Editors' Choice

Ken Ong and Ze'ev Hochberg

New clinical guidelines

Ethical and policy issues in genetic testing and screening of children

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With rapid growth in both genetic knowledge and in consumer interest, the genetic testing and genetic screening of children are now commonplace. The decisions about whether to offer these tests should be driven by the best interests of the child. The growing evidence on the psychosocial and clinical effects of such testing and screening should inform best practices. This policy statement represents recommendations developed collaboratively by the American Academy of Pediatrics and the American College of Medical Genetics and Genomics. They apply to many of the scenarios in which genetic testing and screening may occur, including: diagnostic testing; newborn screening; carrier testing; predictive genetic tests; histocompatibility testing; adoption; disclosure, and direct-to-consumer testing.

For children who present with clinical features of genetic illnesses or with a family history of a genetic condition, pediatric endocrinologists have an increasing number of genetic tests available in our clinics, even without having to consult with our medical genetics colleagues. More and more, we are put under pressure to perform these tests by families. These recommendations from the USA are therefore timely and will be very valuable reading to many pediatricians worldwide. In addition to the wide range of scenarios described above, the authors provide two important general recommendations, which most pediatricians would no doubt whole-heartedly endorse. First, that the decisions regarding the use of testing should be driven by the best interests of the child. Second, that genetic testing is best offered in the context of genetic counseling.

A key strength of these recommendations is that they are informed by the published evidence on the psychosocial, clinical and reproductive harms and benefits of genetic testing and screening, which was comprehensively reviewed and published at the same time [1]. Evidence on key issues such as informed consent or assent, voluntary agreement, privacy and confidentiality are presented and interpreted. An interesting distinction is made between 'predictive' testing, for mutations that will almost certainly give rise to disease, typically childhood-onset conditions, versus 'predispositional' testing, for mutations with incomplete penetrance that may never become manifest, such as adult-onset cancer syndromes.

Recently approved anti-obesity drugs

Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs

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Background: Lorcaserin was recently approved by the US Food and Drug Administration for the treatment of obesity.

Methods: The authors performed a systematic review and meta-analysis of randomized controlled trials (RCTs) to evaluate the effect of lorcaserin on weight loss and its adverse events in obese adults (18–65 years old).

Results: In RCTs of 1 year duration, lorcaserin led to greater mean weight loss (3.23 kg, 95% CI 2.70, 3.75) and BMI reduction (1.16 kg m^{-2} , 95% CI 0.98, 1.34) compared with placebo. The use of lorcaserin for 8 and 12 weeks reduced weight by 1.6 kg (95% CI 0.34, 2.86) and 2.9 kg (95% CI 2.2, 3.5), respectively. Compared to placebo, lorcaserin decreased waist circumference, blood pressure, total cholesterol, low-density lipoprotein-cholesterol and triglycerides, but did not change heart rate or high-density lipoprotein-cholesterol. Headache, nausea and dizziness were significantly more common in the patients receiving lorcaserin than patients receiving placebo, but diarrhea was no more common.

Conclusions: Lorcaserin achieves modest weight loss and appears to be well tolerated. Clinical and pharmaco-vigilance studies with longer study duration are needed to inform of the long-term efficacy and safety of lorcaserin.

In spite of the growing burden of obesity worldwide, anti-obesity drug development and approvals have lagged behind. Lorcaserin is suggested to induce satiety through its selective agonistic effect on the serotonin 5-HT_{2C} receptor. It has been in clinical trials for several years and was recently approved by the US Food and Drug Administration (FDA). Notably, it had been previously rejected by the FDA because of evidence of increased cancer incidence in rats and because of its modest efficacy (3% loss of body weight after 1 year of treatment). The FDA advisory panel has now concluded that the cancer findings do not apply to humans, and our desperation has reached a state when even modest efficacy is welcome.

Since the 2012 *Yearbook*, the FDA has also approved Qsymia, a combination of two older drugs, phentermine and topiramate, which reduces hunger by targeting various neurotransmitter systems, mainly norepinephrine, but also dopamine and serotonin. Qsymia is more potent than lorcaserin (8.9% loss of body weight at the highest dose compared to placebo), yet it may have more serious side effects, including increased heart rate and risk of birth defects. The FDA judged that the benefits outweigh the risks. These are not perfect drugs; healthy lifestyle and carbohydrate-low diets work better when they are introduced, only that we fail to introduce them.

New treatment for hypothalamic obesity

Glucagon-like peptide-1 agonist exenatide leads to reduction of weight and caloric intake in a rat model of hypothalamic obesity

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Horm Res Paediatr 2012;78:47–53

Background: Hypothalamic obesity is caused by damage of the medial hypothalamic nuclei. The glucagon-like peptide-1 agonist exenatide is a synthetic version of exendin-4 (Ex4), is used in the treatment of type 2 diabetes, and causes weight loss via hindbrain signaling.

Methods: The authors tested Ex4 in an established rat model of hypothalamic obesity due to medial hypothalamic lesions. Lesion and control animals were given either daily intraperitoneal injections of 1 µg/kg Ex4 or saline for 9 days.

Results: Significant differences in percent baseline food intake (lesion –20.8%, control –13.6%; $p < 0.001$) and percent change in body weight (lesion –4.9%/9 days, control –3.2%/9 days; $p < 0.05$) were observed during Ex4 treatment compared with saline.

Conclusion: Ex4 reduced food intake and body weight in a rat model of hypothalamic obesity. Follow-up studies are required to further elucidate its effects on energy homeostasis and to establish Ex4 as a potential drug for treatment of hypothalamic obesity.

Treatment with glucagon-like peptide-1 agonist exendin-4 in a patient with hypothalamic obesity secondary to intracranial tumor

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Horm Res Paediatr 2012;78:54–58

Background: Patients with hypothalamic tumors frequently experience hyperphagia and severe obesity. Treatment with diet, exercise, and/or pharmacologic agents has limited effect. The glucagon-like peptide-1 agonist exenatide (exendin-4) is used in the treatment of type 2 diabetes, and causes persistent weight loss via signaling in the brainstem.

Methods: The authors report the case of a 17-year-old patient with severe obesity due to a hypothalamic germ cell tumor. He received chemoradiotherapy and exenatide 5 µg subcutaneously twice daily.

Results: Exenatide resulted in a 29-kg weight loss (BMI reduced from 37.1 to 29.1) after 2.5 years of treatment; significant weight gain occurred soon after exenatide was discontinued.

Conclusion: Exenatide substantially reduced excess body weight in a patient with severe hypothalamic obesity. This novel observation requires follow-up clinical studies in order to establish the effects of exenatide in patients with disrupted hypothalamic energy-regulatory pathways.

Patients with brain tumors in the hypothalamic region are at high risk for severe obesity resulting either directly from the tumor or from treatment-related damage in the ventromedial region which regulates appetite and satiety. Treatment options are limited and are often ineffective, because the degree of hyperphagia is often severe, many patients have other disabilities that limit physical activity, and the hypothalamic appetite-regulatory targets are destroyed. Glucagon-like peptide-1 (GLP-1) agonists are increasingly used to lower hyperglycemia in patients with type 2 diabetes, in whom significant weight loss is a recognized additional benefit of these agents. GLP-1 receptors are present in the gut, exocrine pancreas, brainstem and hypothalamus, and its mechanism on weight loss may therefore involve both central and peripheral actions, for example by reducing the rate of gastric emptying.

The pair of papers reported above provide complementary evidence in a rat model and in humans that GLP-1 agonists can indeed reduce hyperphagia and weight gain through non-hypothalamic mechanisms. Larger case series have also been recently reported [2] and formal trials of exenatide in similar adult and pediatric patient groups are in progress.

New treatment for Cushing's disease

Potential role for retinoic acid in patients with Cushing's disease

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J Clin Endocrinol Metab 2012;97:3577–3583

Background: Cushing's disease, i.e. cortisol excess due to an ACTH-secreting pituitary adenoma, is a rare disorder with considerable morbidity and mortality, but as yet no satisfactory medical treatment. Experimental data have recently shown that retinoic acid restrains ACTH secretion by tumoral corticotropes. The authors aimed to evaluate the efficacy and safety profile of retinoic acid in patients with Cushing's disease.

Methods: The authors performed a single-arm, prospective, multicenter study. Seven patients with Cushing's disease (3 men, 4 postmenopausal women) were started on 10 mg retinoic acid daily and dosage increased up to 80 mg daily for 6–12 months. ACTH, urinary free cortisol (UFC), and serum cortisol as well as clinical features of hypercortisolism and possible side effects of retinoic acid were evaluated at baseline, during retinoic acid administration, and after drug withdrawal.

Results: A marked decrease in UFC levels was observed in 5 patients; mean UFC levels on retinoic acid were 22–73% of baseline values and normalization in UFC was achieved in 3 patients. Plasma

ACTH decreased in the first month of treatment and then returned to pretreatment levels in responsive patients whereas no clear-cut pattern could be detected for serum cortisol. Blood pressure, glycemia, and signs of hypercortisolism, e.g. body weight and facial plethora, were ameliorated to a variable extent on treatment. Patients reported only mild adverse effects, e.g. xerophthalmia and arthralgias.

Conclusions: Long-term treatment with retinoic acid was beneficial and well tolerated in 5 of 7 patients with Cushing's disease. This represents a novel, promising approach to medical treatment in Cushing's disease.

Experimental studies have shown that retinoic acid has antiproliferative actions on tumoral corticotropes and reduces ACTH secretion in a murine tumoral corticotrope cell line. Given these favorable premises, this paper reports the use of retinoic acid in patients with Cushing's disease, and finds a marked decrease in UFC levels in 5/7 patients; plasma ACTH decreased in the first month of treatment and then returned to pretreatment levels in responsive patients whereas no clear-cut pattern could be detected for serum cortisol. More importantly, the phenotype ameliorated in terms of blood pressure, glycemia, body weight and facial plethora. Retinoic acid is currently used in patients with severe acne, in whom it is given for long treatment periods and is, by and large, well tolerated. While this new indication is not to replace the first line of treatment – surgery, it may be useful as an adjunct therapy before surgery or when surgery has failed.

New treatment: from gene mutation to drug

Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia

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N Engl J Med 2012;367:1891–1900

Background: Serum proprotein convertase subtilisin/kexin 9 (PCSK9) binds to low-density lipoprotein (LDL) receptors, increasing the degradation of LDL receptors and reducing the rate at which LDL cholesterol is removed from the circulation. REGN727/SAR236553 is a fully human PCSK9 monoclonal antibody, which increases the recycling of LDL receptors and reduces LDL cholesterol levels.

Methods: The authors performed a phase 2, multicenter, double-blind, placebo-controlled trial involving 92 patients with LDL cholesterol levels of ≥ 100 mg/dl (≥ 2.6 mmol/l) after treatment with 10 mg of atorvastatin for at least 7 weeks. Patients were randomly assigned to receive 8 weeks of treatment with 80 mg of atorvastatin daily + REGN727/SAR236553 once every 2 weeks, 10 mg of atorvastatin daily + REGN727/SAR236553 once every 2 weeks, or 80 mg of atorvastatin daily + placebo once every 2 weeks, and were followed for an additional 8 weeks after treatment.

Results: The percentage reductions in LDL cholesterol levels from baseline were (mean $\pm$ SE) 73.2 ± 3.5 with 80 mg of atorvastatin + REGN727/SAR236553, and 66.2 ± 3.5 with 10 mg of atorvastatin + REGN727/SAR236553, compared to only 17.3 ± 3.5 with 80 mg of atorvastatin + placebo ($p < 0.001$). All patients who received REGN727/SAR236553 attained an LDL cholesterol level of < 100 mg/dl versus 52% of those who received 80 mg of atorvastatin + placebo. At least 90% of patients who received REGN727/SAR236553 attained LDL cholesterol levels of < 70 mg/dl (1.8 mmol/l) versus 17% who received 80 mg of atorvastatin + placebo.

Conclusions: In patients with primary hypercholesterolemia, the addition of REGN727/SAR236553 to either 10 or 80 mg of atorvastatin resulted in greater reduction in LDL cholesterol than that attained with 80 mg of atorvastatin alone. (Funded by Sanofi and Regeneron Pharmaceuticals; ClinicalTrials.gov number, NCT01288469.)

The paper reports the extremely promising results of a new therapeutic agent, an antibody against PCSK9, which achieved a massive 70% reduction in LDL cholesterol levels from baseline levels on routine statin therapy, and far more than was achieved by maximal statin doses.

While statins are highly effective drugs to lower LDL cholesterol levels and have had a major impact on the prevention of cardiovascular disease risk, many patients are unable to reach recommended target levels of LDL cholesterol due to unacceptable side effects with high-dose statins. Statins work by inhibiting the enzyme HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, and thereby induce the expression of LDL receptors in the liver, which in turn increases the catabolism of plasma LDL. PCSK9 binds to LDL receptors to promote their degradation, reducing the rate of removal of LDL cholesterol from the circulation. Consequently, humans with deleterious PCSK9 gene defects have unusually low LDL cholesterol levels, but are otherwise quite healthy. Conversely, gain-of-function mutations in PCSK9 were identified in 2003 as the third genetic cause of autosomal dominant hypercholesterolemia [3].

This paper represents an amazing journey, within only 10 years, from gene discovery to demonstration of therapeutic potential. It also demonstrates the 'power of the extremes', the research approach to study individuals with unusually low (as well as high) disease risk markers. 2% of African-Americans in the Dallas Heart Study had loss-of-function mutations in PCSK9, associated with unusually low levels of LDL cholesterol [4]. Phase III trials are in progress to test the effects of this agent, and other similar therapies [5], to prevent cardiovascular events and to assess their safety profiles with greater rigor.

Two reminders about prolactinomas

Genetic analysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis

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Eur J Endocrinol 2013;168:533–541

Background: Germline mutations in the aryl hydrocarbon receptor interacting protein gene (AIP) have been identified in young patients (age ≤ 30 years) with sporadic pituitary macroadenomas. There are few data on prevalence of multiple endocrine neoplasia type 1 (MEN1) mutations in this population.

Methods: The authors assessed the prevalence of AIP and MEN1 genetic abnormalities in 174 young patients (≤ 30 years old) from endocrinology departments of 15 French university hospital centers, with a diagnosis of sporadic and isolated macroadenoma, without hypercalcemia and/or MEN1-associated lesions. The entire coding sequences of AIP and MEN1 were screened for mutations. In cases of negative sequencing screening, multiplex ligation-dependent probe amplification was performed to identify the presence of large genetic deletions.

Results: 21 out of 174 (12%) patients had AIP ($n = 15$, 8.6%) or MEN1 ($n = 6$, 3.4%) mutations. In pediatric patients (age ≤ 18 years), AIP/MEN1 mutation frequency was nearly 22% ($n = 10/46$). AIPmut and MEN1mut were identified in 8/79 (10.1%) and 1/79 (1.2%) somatotropinoma patients respectively; they each accounted for 4/74 (5.4%) prolactinoma patients with mutations. Half of those patients ($n = 3/6$) with gigantism displayed mutations in AIP. Interestingly, 4/12 (33%) patients with non-secreting adenomas bore either AIP or MEN1 mutations, whereas none of the 8 corticotroph adenomas or the single thyrotropinoma case had mutations. No large gene deletions were observed.

Conclusion: Mutations in MEN1 can be of significance in young patients with sporadic isolated pituitary macroadenomas, particularly prolactinomas. The authors suggest genetic analysis of MEN1 and AIP in such patients.

This paper serves as a reminder of the high incidence of MEN1 and AIP mutations in young patients who present with sporadic isolated pituitary macroadenomas, particularly macroprolactinomas. Positive findings could implicate a possible familial trait.

Changes in heart valve structure and function in patients treated with dopamine agonists for prolactinomas, a two-year follow-up study

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Clin Endocrinol (Oxf) 2012;77:99–105

Background: The use of ergot-derived dopamine agonists (DA) to treat patients with prolactinomas has not been associated with an increased risk of significant heart valve dysfunction. Accordingly, the present study evaluated whether the long-term use of DA for hyperprolactinemia may be associated with increased risk of valvular heart disease.

Methods: A total of 74 patients (mean age 48 ± 1.4 years, 23% male) with prolactinoma treated with DA for at least 1 year were evaluated with repeated 2-dimensional echocardiography at baseline and after 2 years of follow-up to evaluate changes in valvular heart structure (thickening, calcifications and leaflet motion abnormalities) and function (regurgitation or stenosis). 45 patients were treated with cabergoline, and 29 patients were not treated with cabergoline.

Results: At 2-year follow-up, no significant valvular stenosis was observed in any patient. In addition, the prevalence of any significant valve regurgitation did not change from baseline (from 12 to 15%, $p = \text{NS}$). However, there was an increase in the prevalence of valvular calcifications (from 48 to 58%, $p = 0.004$) and, specifically, in the prevalence of aortic valve calcifications (from 39 to 53%, $p = 0.002$). In a per-treatment-based analysis, patients treated with cabergoline had a higher prevalence of aortic valve calcification at 2 years' follow-up as compared to patients not treated with cabergoline (63 vs. 38%, $p = 0.016$).

Conclusions: The long-term therapy with DA (cabergoline) in patients with prolactinoma is associated with an increased prevalence of valvular calcification. However, these structural changes were not accompanied by an increased prevalence of valvular dysfunction.

This follow-up study of patients with prolactinoma treated with ergot-derived DA aimed to investigate whether the long-term use of these therapies increased the risk of significant valvular heart disease. The authors report a rise in the prevalence of valvular calcification after 2 years of follow-up, specifically, owing to an increase in aortic valve calcification. Furthermore, in a per-treatment-based analysis, the prevalence of aortic valve calcification at 2 years' follow-up was significantly higher in patients treated with cabergoline compared with those not treated with cabergoline. However, this change in valve morphology was not associated with an increase in valvular dysfunction. While the authors do not recommend the withdrawal of cabergoline for the long-term treatment of hyperprolactinemia, its safety should be considered on an individual basis. In any case, microprolactinomas do not need treatment if they are asymptomatic.

New concerns about old treatments

Tight glycemic control versus standard care after pediatric cardiac surgery

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Background: In some studies, tight glycemic control improved outcomes in adults undergoing cardiac surgery, but these benefits are unproven in critically ill children at risk for hyperinsulinemic hypoglycemia.

Methods: The authors performed a two-center, randomized trial, involving 980 young children, 0–36 months of age, undergoing cardiac surgery with cardiopulmonary bypass. Patients were randomly assigned to either tight glycemic control (with an insulin-dosing algorithm targeting a blood glucose level of 80–110 mg/dl (4.4–6.1 mmol/l)) or standard care in the cardiac intensive care unit (ICU). Continuous glucose monitoring was used to guide the frequency of blood glucose measurement and to

detect impending hypoglycemia. The primary outcome was the rate of healthcare-associated infections in the cardiac ICU. Secondary outcomes included mortality, length of stay, organ failure, and hypoglycemia.

Results: 444 (91%) of the 490 children assigned to intervention received insulin versus only 9 (2%) of the 490 children assigned to standard care. Normoglycemia was achieved earlier in intervention children (6 vs. 16 h, $p < 0.001$) and was maintained for a greater proportion of the critical illness period (50 vs. 33%, $p < 0.001$). However, intervention did not significantly decrease the rate of healthcare-associated infections (8.6 vs. 9.9 per 1,000 patient-days, $p = 0.67$), nor improve any secondary outcomes. Only 3% of intervention children had severe hypoglycemia (blood glucose <40 mg/dl (2.2 mmol/l)).

Conclusions: Tight glycemic control with a low hypoglycemia rate is achievable in children postcardiac surgery, but it does not improve the infection rate or other important clinical outcomes.

Hypoglycemia and risk of death in critically ill patients

NICE-SUGAR Study Investigators, Finfer S, Liu B, Chittock DR, Norton R, Myburgh JA, McArthur C, Mitchell I, Foster D, Dhingra V, Henderson WR, Ronco JJ, Bellomo R, Cook D, McDonald E, Dodek P, Hébert PC, Heyland DK, Robinson BG *N Engl J Med* 2012;367:1108–1118

Background: Whether hypoglycemia leads to death in critically ill patients is unclear.

Methods: The authors examined the associations between moderate and severe hypoglycemia (blood glucose 41–70 mg/dl (2.3–3.9 mmol/l) and ≤ 40 mg/dl (2.2 mmol/l), respectively) and death among 6,026 critically ill patients in intensive care units. Patients were randomly assigned to intensive or conventional glucose control. Cox regression analysis was performed, with adjustment for treatment assignment and other covariates.

Results: 2,714 (45.0%) of the 6,026 patients had moderate hypoglycemia, of whom 2,237 (82.4%) were in the intensive-control group. 223 (3.7%) patients had severe hypoglycemia, of whom 208 (93.3%) were in the intensive-control group. Of the 3,089 patients without hypoglycemia, 726 (23.5%) died, compared to 774 (28.5%) of the 2,714 with moderate hypoglycemia and 79 (35.4%) of the 223 with severe hypoglycemia. Compared with those without hypoglycemia, the adjusted hazard ratios for death among patients with moderate or severe hypoglycemia were 1.41 (95% CI 1.21–1.62; $p < 0.001$) and 2.10 (95% CI 1.59–2.77; $p < 0.001$), respectively.

Conclusions: In critically ill patients, both moderate and severe hypoglycemia are associated with increased mortality. The dose-response relationship between hypoglycemia and mortality is strongest for death from distributive shock. However, these observational analyses cannot prove a causal relationship.

The original findings by van den Berghe et al. [6] of a 30% reduction in mortality by normalizing blood glucose levels in critically ill adults in the ICU setting have proved hard to replicate. The RCT analyses of the large international trial NICE-SUGAR Study reported in 2009 that tight glucose control increased, rather than decreased, mortality. However the mechanism linking hypoglycemia to increased mortality has not been explained. The authors' observation that mortality was particularly high in those patients with severe hypoglycemia in the absence of insulin treatment (hazard ratio 3.84; 95% CI 2.37–6.23; $p < 0.001$) is suggestive of reverse causality, i.e. that at least in some cases hypoglycemia may occur as a result of more severe disease processes leading to death. However, the authors argue that their observed dose-response associations with increasing severity of hypoglycemia, and higher mortality in patients who had moderate hypoglycemia on more than 1 day (>1 vs. 1 day, $p = 0.01$), both support some causal adverse effects of hypoglycemia on mortality.

In contrast to the emerging evidence of harm in adults, in the pediatric intensive care setting Vlasselaers et al. [7] previously reported that lowering blood glucose concentrations to age-adjusted normal fasting values lowered the inflammatory response and reduced mortality from 6 to 3%. While the recent pediatric study by Agus and colleagues could not find any benefits of glucose lowering, at least no evidence of harm was shown and tight glucose control was achievable with a low rate of hypoglycemia. Does this mean that the potential benefit of tight glucose control in critically ill children is still an open question? Possibly – but the answer may require an even larger trial to establish! Meanwhile, avoidance of hypoglycemia may be of at least equal importance in critically ill children.

The relationship of sugar to population-level diabetes prevalence: an econometric analysis of repeated cross-sectional data

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Background: While experimental and observational studies suggest that sugar intake is associated with the development of type 2 diabetes, independent of its role in obesity, it is unclear whether alterations in sugar intake can account for differences in diabetes prevalence between populations.

Methods: The authors performed econometric modeling of repeated cross-sectional data on diabetes and nutritional components of food from 175 countries.

Results: A 150 kcal/person/day increase in sugar availability (equivalent to one can of soda/day) was associated with a 1.1% higher diabetes prevalence ($p < 0.001$) after testing for potential selection biases and controlling for other food types (including fibers, meats, fruits, oils, cereals), total calories, overweight and obesity, period effects, and several socioeconomic variables (such as aging, urbanization and income). No other individual food types yielded significant associations with diabetes prevalence after controlling for obesity and other confounders. The impact of sugar on diabetes was independent of sedentary behavior and alcohol use, and the effect was modified but not confounded by obesity or overweight. Duration and degree of sugar exposure correlated with diabetes prevalence in a dose-dependent manner, while declines in sugar exposure correlated with subsequent declines in diabetes rates, independently of other socioeconomic, dietary and obesity prevalence changes.

Conclusions: Differences in sugar availability statistically explain variations in diabetes prevalence rates at a population level that are not explained by physical activity, overweight or obesity.

We tell our patients that eating sugar does not cause diabetes. These results show that sugar availability is a significant statistical determinant of diabetes prevalence rates worldwide. As the fat and calorie hypothesis is falling out of favor, the sugar hypothesis is gaining ground. It is now thought that the mismatch between our Paleolithic genes and our modern carbohydrate-rich diet is the foundation of the obesity epidemic. While obesity remains the common claim for the T2D epidemic, several countries with high diabetes prevalence rates have low obesity rates, including Philippines, Romania, France, Bangladesh and Georgia. Here, Basu and colleagues reported that each 150 kcal/person/day increase in total food energy availability had no impact on diabetes prevalence; rather, a 150 kcal/person/day rise in sugar availability was associated with a 1.1% rise in diabetes prevalence. These findings suggest that sugar should be investigated for its role in diabetes pathogenesis apart from its contributions to obesity.

Viral obesity

Long-term changes in adiposity and glycemic control are associated with past adenovirus infection

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Diabetes Care 2013;36:701–707

Background: In animal models, human adenovirus 36 (Ad36) increases adiposity but improves glycemic control. Similarly, in humans, natural Ad36 infection shows cross-sectional associations with greater adiposity and better glycemic control in humans. The authors aimed to compare longitudinal measures

of adiposity (BMI and body fat percentage) and glycemic control (fasting glucose and insulin) in Ad36-infected versus uninfected adults.

Methods: Baseline sera from Hispanic men and women (n = 1,400) were screened post hoc for the presence of Ad36-specific antibodies. Indices of adiposity and glycemic control at baseline and ~10 years later were compared between seropositive and seronegative subjects, with adjustment for age and sex. Indices of glycemic control were further adjusted for baseline BMI and were analyzed only in non-diabetic subjects.

Results: Seropositive subjects (14.5%) had greater adiposity at baseline, compared with seronegative subjects. Longitudinally, seropositive subjects showed greater adiposity indices but lower fasting insulin levels. Subgroup analyses revealed that Ad36 seropositivity was associated with better baseline glycemic control and lower fasting insulin levels over time in the normal-weight group (BMI ≤ 25 kg/m²) and longitudinally, with greater adiposity in the overweight (BMI 25–30 kg/m²) and obese (BMI >30 kg/m²) men. Statistically, the differences between seropositive and seronegative individuals were modest in light of the multiple tests performed.

Conclusions: This study strengthens the plausibility that in humans, Ad36 increases adiposity and attenuates deterioration of glycemic control. Panoptically, the study raises the possibility that certain infections may modulate obesity or diabetes risk. A comprehensive understanding of these under-recognized factors is needed to effectively combat such metabolic disorders.

Nikhil Dhurandhar, the senior author, has long argued that fat deposition is higher in infected chickens, but that their blood cholesterol and fat levels were lower than in uninfected chickens. He then showed that 20% of obese people had antibodies to human adenovirus-36 (Ad36), a common infection associated with cold-like symptoms, suggesting they had been infected at some point. Specifically, this virus targets fat cells, and when infected onto stem cells they differentiate into fat cells. His research team now shows that seropositive subjects (14.5%) show larger longitudinal gains in adiposity than seronegative subjects, but have lower fasting insulin levels. These results are also recently confirmed by other investigators from Japan [8] and Sweden [9].

New mechanisms: mTOR and insulin sensitivity

PTEN mutations as a cause of constitutive insulin sensitivity and obesity

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N Engl J Med 2012;367:1002–1011

Background: Epidemiologic and genetic evidence links type 2 diabetes, obesity, and cancer. The tumor-suppressor phosphatase and tensin homologue (PTEN) regulates both cellular growth and metabolic signaling. Germline PTEN mutations cause the cancer-predisposing Cowden syndrome, which provides an opportunity to study the metabolic effects of PTEN haploinsufficiency in humans.

Methods: The authors measured insulin sensitivity and β -cell function in 15 PTEN mutation carriers and 15 matched controls. Insulin signaling was measured in muscle and adipose-tissue biopsy specimens from 5 mutation carriers and 5 well-matched controls. The effect of PTEN haploinsufficiency on obesity was assessed by comparing the 15 patients to data on 2,097 healthy adult controls from a population-based study. Body composition was assessed by dual-emission x-ray absorptiometry and skinfold thickness.

Results: PTEN mutation carriers were more insulin-sensitive than controls (e.g. mean fasting plasma insulin level, 29 pmol/l (range 9–99) vs. 74 pmol/l (range 22–185); $p = 0.001$); similarly on hyperinsulinemic euglycemic clamping, PTEN mutation carriers required double the glucose infusion rate than controls ($p = 0.009$). Higher insulin sensitivity could be explained by enhanced insulin signaling through the PI3K-AKT pathway, as evidenced by increased AKT phosphorylation. PTEN mutation carriers were more obese than controls, as evidenced by higher mean body mass index 32 (range 23–42) vs. 26 (range 15–48) ($p < 0.001$) and greater adiposity, but similar fat distributions.

Conclusions: PTEN haploinsufficiency is a monogenic cause of profound constitutive insulin sensitivity that is apparently obesogenic. PTEN mutations have apparently divergent effects on increased risks of obesity and cancer but a decreased risk of type 2 diabetes due to enhanced insulin sensitivity.

Last year's Editor's Choice chapter described mosaic-activating mutations in AKT1 (resulting in the Proteus syndrome) and AKT2 (severe hypoglycemia), thereby linking insulin and IGF-I signaling through the phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway to metabolic and somatic effects [10, 11]. Those findings naturally turned the spotlight onto PTEN, a protein and lipid phosphatase, which is a known antagonist of PI3K signaling. Consequently, it is entirely consistent to find that human PTEN loss-of-function mutation carriers, who all have the rare Cowden syndrome, have increased PI3K/AKT/mTOR pathway signaling, increased cancer risk and are now shown to have increased insulin signaling activity.

An interesting observation is the specific increase in adiposity, but not in lean or bone mass. PTEN mutation carriers also had higher fasting levels of AKT phosphorylation in adipose tissue, but not in muscle. The authors state that this finding is consistent with the notion that adipose tissue is exquisitely sensitive to insulin, and suggest that adipose tissue may govern insulin sensitivity in the fasting state. In addition to providing a mechanistic link to explain the established epidemiological associations between insulin resistance and risk of cancer, activators and regulators of the PI3K/AKT/mTOR signaling pathway are likely to have key roles linking adiposity to risk of type 2 diabetes.

A new obesity protein

Circulating growth arrest-specific 6 protein is associated with adiposity, systemic inflammation, and insulin resistance among overweight and obese adolescents

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Background: Growth arrest-specific 6 (Gas6) is a vitamin K-dependent protein secreted by immune cells, endothelial cells, vascular smooth muscle cells, and adipocytes. Preclinical studies indicate that Gas6 and its receptors of the TAM (Tyro-3, Axl, Mer) family may be involved in the pathogenesis of obesity and its complications, including systemic inflammation and insulin resistance. Until now, little has been known about the clinical significance of the Gas6/TAM system in childhood obesity. This study aimed to determine whether circulating Gas6 and soluble Axl (sAxl) levels are associated with adiposity, inflammation, and insulin resistance status among Taiwanese adolescents.

Methods: The authors performed a cross-sectional analysis of data from the Taipei Children Heart Study-III. A total of 832 adolescents (average age 13.3 years) were included; they were divided into three groups – lean, overweight, and obese. Circulating Gas6 and sAxl levels, adiposity, inflammatory markers, and insulin resistance status were examined.

Results: Levels of circulating Gas6 and sAxl were significantly higher in overweight and obese adolescents than in the lean group (both $p < 0.05$). Circulating Gas6 levels were significantly positively correlated with body mass index Z-score ($p = 0.045$), waist circumference ($p < 0.001$), waist-to-hip circumference ratio ($p < 0.001$), body fat mass ($p = 0.02$), serum high-sensitivity C-reactive protein ($p = 0.005$), and tumor necrosis factor- α levels ($p = 0.039$) among overweight and obese adolescents. The correlations remained significant after adjusting for age, gender, Tanner stage, smoking status, and drinking status. In addition, every 1 ng/ml increase in circulating Gas6 concentration corresponded to a 15–19% increase in the risk of developing insulin resistance among overweight and obese adolescents.

Conclusions: Circulating Gas6 levels are strongly associated with adiposity, inflammation, and insulin resistance status among overweight and obese adolescents. The potential role of the Gas6/TAM system in the initiation of childhood obesity and obesity-associated complications deserves further attention.

Growth arrest-specific 6 (Gas6) was cloned in 1988 and shown in 1993 to be upregulated in growth-arrested fibroblasts, which suggested a role in protection from cellular stress and apoptosis. It now gets a clinical significance with the finding in a community-based study that it is strongly associated with adiposity, inflammation, and insulin resistance status among overweight and obese adolescents. Interestingly, transgenic animals that ectopically express the TAM family of receptors also develop progressive obesity with elevated circulating TNF- α concentrations and severe systemic insulin resistance. This preclinical evidence indicates that the Gas6/TAM system likely represents an important pathogenic mechanism for obesity and obesity-associated inflammation and insulin resistance. The potential role of the Gas6/TAM system in the initiation of childhood obesity and obesity-associated complications deserves further attention.

A developmental model of PCOS

Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of polycystic ovarian syndrome

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Background: Gonadal steroid hormone feedback is impaired in polycystic ovarian syndrome (PCOS), a common endocrine disorder characterized by hyperandrogenism and an increase in LH pulse frequency.

Methods: The authors used a prenatal androgen (PNA)-treated mouse model of PCOS to investigate negative and positive feedback effects of estrogens on the hypothalamic-pituitary axis regulation of LH.

Results: PNA-treated mice exhibited severely disrupted estrous cycles, hyperandrogenism, reduced fertility, and altered ovarian morphology. To assess the negative feedback effects of estrogens, LH was measured before and after ovariectomy and after estradiol (E₂) administration. Compared with controls, PNA-treated mice exhibited a blunted post-castration rise in LH ($p < 0.001$) and an absence of LH suppression after E₂ administration. To assess E₂-positive feedback, control and PNA-treated GnRH-green fluorescent protein transgenic mice were subjected to a standard ovariectomy with E₂-replacement regimen, and both plasma and perfusion-fixed brains were collected at the time of the expected GnRH/LH surge. Immunocytochemistry and confocal imaging of cFos and green fluorescent protein were used to assess GnRH neuron activation and spine density. In the surged group, both control and PNA-treated mice had increased LH and cFos activation in GnRH neurons ($p < 0.05$) compared with non-surged animals. Spine density was quantified in cFos-positive and -negative GnRH neurons to examine whether there was an increase in spine density in cFos-expressing GnRH neurons of surged mice as expected. An increase in spine density in cFos-expressing GnRH neurons was evident in control animals, however no increase was observed in the PNA-treated mice because spine density was elevated across all GnRH neurons.

Conclusions: These data support the contention that PNA exposure results in a PCOS-like phenotype that includes impaired E₂-negative feedback. Additionally, although E₂-positive feedback capability is retained in PNA mice, elevated GnRH neuron spine density may reflect altered synaptic regulation.

Although the etiology of PCOS remains unknown and is widely debated, abnormally high prenatal androgen (PNA) exposure, as seen in congenital adrenal hyperplasia and Cushing's disease, has been shown to lead to the cardinal ovarian morphology and reproductive and endocrine features of PCOS in adult life. Therefore, it is not surprising that experimental prenatal androgen exposure leads to many of the reproductive features of PCOS in animals. The mouse model provides the unique benefit of being amenable to genetic manipulations. These observations in the PCOS mouse model suggest that although there is some gonadal steroid hormone feedback intact in PNA-treated mice, the sensitivity of GnRH neurons to suppression by E₂ is impaired. It remains unclear whether these differences in neuronal regulation of GnRH neurons are programmed during development or induced by circulating androgens in adulthood.

Formation of a human β -cell population within pancreatic islets is set early in life

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Background: Insulin resistance is usually compensated by an increase in functional pancreatic β -cell mass, otherwise diabetes ensues. The degree of compensation depends on the baseline β -cell mass from which the expansion originates. However, little is known about the establishment of baseline β -cell mass in humans.

Methods: The authors examined the formation of β -cell populations relative to other pancreatic islet cell types and associated neurons throughout the normal human lifespan. Human pancreatic sections derived from normal cadavers aged 24 weeks premature to 72 years were examined by immunofluorescence. Insulin, glucagon, and somatostatin were used as markers for β -, α -, and δ -cells, respectively. Cytokeratin-19 was used as a marker of ductal cells, Ki-67 a marker of cell proliferation, and Tuj1 (neuronal class III β -tubulin) a marker of neurons.

Results: Most β -cell neogenesis was observed preterm with a burst of β -cell proliferation peaking within the first 2 years of life. Thereafter, little indication of β -cell growth was observed. Postnatal proliferation of α - and δ -cells was rare, but a wave of ductal cell proliferation was found mostly associated with exocrine cell expansion. The β -cell to α -cell ratio doubled during the neonatal period, reflecting increased growth of β -cells. During childhood, there was a 7-fold change in the β -cell to δ -cell ratio, reflecting loss of δ -cells. A close association of neurons to pancreatic islets was noted throughout development and adulthood. Conversely, negligible neuronal association to exocrine pancreas was observed.

Conclusion: The human baseline β -cell population and its appropriate association with other islet cell types is established before the age of 5 years.

Maintaining an adequate β -cell mass is critical to the avoidance of glucose intolerance and type 2 diabetes. The baseline β -cell mass is the critical starting population from which controlled β -cell hyperplasia and hypertrophy may occur to compensate for insulin resistance, for example if you become obese, or during pregnancy. An adequate baseline β -cell mass can potentially be established from the formation of new β -cells from ductal epithelial progenitor cells (i.e. β -cell neogenesis) or from proliferation of existing β -cells, or both.

In a remarkable study of 40 human autopsy specimens from individuals who died at ages ranging from 24 weeks premature to 72 years, the authors examine rates of β -cell neogenesis, proliferation (replication), and survival (indicated by rate of apoptosis). Not surprisingly, β -cell neogenesis was far more common during fetal development compared to later periods, although significant levels were seen in occasional postnatal specimens. A burst of β -cell proliferation occurred in the early postnatal period, up to age 2 years, and to a lesser extent during childhood, but adult human β -cells proliferate very slowly. Conversely the rate of β -cell apoptosis was low and constant throughout life, suggesting that apoptosis does not precede the postnatal proliferation in β -cell mass. These findings are consistent with previous studies in rodents, which showed that a neonatal rapid expansion of β -cell mass arises largely due to β -cell proliferation. It is noteworthy that the observed critical period for establishing the baseline β -cell mass coincides directly with the epidemiological and experimental links between fetal development, and infancy growth and nutrition, and risk of type 2 diabetes in adulthood.

References

1. Ross LF, Saal HM, David KL, et al: Technical report: ethical and policy issues in genetic testing and screening of children. *Genet Med* 2013;15:234–245.
2. Zoicas F, Droste M, Mayr B, et al: GLP-1 analogues as a new treatment option for hypothalamic obesity in adults: report of nine cases. *Eur J Endocrinol* 2013;168:699–706.
3. Abifadel M, Varret M, Rabes JP, et al: Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003;34:154–156.
4. Cohen J, Pertsemlidis A, Kotowski IK, et al: Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat Genet* 2005;37:161–205.

5. Koren MJ, Scott R, Kim JB, et al: Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study. *Lancet* 2012;380:1995–2006.
6. Van den Berghe G, Wouters P, Weekers F, et al: Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001;345:1359–1367.
7. Vlasselaers D, Milants I, Desmet L, et al: Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. *Lancet* 2009;373:47–56.
8. Yamada T, Hara K, Kadowaki T: Association of adenovirus 36 infection with obesity and metabolic markers in humans: a meta-analysis of observational studies. *PloS One* 2012;7:e42031.
9. Almgren M, Atkinson R, He J, et al: Adenovirus-36 is associated with obesity in children and adults in Sweden as determined by rapid ELISA. *PloS One* 2012;7:e41652.
10. Lindhurst MJ, Sapp JC, Teer JK, et al: A mosaic activating mutation in AKT1 associated with the Proteus syndrome. *N Engl J Med* 2011;365:611–619.
11. Hussain K, Challis B, Rocha N, et al: An activating mutation of AKT2 and human hypoglycemia. *Science* 2011;334:474.