversion, and stress responses are mediated by the GLP-1R, presumably with different responses mediated in different areas of the brain, leading D'Alessio to comment, "GLP-1 sits right in the middle of an illness- and stress-integrative system." Central GLP-1R activation may be indirect, via activation of hindbrain GLP-1 neurons, as seen following psychogenic stress, may involve GLP-1R activation in the circumventricular organs, may involve GLP-1 transport across the BBB, or may occur via activation of peripheral afferent nerves. A GLP-1–albumin conjugate, which does not cross the BBB, does activate central GLP-1 response when administered to the circumventricular organs, suggesting their importance. Exenatide may be transported across the BBB and therefore may act at a number of central nervous system sites. An additional aspect of GLP-1 action is that the adaptive responses seen with many other factors do not occur with long-term administration of central GLP-1R agonists, so that ongoing suppression of appetite and weight loss can be expected to occur, although the mechanisms of these chronic effects may differ from those of GLP-1 administered acutely.

Peripheral infusions of supraphysiologic levels of GLP-1 in humans decrease food intake with increased fullness and satiety in a dose-dependent fashion. These effects may be mediated by delay in gastric emptying, but the relationship between gastric emptying and the appetite-regulating functions of central GLP-1 is not known. There is evidence that visceral signals are mediated through the vagus nerve. The GLP-1-R is expressed in the vagus nerve, so that signals originating in the gastrointestinal tract or portal vein may be responsible for some of central effects of peripheral GLP-1. The extent to which intestinal GLP-1 plays a role in satiety is uncertain, with D'Alessio commenting that DPP-IV inhibitors, which may be considered to increase GLP-1 within the upper physiologic range, have much less weight loss effect than GLP-1-R agonists.

Bo Ahrén (Lund, Sweden) presented a perspective on the potential of GLP-1 analogs and of DPP-IV inhibitors in the treatment of diabetes. GLP-1 has multiple desirable effects and targets key defects in type 2 diabetes, stimulating insulin secretion in a glucose-dependent manner, reducing the likelihood of hypoglycemia, increasing β-cell mass in animal models with the potential to modify long-term disease progression, decreasing glucagons secretion, leading to reduction in hepatic glucose output, delaying gastric emptying with consequent inhibition of glucose influx from the gastrointestinal tract, possibly increasing insulin sensitivity after chronic administration, and decreasing food intake. The stimulation of insulin in a glucose-dependent fashion, the inhibition of glucagon, and the ability to increase β-cell mass are functions not achievable by other existing diabetes treatments.

Studies more than a decade ago showed that GLP-1 reduces the meal-related exogenous insulin requirement in type 2 diabetes, presumably related to effects on glucagon and on gastric emptying (4). Infusion of GLP-1 over a several-hour period to persons with type 2 diabetes decreases glucose and increases C-peptide levels during hyperglycemia, but not when the glucose level is normal (5). Following a 6-week period of GLP-1 infusion, glucose levels are reduced, with suggestion of improvement in insulin sensitivity. As GLP-1 is rapidly inactivated by DPP-IV, which cleaves alanine or proline at the second amino acid of the NH_2-terminal of the peptide, the two strategies that have been employed have been to use GLP-1 R agonists, including exenatide, liraglutide, and CJC-1131, and DPP-IV inhibitors, those in development being vildagliptin (Novartis), sitagliptin (Merck), and saxagliptin (Bristol-Myers Squibb).

Exenatide, the pharmaceutical name given to the peptide exendin-4, has >50% homology to GLP-1, acting as a GLP-1 agonist, but with glycine as the second NH_2-terminal amino acid, so that it is not inactivated by DPP-IV. In three 30-week studies, combination therapy with sulfonylureas, with metformin, and with both was associated with decrease in HbA_{1c} (A1C) by ~1% from starting levels of ~8.5%. The proinsulin-to-insulin ratio decreases with exenatide administration, suggesting improvement in β-cell function. In open-label extension studies with 392 persons, A1C decreased 1.2% and body weight decreased progressively by 4–5 kg from ~100 kg, with A1C remaining suppressed over 82 weeks in 265 persons. HDL cholesterol and triglyceride levels also decreased, presumably at least in part related to the reduction in body weight. Preliminary 2-year data suggest ongoing benefit with 5.5-kg weight loss and stable A1C lowering. Nausea occurs in approximately half of patients on initiation of treatment, but treatment discontinuation is required in only ~4%. Hypoglycemia was seen in combination with sulfonylurea. Low-titer antibodies are found but do not affect the efficacy of the agent.

Liraglutide is a GLP-1 analog containing a fatty acid moiety, not degraded by DPP-IV, leading to slower absorption and prolongation of action. Ahrén described a 5-week study with or without metformin showing reduction in fasting glucose and 1% decrease in A1C (despite the short duration of study) with reduction in body weight by 1–2 kg. Usually, this agent also causes transient nausea in approximately one-quarter of patients and does not lead to antibody formation. CJC-1131 is a GLP-1 analog covalently bound to albumin, leading to a 10-day half-life, with evidence of therapeutic benefit in persons with type 2 diabetes.

Reviewing studies of DPP-IV inhibitors, which appear to be weight-neutral

rather than causing weight loss, but which are administered orally and do not cause nausea, Ahrén noted that vildagliptin binds with high affinity to DPP-IV, increasing GLP-1 levels approximately threefold. In patients with baseline A1C 7.2%, the GLP-1 response to a standard meal increased with an 18-mg/dl reduction in fasting glucose levels and with improvement in the 24-h glucose profile following a 4-week treatment period, with lower glucagon and without change in circulating insulin levels. In a 52-week study of 107 persons receiving metformin, with baseline A1C 7.7%, the fasting glucose decreased 10 mg/dl and A1C decreased 0.5%, while the A1C levels increased 0.6% with placebo, without change in body weight. Ahrén showed similar effect of the other DPP-IV inhibitors being developed, with sitagliptin (MK-0431) the best studied. In studies of this agent reported at the meeting, Brazg et al. (abstract 11) administered 50 mg MK-0431 twice daily versus placebo to 28 patients in a 4-week cross-over study, with 24-h glucose 125 vs. 158 mg/dl and fasting glucose decreasing 24 vs. 3 mg/dl. Herman et al. (abstract 541) treated 552 persons with placebo, or MK-0431 25, 50, or 100 mg daily, or 50 mg twice daily, finding a modest dose-response effect with placebo-adjusted decrease in A1C from baseline 7.6–7.8% by 0.4–0.7%, and with decrease in fasting glucose by 11–17 mg/dl. The fall in A1C in the 100-mg daily group was 0.4, 0.6, and 0.8% for baseline A1C <7, 7–8.5, and 8.5–10%, respectively. Scott et al. (abstract 41) reported a 12-week study of monotherapy with the agent in 743 persons, with placebo-corrected A1C decrease from 7.9 by 0.8%, without change in body weight as opposed to a 1.1-kg weight gain with 5–20 mg glipizide daily. Two vs. 21 patients treated with MK-0431 vs. glipizide experienced one or more hypoglycemic event.

A number of studies presented at the ADA meeting illustrated further aspects of GLP-1 physiology and its therapeutic potential. Parsons et al. (abstract 589) reported that in a type 2 diabetes animal model, overexpression of GLP-1 was associated with evidence of improvement in islet cell function as well as in glucose levels. Bodvarsdottir et al. (abstract 500) administered the GLP-1 analog liraglutide in a type 2 diabetes animal model, showing dose-related normalization of glycemia. Mu et al. (abstract 53) found that glucagon receptor deletion in *ob/ob* mice caused 25–30% lowering of adult body weight, suggesting that glucagon suppression may contribute to the weight loss occurring with GLP-1 agonist treatment. Duttaroy et al. (abstract 267) administered the DPP-IV inhibitor vildagliptin orally or exendin-4 parenterally beginning 5 days before streptozotocin administration in mice, showing similar improvement in glycemia, with evidence of enhanced differentiation of pancreatic progenitor cells after both treatments. Duttaroy et al. (abstract 572) reported that vildagliptin increased islet cell mass 50% and increased islet insulin content 23% in a 3-week neonatal mouse model, with β-cell mass continuing to be 43% above baseline 1 week following discontinuation of treatment. Carter et. al (abstract 270) administered exenatide and/or lisofylline, an anti-inflammatory compound, in a type 1 diabetic mouse model, showing that with the combination of both agents diabetes could be reversed, with histology showing development of new islet-like groups of insulin-producing cells.

Heine et al. (abstract 9) compared exenatide versus insulin glargine in a 26-week study of 283 vs. 268 persons treated with metformin and/or a sulfonylurea, showing fall in A1C from 8.2 and 8.3% to 7.2 vs. 7.1%, with a 2.3-kg weight loss vs. a 1.8-kg weight gain. Exenatide reduced postprandial glucose excursions following breakfast and dinner, while glargine predominantly reduced fasting glucose, suggesting that the two agents may offer complementary approaches to treatment. Kendall et al. (abstract 16) and Blonde et al. (abstract 477) reported 82-week, open-label, extension study results with exenatide in 256 persons, as well as effects in 128 persons receiving placebo for the first 30 weeks, showing weight loss averaging 4.6 kg, sustained A1C reduction of 1.2%, and weight loss–related decreased triglyceride, diastolic blood pressure, and LDL cholesterol, as well as increase in HDL cholesterol. Linnebjerg et al. (abstract 469) reported that exenatide, which is cleared by the kidneys, has half-life averaging 1.5 h in persons with normal renal function, 2.1 h in those with creatinine clearance 60–80 ml/min, 3.2 h in those with clearance 35–50 ml/min, and 6 h in those with clearance <30 ml/min, with worse gastrointestinal side effects in the latter group, leading to the suggestion that the agent can be administered without dose adjustment to persons with clearance >35 ml/min. Mari et al. (abstract 482) reported that analysis of metformin and metformin- plus sulfonylurea-treated persons given exenatide showed that the agent improved insulin secretion. Maggs et al. (abstract 485) reported no correlation of the reduction in A1C or weight loss on exenatide with either early or late experience of gastrointestinal side effects. Ratner et al. (abstract 10) administered the albumin-bound GLP-1 conjugate CJC-1131 at two doses versus placebo for 12 weeks to 81 persons treated with metformin, with baseline A1C 7.9% decreasing 0.6 and 1.1% vs. placebo with lower and higher dosages.

Karl et al. (abstract 48) administered 120 μg pramlintide two to three times daily to 166 insulin-treated type 2 diabetic persons, showing that weight decreased 2.8 kg and that A1C decreased 0.6% from the initial level of 8.3%, with a 10% dose-related decrease in prandial insulin requirement. Guthrie et al. (abstract 478) reported an open-label study of persons with type 1 diabetes receiving 30 or 60 μg pramlintide three to four times daily, with a decrease in A1C from 8 to 7.8%, a 3-kg decrease in body weight, and a 22% reduction in rapid-acting insulin doses after 26 weeks. Nausea and vomiting occurred in 40 and 8% of patients, respectively, and 23% experienced at least one episode of severe hypoglycemia during the study period.

Leptin and adiponectin

Several presentations at the meeting based on the Atherosclerosis Risk in Communities (ARIC) study suggested that substances produced by adipocytes are relevant to the development of diabetes. Duncan et al. (abstract 364) studied complement C3, which is produced by adipocytes, and which, in addition to its immune actions, is a precursor to acylation-stimulating protein, stimulating triglyceride synthesis. In 581 persons developing diabetes and 572 control subjects in the ARIC study, the risk of diabetes in the second through fourth C3 quartiles was 1.6- to 2-fold greater than that in the lowest quartile, adjusting for age, sex, ethnicity, study center, parental history of diabetes, hypertension, BMI, waist-to-hip ratio, fasting glucose, and insulin at baseline. Schmidt et al. (abstract 1052) similarly presented a case-control analysis from ARIC participants, showing that there was an association between leptin and diabetes risk, with the second, third, and fourth sex-specific quartiles of leptin having 1.6-, 2.3-, and 4-fold

greater risk than the lowest quartile. After adjustment for BMI, waist, inflammation score, hypertension, triglycerides, insulin, and fasting glucose, leptin was seen to be protective, with the second through fourth quartiles having 7, 42, and 61% lower risk of diabetes, respectively, suggesting that leptin resistance is the risk factor, with leptin itself having antidiabetic effects. The implications of these studies and of much related research were the topic of a number of fascinating presentations.

Jeffrey Flier (Boston, MA) gave the Banting Lecture on the interrelationship between fat and the brain, as well the regulation of energy balance. He noted the interaction of obesity and diabetes and the rising prevalence of obesity in the U.S. and worldwide, with consequent increase in cardiovascular disease and malignancy. In the Nurses Health Study, the risk of diabetes increased nearly 100-fold comparing a BMI of 20 with that of 35 kg/m^2 (6). From an evolutionary perspective, starvation is a greater threat to survival than obesity, resulting in the growing epidemic of obesity with excess food availability. Obesity results both from genes and from the environment. The most common monogenic abnormality associated with obesity is the loss-of-function mutation in the melanocortin (MC)-4 gene, accounting for 3–4% of severe obesity. There are likely to be many polygenic abnormalities, most of which have not been identified. Environmental factors include the easy and inexpensive availability of food in much of the world, particularly that of foods increasing the development of obesity, in the setting of reduced activity.

The brain controls many aspects of appetite, food intake, physical activity, thermogenesis, metabolic fluxes, reproduction, growth, and thyroid activity, with the hypothalamus particularly involved in regulation of energy balance, with multiple brain signaling mechanisms both increasing and limiting food intake, involving olfaction, taste, visual signals, and the more complex memories and associations involved in central reward circuitry. The challenge, Flier suggested, is to understand the integration of these diverse signals.

Adipose tissue acts not only as a site of energy storage but also as an endocrine gland, producing substances including free fatty acids (FFAs), estrogen, adipsin (a complement-related protein that is suppressed in obesity), cytokines such as tumor necrosis factor (TNF)-α, angiotensinogen, and adipokines including adiponectin and resistin. An additional adipocyte process is the intracellular production of active glucocorticoids, as seen in the action of 11-β hydroxysteroid dehydrogenase-1 on cortisone to increase levels of cortisol. An important adipocyte secretory product is leptin, which rises as adipocyte mass increases and signals the brain to decrease food intake and increase energy expenditure. It is likely that reduction in the level of leptin with starvation serves as a starvation signal (7), causing hunger, suppression of energy expenditure, and changes in thyroid and reproductive hormones (8,9), leading to weight regain.

Most obese humans have increased levels of leptin but are resistant to its central actions. Flier explored the mechanism of this phenomenon. There is no evidence of circulating antagonists. The blood-brain leptin barrier may play a role in leptin resistance. Leptin resistance may also be a function of decreased postreceptor response. The hormone acts at two neuronal systems, one producing proopiomelanocortin (POMC) and cocaine-amphetamine–regulated transcript (CART), causing catabolic effects and weight loss and activated by leptin, and the other producing agouti-related peptide (AGRP) and neuropeptide Y (NPY), causing food intake and inhibited by leptin. POMC stimulates and AGRP inhibits the MC-4 receptor in the paraventricular nucleus and ventromedial hypothalamus. Other circulating factors related to central control of food intake and energy balance include insulin, gastrointestinal peptides such as ghrelin and peptide YY, serotonin, cannabinoids, glucose, and FFAs. Multiple sites in the hypothalamus and elsewhere in the brain are involved in the process.

Leptin receptor activation leads to its tyrosine phosphorylation via Janus kinase 2, leading to signal transducers and activators of transcription (STAT)3 activation, with subsequent nuclear transcription effects essential for appetite/metabolism regulation. In a negative feedback loop, STAT3 activates the suppressor of cytokine signaling (SOCS)3 in both POMC/CART and in NPY/AGRP neurons, leading to inhibition of leptin action, and likely responsible for the leptin resistance associated with obesity. Heterozygotes with partial lack of SOCS3 expression have reduced fat mass and increased leptin sensitivity, with protection against high-fat diet–induced obesity, at least in part due to increased hypothalamic leptin-mediated STAT3 activation. SOCS3, then, appears to be rate limiting, with mice having neuron-specific deletion of SOCS3, despite reduced leptin levels, more sensitive to leptin and protected against diet-induced obesity, showing improved glucose homeostasis.

Flier speculated that SOCS3 may also play a role in insulin action, as its overexpression decreases insulin signal transduction and as adipocytes with SOCS3 deletion showed increased insulin-stimulated glucose uptake and reduced ability of TNF-α to suppress insulin action. SOCS3 appears, then, to be induced both by leptin and by insulin in some of their target cells and to antagonize their actions. SOCS3 is induced by FFAs and by resistin and appears to be necessary for resistin-induced insulin resistance. The mechanism of FFA-induced insulin resistance is more complex, with SOCS3, protein kinase C θ, Janus kinase, peroxisome proliferator–activated receptors (PPARs), and the inhibitor of nerve factor-κB kinase all involved. FFA uptake interacts with toll-like receptor (TLR)4, the obligatory receptor for lipopolysaccharide (endotoxin), and activates the macrophage innate immune response. TLR4 may be present on adipocytes and mediate FFA effects, with evidence that mice not expressing TLR4 show reduced response to high-fat diets and to lipid infusion, suggesting TLR4 to be a link between the innate immune system and the inflammatory signals contributing to insulin resistance.

Returning to the response of central circuits regulating energy balance to peripheral signals such as leptin, Flier pointed out the important concept of neural plasticity, so that, for example, the number and direction of neural projections at hypothalamic sites can be regulated by leptin, the number of synaptic inputs can be regulated by ghrelin, and the number of functional neurons can be modified by ciliary neurotrophic factor (CNTF). CNTF is a 22,000–molecular weight protein expressed in astrocytes, acting as a trophic factor for motor neurons in the ciliary ganglion and stimulating cell survival. CNTF ameliorates obesity in leptin-deficient and diet-induced models, activating STAT3 signaling, with clinical studies suggesting weight loss that persists after completion of treatment, perhaps because of stimulation of hypothalamic neurogenesis. Neu-

ral stem cells do give rise to new neurons throughout life, as has particularly been found in the dentate gyrus of the hippocampus, playing a role in memory, and in the subventricular zone, having effect on olefaction. Direct central administration of CNTF causes neurogenesis with expression of neuropeptides including NPY and POMC, potentially both activating neural signaling and causing growth of new leptin-responsive cells. Flier commented on another peptide, melanin-concentrating hormone (MCH), which stimulates food intake, with MCH antagonists having potential benefit in the treatment of obesity.

Philipp Scherer (Bronx, NY) received the ADA Outstanding Scientific Achievement Award for his research on adipose tissue, which he placed in clinical context by reviewing the increasing prevalence of obesity in the U.S. and worldwide. He addressed new understanding of the adipocyte based on a series of mouse models with relevance to clinical disease. Excess adipose tissue, but also lipoatrophy and lipodystrophy, are associated with insulin resistance. The absence of adipose tissue leads to dysregulation of triglyceride and fatty acids and fat deposition in muscle and liver, and to abnormalities of adipokines, suggesting the importance of this tissue in maintaining normal insulin sensitivity. Adipose tissue also has effects on inflammation, leading to effects on energy metabolism, metabolic syndrome, cardiovascular disease, and malignancy. Adipose tissue is the only organ with unlimited growth potential at every stage of life, producing factors with effects on the vasculature, liver, muscle, brain, reproductive tract, and β-cells.

Adiponectin, Scherer noted, strongly resembles the proinflammatory cytokine TNF-α. Adiponectin infusion decreases hepatic glucose production by increasing the hepatocyte response to insulin, suggesting that it acts as an endogenous insulin sensitizer. Despite its exclusive production in adipocytes, adiponectin levels are lowest in persons with high fat mass, and levels also tend to be low in persons with evidence of inflammation or of atherosclerosis. Adiponectin has antiatherosclerotic effects in suppressing the endothelial inflammatory response and inhibiting vascular smooth muscle cell proliferation. Adiponectin levels are higher in women than in men, lower in lipodystrophy and in type 2 diabetes, higher in type 1 diabetes, and increase with PPARγ agonist treatment. There are a number of adiponectin polymorphisms associated with diabetes, and the susceptibility locus for type 2 diabetes and the metabolic syndrome on chromosome 3q27, where the adiponectin gene is located, appears to act by decreasing circulating adiponectin levels.

Adiponectin circulates in forms made up of different numbers of adiponectin monomers, making it difficult to determine the circulating biologically relevant level. The higher levels in females appear due to higher–molecular weight forms, which appear to be metabolically active, with PPARγ agonists primarily increasing these forms of adiponectin (10). Conversely, type 2 diabetes is associated with particular reduction in the high–molecular weight forms, with levels decreasing progressively as glycemia worsens. The ratio of low-to-high molecular weight forms appears to show particularly close correlation with insulin resistance (11).

In transgenic mouse models overexpressing adiponectin, insulin sensitivity is increased, lipids improved, and the adverse effect of high-fat diet is lessened, with increased brown adipose tissue depots, perhaps increasing the metabolic rate. In leptin-deficient *ob/ob* mice, overexpression of adiponectin improves glycemia, islet morphology, and β-cell function, with more efficient triglyceride clearance due in part to higher levels of adipocyte lipoprotein lipase. Smaller adipocytes are seen, concordant with improved metabolism. Administration of neutralizing adiponectin antibodies reverses these improvements. However, adiponectin overxpression in these mice increases their fat mass despite the improved metabolic health, suggesting potential adverse effects of increased adiponectin, although depositing lipids in adipocytes appears far more desirable than that in sites such as muscle and liver, which worsen insulin sensitivity. The effects of adiponectin are remarkably similar, then, to those of the PPARγ agonists. In mice not expressing adiponectin, the response to PPARγ agonists is reduced, and the agents fail to improve glucose tolerance or to increase hepatic AMP-activated protein kinase activity. Scherer pointed out that visceral fat stores are major sites of adiponectin production, so that hepatic exposure to adiponectin may be particularly high because of higher portal venous levels. Visceral obesity may therefore be particularly damaging in insulin-resistant states, leading to low portal adiponectin and to high portal FFAs, both of which have important effects on hepatic metabolism.

A novel family of cytokines includes resistin and resistin-like molecules, which have effects opposite to those of adiponectin on hepatic insulin sensitivity. Resistin has highly exposed disulfide bonds, with forms of resistin not able to form disulfide bonds having decreased activity, a phenomenon also seen with adiponectin. The redox potential of the endoplasmic reticulum is determined by cellular glutathione, which has important effects on the formation of these disulfide bonds. As adipocyte glucose uptake does not decrease with increasing glucose exposure, hyperglycemia leads to increased adipocyte reactive oxygen species production, contributing to inflammatory adipocyte changes, with activation of nerve factor-κB triggering the inflammatory cascade and worsening insulin sensitivity, as well as lowering glutathione levels. The insulin resistance further worsens adipocyte oxidative stress, producing a positive feedback cycle. PPARγ agonists decrease glutathione molecular chaperones in addition to having direct antioxidant effects.

Examining a lipodystrophic fat model termed FAT-ATTAC (fat apoptosis through triggered activation of caspase 8), leading to the ability to induce acute complete fat loss after maturation, with the *ob/ob* mutation, FAT-ATTAC mice have low insulin levels, loss of β3 agonist insulin response, and dramatically increased food intake. The mice have increased body core temperature and decreased inflammatory markers, with reduction in response to endotoxin. There is evidence that macrophages present in adipose tissue mediate much of the increase in circulating inflammatory markers in obesity, perhaps in response to paracrine signals from adipocytes. Thus, a number of questions remain to be addressed in understanding adipocyte physiology, including fuller characterization of adipocyte secretory factors and receptors, in particular those for resistin and resistin-like molecule. Scherer also touched on the importance of determining the mechanism of leptin action, and the role of the macrophage in adipose tissue. He noted that angiogenesis inhibitors have marked effects on adipocytes, suggesting a future direction for the treatment of obesity.

Streamson Chua (New York, NY) discussed additive and synergistic interactions of leptin-sensitive neurons,

beginning with an overview of the neural connections from leptin-sensitive neurons. Leptin is produced by white and brown fat, signaling both orexigenic neurons (NPY, AGRP, MCH, and hypocretin), and anorexogenic neurons (melanocyte-stimulating hormone, CART, corticotropin-releasing hormone, and thyrotropin-releasing hormone), in the arcuate nucleus, NPY/AGRP and POMC/CART neurons, in the paraventricular nucleus corticotropin-releasing hormone and thyrotropin-releasing hormone neurons, and in the lateral hypothalamic area MCH and hypocretin neurons. These neurons in turn have multiple interconnections, suggesting both direct and indirect effects of leptin on these and other neurons.

The leptin receptor is a class I cytokine receptor of the interleukin-6 family. Animals with a defective leptin receptor develop hyperphagia, obesity, insulin resistance, glucose intolerance, brown fat atrophy, cold intolerance, hypothalamic hypogonadism, and adrenal hyperfunction. Chua described studies utilizing three different receptor alleles, Lepr-neo, Lepr-flox, and Lepr-d17, the latter lacking the STAT3 activation site, allowing study of the effects of defective leptin receptors at the POMC/CART, NPY/AGRP, or both sets of neurons. Body weight was increased in the mice lacking receptors at both sites and to a lesser extent in those lacking receptor at one site, particularly without leptin receptors at AGRP neurons. Lean tissue mass particularly increased in females, due to increased linear growth, while fat mass tripled in females and doubled in males with the dual receptor deletion. Adrenal hyperfunction and hyperinsulinemia were seen with the dual receptor defect, although insulin levels are higher in *db/db* mice completely lacking the leptin receptor, and fasting glucose was not increased, suggesting that other leptin-sensitive neurons, presumably in the ventral hypothalamic nucleus and the lateral hypothalamic area, also contribute to the actions of leptin on body composition, insulin sensitivity, and fertility.

Michael Rosenbaum (New York, NY) discussed aspects of regulation of body weight, reviewing the concept that "the brain knows how many calories " to store and suggesting that leptin constitutes the signal and that relative leptin deficiency must be present in obesity. Body fatness is more strongly heritable than schizophrenia, hypertension, seizure disorders, coronary artery disease, or breast cancer. There is a relatively linear relationship of 24-h energy expenditure to body mass across all mammalian species (12), and Rosenbaum described a linear relationship between fat-free mass and 24-h energy expenditure in human studies. During 6- to 8-week periods of fixed composition liquid diets at basal, 90%, and 80% weight, however, weight loss reduces the expected energy expenditure, with persons who lose 10% of body weight having ~20% lower fat-free mass–adjusted caloric requirement, a phenomenon that persists over years. Energy expenditure includes the resting energy expenditure (REE), the non-REE, caloric expenditure required for physical activity, and the thermic effect of feeding. With 10% weight loss, Rosenbaum described reductions not only in REE but also a 40% decrease in non-REE, together accounting for 89% of the change in total energy expenditure. Thus, after weight is lost, skeletal muscle work efficiency at low levels of activity increases by 15–20%, associated with preferential use of fatty acids as fuel, and accounting for 35% of the reduction in nonresting nervous expenditure. Rosenbaum noted that these changes are similar to those occurring with leptin deficiency, with both weight–reduced and leptin-deficient persons being hypometabolic and hyperphagic, and showing decreased sympathetic and increased parasympathetic tone. He suggested that leptin is a negative regulator defending body fatness rather than increasing with weight gain to preserve thinness, noting that from an evolutionary perspective the imperative is to decrease energy expenditure and fertility during periods of undernutrition, so that leptin exhibits "asymmetric regulation, " with reduction in levels causing hyperphagia, hypometabolism, infertility, hypothyroidism, and hypercortisolemia. The weight-reduced state, then, is perceived by the brain as one of relative leptin insufficiency, leading to adaptations aimed at preservation of fat mass. This concept predicts that leptin administration will have little or no effect in states of leptin sufficiency, but might have significant impact following weight loss. To study this, leptin was administered to restore baseline levels in persons who had had 10% weight loss. Energy expenditure in skeletal muscle decreased with weight loss and was restored almost to baseline with leptin administration. The work efficiency of skeletal muscle increased with weight loss, and this reversed with leptin, the decrease in glucose utilization and increase in FFA utilization also returning to baseline. Weight loss decreases thyroxin and triiodothyronine, with both normalized by leptin, although the decrease in thyroid-stimulating hormone with weight loss is not restored by leptin. Urinary catecholamine excretion and sympathetic nervous system activity were decreased by weight loss and increased by leptin replacement. These actions of leptin are concordant with its known effects, then, and suggest that the hormone may have therapeutic benefit following weight loss in obese persons.

Christos Mantzoros (Boston, MA) extended these observations, discussing potential therapeutic uses of leptin in low-leptin states. He noted that leptin acts in muscle, brown and white adipose tissue, liver, and macophages, as well as in brain. Leptin administration to children with leptin deficiency restores normal weight. In a study of leptin administration to 73 leptin-deficient heterozygotes, there was a dose-response weight loss effect, suggesting potential benefit for these individuals as well. This is not, however, a common cause of severe obesity in humans. Leptin has a direct relationship to body fat, so that in the vast majority of persons' obesity is associated with leptin resistance rather than deficiency, while low leptin "is the signal . . . that there are not enough calories, " and is associated with energy deficiency, with low leptin in turn reducing levels of reproductive hormones, thyroid hormone, and IGF-1. Leptin-deficient mice and humans have not only extreme obesity but also delayed pubertal development, with replacement increasing gonadotropins and leading to puberty onset in leptin-deficient persons. Leptin plays a role in the initiation of normal puberty (13). Women with hypothalamic amenorrhea have low estrogen, low or normal gonadotropins, low thyroid hormone, increased growth hormone but decreased IGF-1, increased cortisol, and infertility and bone loss with increased risk for stress fracture. This occurs in women during stress, weight loss, low weight for height, and/or strenuous exercise, with the set of abnormalities seen in ~5% of college-age women. These women have low leptin levels and absence of the normal diurnal leptin pattern. Administration of leptin to six such women for 2 months led to increase in leptin levels, with associated ovulation and improvement in ovarian and endometrial morphology and improvement of hypothalamic function. Increased bone forma-

tion was suggested by increased osteocalcin and bone alkaline phosphatase (9).

Lipoatrophy, both the spontaneous form and that caused by antiretroviral treatment of HIV, is associated with insulin resistance. In persons with congenital lipoatrophy and diabetes, leptin treatment lowered triglyceride, A1C (by ~3%), and liver fat. In a controlled trial of HIV-infected persons with lipoatrophy and insulin resistance, leptin replacement improved insulin resistance and hyperlipidemia. Two studies addressing leptin treatment of lipodystrophy were presented at the ADA meeting. Javor et al. (abstract 47) administered twice-daily recombinant methionyl human leptin for 12 months to 15 persons with generalized lipodystrophy, showing decrease in fasting glucose from 205 to 126 mg/dl, in A1C from 9 to 7.1%, in triglycerides from 1,380 to 516 mg/dl, and in LDL cholesterol from 139 to 85 mg/dl, with a 40% reduction in liver volume and a 4-kg weight loss. In a similar study with this treatment for up to 30 months, Kusakabe et al. (abstract 610) showed early reduction in glycemia and reduction of urinary albumin excretion in patients with overt albuminuria.

Thus, leptin is the peripheral signal of adequate energy stores required for normal body weight regulation, insulin sensitivity, and immune, reproductive, and neuroendocrine function. It may represent a better treatment for hypothalamic amenorrhea than estrogen, with other disease states potentially responding to leptin including anorexia nervosa, delayed puberty, and a variety of related conditions. We appear ready to enter a new stage in the therapy of obesity and related conditions, applying seemingly disparate areas of research regarding peptides derived from the gastrointestinal tract, the adipocyte, and the brain. The knowledge gained may have important benefits in a number of states related to diabetes and insulin resistance, as well as having potential applicability in understanding and treatment of adverse effects of hypocaloric states. It is intriguing to speculate that some of the mechanisms of benefit of agents acting at the level of GLP-1 may be better understood with understanding of the effects of leptin and of adiponectin, and that the discovery of additional signaling processes derived from these three tissues will have profound effects on future therapy of metabolic disorders.

References

1. Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824–830, 2002
2. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72, 1996
3. Thiele TE, Van Dijk G, Campfield LA, Smith FJ, Burn P, Woods SC, Bernstein IL, Seeley RJ: Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats. *Am J Physiol* 272:R726–R730, 1997
4. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316–1322, 1992
5. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744, 1993
6. Colditz GA, Willett WC, Rotnitzky A, Manson JE: Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med* 122:481–486, 1995
7. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS: Role of leptin in the neuroendocrine response to fasting. *Nature* 382:250–252, 1996
8. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS: The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111:1409–1421, 2003
9. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS: Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 351:987–997, 2004
10. Tonelli J, Li W, Kishore P, Pajvani UB, Kwon E, Weaver C, Scherer PE, Hawkins M: Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. *Diabetes* 53:1621–1629, 2004
11. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE: Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 279:12152–12162, 2004
12. Kleiber M: Metabolic turnover rate: a physiological meaning of the metabolic rate per unit body weight. *J Theor Biol* 53: 199–204, 1975
13. Mantzoros CS, Flier JS, Rogol AD: A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab* 82:1066–1070, 1997