Editors' Choice

Jean-Claude Carel and Ze'ev Hochberg

Clinical trials: turn off the sugar high but do no harm

Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study

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Lancet 2009;373:547–556

Background: Critically ill infants and children often develop hyperglycemia, which is associated with adverse outcome; however, whether lowering blood glucose concentrations to age-adjusted normal fasting values improves outcome is unknown. We investigated the effect of targeting age-adjusted normoglycemia with insulin infusion in critically ill infants and children on outcome.

Methods: In a prospective, randomized controlled study, this project enrolled 700 critically ill patients, 317 infants (aged <1 year) and 383 children (aged ≥1 year), who were admitted to the pediatric intensive care unit (PICU) of the University Hospital of Leuven, Belgium. Patients were randomly assigned by blinded envelopes to target blood glucose concentrations of 2.8–4.4 mmol/l in infants and 3.9–5.6 mmol/l in children with insulin infusion throughout PICU stay (intensive group [n = 349]), or to insulin infusion only to prevent blood glucose from exceeding 11.9 mmol/l (conventional group [n = 351]). Patients and laboratory staff were blinded to treatment allocation. Primary endpoints were duration of PICU stay and inflammation. Analysis was by intention-to-treat. This study is registered with ClinicalTrials.gov, number NCT00214916.

Findings: Mean blood glucose concentrations were lower in the intensive group than in the conventional group (infants: 4.8 [SD 1.2] vs. 6.4 [1.2] mmol/l, $p < 0.0001$; children: 5.3 [1.1] vs. 8.2 [3.3] mmol/l, $p < 0.0001$). Hypoglycemia (defined as blood glucose ≤2.2 mmol/l) occurred in 87 (25%) patients in the intensive group ($p < 0.0001$) versus 5 (1%) patients in the conventional group; hypoglycemia defined as blood glucose <1.7 mmol/l arose in 17 (5%) patients versus 3 (1%; $p = 0.001$). Duration of PICU stay was shortest in the intensively treated group (5.51 [95% CI 4.65–6.37] vs. 6.15 days [5.25–7.05], $p = 0.017$). The inflammatory response was attenuated at day 5, as indicated by lower C-reactive protein in the intensive group compared with baseline (−9.75 [95% CI −19.93 to 0.43] vs. 8.97 mg/l [−0.9 to 18.84], $p = 0.007$). The number of patients with extended (>median) stay in PICU was 132 (38%) in the intensive group versus 165 (47%) in the conventional group ($p = 0.013$). Nine (3%) patients died in the intensively treated group versus 20 (6%) in the conventional group ($p = 0.038$).

Conclusion: Targeting of blood glucose concentrations to age-adjusted normal fasting concentrations improved short-term outcome of patients in PICU. The effect on long-term survival, morbidity, and neurocognitive development needs to be investigated.

Early insulin therapy in very-low-birth-weight infants

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N Engl J Med 2008;359:1873–1884

Background: Studies involving adults and children being treated in intensive care units indicate that insulin therapy and glucose control may influence survival. Hyperglycemia is also associated with morbidity and mortality. An international randomized, controlled trial aimed to determine whether early insulin replacement reduced hyperglycemia and affected outcomes in such very-low-birth-weight neonates.

Methods: In this multicenter trial, 195 infants were assigned to continuous infusion of insulin at a dose of 0.05 U/kg/h with 20% dextrose support and 194 to standard neonatal care on days 1–7. The efficacy of glucose control was assessed by continuous glucose monitoring. The primary outcome was mortality at the expected date of delivery. The study was discontinued early because of concerns about futility with regard to the primary outcome and potential harm.

Results: As compared with infants in the control group, infants in the early-insulin group had lower mean ($\pm$ SD) glucose levels (6.2 ± 1.4 vs. 6.7 ± 2.2 mmol/l, $p = 0.007$). Fewer infants in the early-insulin group had hyperglycemia for more than 10% of the first week of life (21 vs. 33%, $p = 0.008$). The early-insulin group had significantly more carbohydrate infused (51 ± 13 vs. 43 ± 10 kcal/kg/day, $p < 0.001$) and less weight loss in the first week (standard-deviation score for change in weight, -0.55 ± 0.52 vs. -0.70 ± 0.47 ; $p = 0.006$). More infants in the early-insulin group had episodes of hypoglycemia (defined as a blood glucose level of <2.6 mmol/l for >1 h; 29% in the early-insulin group vs. 17% in the control group, $p = 0.005$), and the increase in hypoglycemia was significant in infants with birth weights of more than 1 kg. There were no differences in the intention-to-treat analyses for the primary outcome (mortality at the expected date of delivery) and the secondary outcome (morbidity). In the intention-to-treat analysis, mortality at 28 days was higher in the early-insulin group than in the control group ($p = 0.04$).

Conclusions: Early insulin therapy offers little clinical benefit in very-low-birth-weight infants. It reduces hyperglycemia but may increase hypoglycemia.

Hyperglycemia is prevalent in patients in pediatric ICUs and in premature very low birth weight infants. The extent of hyperglycemia correlates with adverse outcome of a critical illness. Whether these associations are an indicator of the severity of the underlying illness, or rather that hyperglycemia or hypoglycemia in themselves are risk factors, is unclear. Two large studies this year postulated that targeting age-adjusted normoglycemia with insulin infusion in critically ill infants and children in the ICU would improve outcome, and designed randomized controlled studies to test this hypothesis. Quite interestingly, the 2 studies yielded quite radically different results. In the pediatric ICU, intensive insulin therapy protected the cardiovascular system, prevented secondary infections, and attenuated the inflammatory response, reducing the need for extended stay in the ICU. Mortality was lowered by 3.1% due to prevention of lethal neurological and pulmonary damage, and possibly prevention of infection. In contrast, in very low birth weight premature babies, although glucose targets were nicely reached, the overall outcome was unfavorable and the study had to be discontinued due to concerns about potential harm, although a preliminary study had given positive results [1]. The risk of hypoglycemia with insulin therapy in these young patient populations necessitates a long-term follow-up study to quantify late survival and morbidity, especially the effect of hyperglycemia and hypoglycemia on the developing brain. Overall these 2 studies illustrate the difficulties of addressing important questions regarding management through ambitious randomized controlled trials. Unavoidable biases (for instance the study by Vlasselaers et al. had 75% of the patients in the ICU after heart surgery) might affect the outcomes and practical problems with managing insulin and glucose infusion might also affect the outcomes. In any case, the authors of the positive study admit that the findings cannot be generalized at this time and that tight blood glucose control requires exceptional expertise of the nursing team.

Mechanism of the year: on the benefits of eating oneself

Autophagy regulates lipid metabolism

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Context: The intracellular storage and utilization of lipids are critical to maintain cellular energy homeostasis. During nutrient deprivation, cellular lipids stored as triglycerides in lipid droplets are hydrolyzed into fatty acids for energy. A second cellular response to starvation is the induction of autophagy, which

delivers intracellular proteins and organelles sequestered in double-membrane vesicles (autophagosomes) to lysosomes for degradation and use as an energy source. Lipolysis and autophagy share similarities in regulation and function but are not known to be interrelated.

Aim: The authors show a previously unknown function for autophagy in regulating intracellular lipid stores (macrolipophagy).

Results: Lipid droplets and autophagic components associated during nutrient deprivation, and inhibition of autophagy in cultured hepatocytes and mouse liver increased triglyceride storage in lipid droplets.

Conclusions: This study identifies a critical function for autophagy in lipid metabolism that could have important implications for human diseases with lipid over-accumulation such as those that comprise the metabolic syndrome.

When starved, cells degrade proteins, lipids and even whole organelles. Autophagy targets all three cellular components. Fatty acids are stored as triglycerides in dynamic lipid droplets, and, when necessary, are released by lipolysis. Established models of lipid storage and breakdown have undergone substantial revision in recent years. For many years it was believed that hormone-sensitive lipase acted alone lipolysis. The discovery of the regulatory proteins of the fat drop perilipin and CGI-58 encouraged a new look at lipolysis.

We now learn that autophagy, the pathway by which excess or damaged organelles and proteins are degraded, also mediates fat mobilization and breakdown in hepatocytes. In autophagy, destined cellular organelles are trapped in double-membrane-bound autophagosomes, and are then broken down in lysosomes. Singh et al. show that, under fasting conditions, specific cytoplasmic proteins are recruited to lipid droplets, where they form a double membrane that encloses droplet parts that fuse with lysosomes, and their contents are degraded. They also show that feeding the animals a high-fat diet impairs autophagy-mediated breakdown, so that increased fat consumption may be associated with decreased fat removal and excessive lipid deposition. A similar response in obese people would explain how they might develop fatty-liver disease.

A new hormone: a lipid posing

N-acylphosphatidylethanolamine, a gut-derived circulating factor induced by fat ingestion, inhibits food intake

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Cell 2008;135:813–824

Context: N-acylphosphatidylethanolamines (NAPEs) are a relatively abundant group of plasma lipids of unknown physiological significance.

Results: NAPEs were secreted into circulation from the small intestine in response to ingested fat. Systemic administration of the most abundant circulating NAPE, at physiologic doses, decreased food intake in rats without causing conditioned taste aversion. Radiolabeled NAPE entered the brain and was concentrated in the hypothalamus. Intracerebroventricular infusions of nanomolar amounts of NAPE reduce food intake. Chronic NAPE infusion results in a reduction of both food intake and body weight,

Conclusions: NAPE effects may be mediated through direct interactions with the central nervous system. NAPE and long-acting NAPE analogs may be novel therapeutic targets for the treatment of obesity.

Neuronal sensing of fatty acids (FAs) has been implicated in the regulation of feeding behavior by studies demonstrating that food intake can be reduced through inhibition of FA synthase or intrathecal oleic acid infusion. In contrast to plasma glucose and amino acids, which increase after meals, plasma FAs typically decrease with feeding and rise with fasting, contrary to what is expected of a negative regulator of appetite. Furthermore, intravenous infusions of lipid do not affect food intake, and thus it was unclear how either circulating triglyceride or FA signal lipid surfeit to the CNS under

physiological conditions. This group hypothesized that food intake could be modified by circulating nutrient signals reflective of meal fat content. Screening lipid derivatives that increased in plasma after high-fat feeding, they discovered a class of phospholipids, the N-acylphosphatidylethanolamines (NAPEs), of previously unknown physiologic function in plasma, whose hydrolysis products, the N-acylethanolamines, have been implicated in the peripheral control of food intake. They found that, at physiologic doses, intraperitoneal and intravenous injection of the most abundant plasma NAPE reduced food intake. NAPE levels increased consistently in rats and mice that had eaten a fatty meal. When synthesized and injected into the laboratory rodents, NAPEs concentrated in the hypothalamus and shut down the rodent's food intake, with one dose lasting 12 h or longer. A curious property of C16:0 NAPE was its ability to reduce locomotor activity in rodents when given systemically or centrally. This may play a physiologic role in mediating the lethargy typically observed in rodents (and humans) during the postabsorptive state. There is precedent for interactions between circulating regulators of appetite and the motor circuits in the brain; acute knockdown of the leptin receptor has been shown to increase locomotor activity in rodents, and the association of lassitude and voracious appetite is the whole mark of hypothalamic obesity. NAPEs is a new circulating FA, synthesized in the small intestine from ingested fat, and may be part of an important physiologic negative feedback loop that serves to reduce food intake and arousal after a fat-containing meal.

New therapy mode: Marfan's syndrome

Angiotensin II blockade and aortic-root dilation in Marfan's syndrome

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Context: Progressive enlargement of the aortic root, leading to dissection, is the main cause of premature death in patients with Marfan's syndrome. Recent data from mouse models of Marfan's syndrome suggest that aortic-root enlargement is caused by excessive signaling by TGF- β that can be mitigated by treatment with TGF- β antagonists, including angiotensin II-receptor blockers (ARBs).

Aim: The authors evaluated the clinical response to ARBs in pediatric patients with Marfan's syndrome who had severe aortic-root enlargement.

Methods: They identified 18 pediatric patients with Marfan's syndrome who had been followed during 12–47 months of therapy with ARBs after other medical therapy had failed to prevent progressive aortic-root enlargement. The ARB was losartan in 17 patients and irbesartan in 1 patient. They evaluated the efficacy of ARB therapy by comparing the rates of change in aortic-root diameter before and after the initiation of treatment with ARBs.

Results: The rate of change in aortic-root diameter decreased significantly from 3.54 ± 2.87 mm/year during previous medical therapy to 0.46 ± 0.62 mm/year during ARB therapy ($p < 0.001$). The deviation of aortic-root enlargement from normal, as expressed by the rate of change in z scores, was reduced by a mean difference of 1.47 z scores/year (95% CI 0.70–2.24; $p < 0.001$) after the initiation of ARB therapy. The sinotubular junction, which is prone to dilation in Marfan's syndrome as well, also showed a reduced rate of change in diameter during ARB therapy ($p < 0.05$), whereas the distal ascending aorta, which does not normally become dilated in Marfan's syndrome, was not affected by ARB therapy.

Conclusions: In a small cohort study, the use of ARB therapy in patients with Marfan's syndrome significantly slowed the rate of progressive aortic-root dilation.

Studies in a mouse model of Marfan's syndrome have shown that a deficiency of fibrillin-1 in the extracellular matrix leads to excessive signaling by TGF- β , an event that probably contributes to the pathogenesis of multiple phenotypic features of Marfan's syndrome, including progressive enlargement of the aortic root. These genetically engineered mice, in which the pathologic changes in the aortic root closely mimic those seen in humans, were subsequently used to demonstrate the therapeutic benefit of treatment with TGF- β antagonists in vivo. The development of pathologic changes in the aortic wall and the progressive dilation of the aortic root were attenuated or prevented by

systemic treatment with a TGF- β -neutralizing antibody or the angiotensin-II-receptor blocker losartan, an antihypertensive medication known to inhibit TGF- β signaling. In comparison, mutant mice treated with the β -blocker propranolol continued to show substantial aortic-wall pathologic changes and had only a moderate reduction in the rate of aortic-root dilation. These findings led to this clinical trial of losartan in patients with Marfan's syndrome. The current study provides early evidence suggesting that the addition of losartan to the traditional regimen used to treat aortic aneurysm in patients with Marfan's syndrome may be beneficial. Therapy resulted in a significant reduction in the rate of change in aortic-root diameter as compared with β -blocker therapy alone. The therapeutic effect extended to the sinotubular junction, a site also affected by Marfan's syndrome. In comparison, aortic segments not typically affected in Marfan's syndrome (e.g., the ascending aorta above the sinotubular junction) continued to show an annual rate of change in diameter that was appropriate for age and body size. Thus, losartan does not arrest aortic growth but specifically reduces the pathologic rate of increase in the diameter of aortic segments that are already of sufficient size to accommodate the physiologic demands of the tissues for blood flow. The authors suggest that despite the encouraging results of this observational study, equipoise is maintained regarding a role for this therapy in the treatment of patients with Marfan's syndrome; these findings must be confirmed by a prospective, randomized trial. Indeed, a trial coordinated by the Pediatric Heart Network of the National Heart, Lung, and Blood Institute, comparing losartan with atenolol in patients with Marfan's syndrome, began enrolling patients.

Concept recentered: adapt to starvation by stunting

Inhibition of growth hormone signaling by the fasting-induced hormone FGF21

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Cell Metab 2008;8:77–83

Aim: The authors investigated fibroblast growth factor 21 (FGF21) effect on possible GH resistance.

Results: In liver, FGF21 reduced concentrations of the active form of STAT5, and caused corresponding decreases in the expression of its target genes, including IGF-1. FGF21 also induces hepatic expression of IGF-1-binding protein 1 and suppressor of cytokine signaling 2, which blunt GH signaling. Chronic exposure to FGF21 markedly inhibited growth in mice.

Conclusion: These data suggest a central role for FGF21 in inhibiting growth as part of its broader role in inducing the adaptive response to starvation.

Fibroblast growth factor 21 (FGF21) has recently taken center stage as a key metabolic hormone that helps the body to adapt to starvation [Yearbook 2008]. One way to adapt is to stunt growth. When mice are fasted, FGF21 expression levels in the liver rise, and in turn, FGF21 stimulates the release of fatty acids from adipose tissue and promotes their conversion in the liver to ketone bodies, which can be used as an energy source when carbohydrates are scarce. This study reports that FGF21 inhibits STAT5 signaling and the expression of IGF-1 through its PPAR effect, and thereby blunts GH action as part of its broader role in promoting energy conservation during starvation. Cross-talk between PPAR α and STAT5B has been previously described in cell-based assays, with GH-activated STAT5B inhibiting PPAR α -regulated gene transcription, and conversely, ligand-activated PPAR α inhibiting STAT5B-regulated transcription. Thus, cross-talk between PPAR α and STAT5 may occur at multiple levels. Notably, in clinical studies the PPAR α agonist bezafibrate significantly lowers IGF-1 levels in patients. This finding together with data showing that FGF21 expression is induced by PPAR α agonists in primary human hepatocytes suggest that the PPAR α /FGF21 pathway may be operative and affect IGF-1 signaling in humans.

Aquaporin 1 is important for maintaining secretory granule biogenesis in endocrine cells

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Context: Aquaporins (AQPs), a family of water channels expressed in epithelial cells, function to transport water in a bidirectional manner to facilitate transepithelial fluid absorption and secretion. Additionally, AQP1 and AQP5 are found in pancreatic zymogen granules and synaptic vesicles and are involved in vesicle swelling and exocytosis in exocrine cells and neurons.

Aim: The authors show AQP1 in dense-core secretory granule (DCSG) membranes of endocrine tissue: pituitary and adrenal medulla.

Methods and Results: The need for AQP1 in endocrine cell function was examined by stable transfection of AQP1 antisense RNA into AtT20 cells, a pituitary cell line, to downregulate AQP1 expression. These AQP1-deficient cells showed more than 60% depletion of DCSGs and significantly decreased DCSG protein levels, including proopiomelanocortin/pro-ATCH and prohormone convertase 1/3, but not non-DCSG proteins. Pulse-chase studies revealed that whereas DCSG protein synthesis was unaffected, approximately 50% of the newly synthesized proopiomelanocortin was degraded within 1 h. Low levels of ACTH were released upon stimulation, indicating that the small number of DCSGs that were made in the presence of the residual AQP1 were functionally competent for exocytosis. Analysis of anterior pituitaries from AQP1 knockout mice showed reduced prohormone convertase 1/3, carboxypeptidase E, and ACTH levels compared to wild-type mice demonstrating that our results observed in AtT20 cells can be extended to the animal model.

Conclusion: AQP1 is important for maintaining DCSG biogenesis and normal levels of hormone secretion in pituitary endocrine cells.

Secretory granules are key organelles for secretion of hormones in endocrine cells in response to stimulation. They are stored within the cell, and as water enters the granules, causing them to swell during exocytosis, they release their contents upon stimulation. AQP1 present on the granule membrane has been shown to participate in the rapid vesicular water gating and swelling during exocytosis. This study investigated the role of AQP1 in pituitary endocrine cell function. We found that induction of DCSG granule biogenesis by chromogranin A (CgA) in a mutant pituitary cell line lacking granules, which led to a significant increase in expression of AQP1 mRNA and protein. They show that AQP1 is present in the membrane fraction of pituitary secretory granules and adrenal chromaffin granules. Furthermore, downregulation of AQP1 expression led to attenuated regulated secretion of ACTH. These results corroborate with decreased ACTH levels in the pituitary and serum in the AQP1 KO mice. The findings demonstrate that AQP1 is important for maintaining secretory function and granule biogenesis, and hence normal hormone sequestration in endocrine cells.

Food for thought: count the hours

Physiological significance of a peripheral tissue circadian clock

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Context: Mammals have circadian clocks in peripheral tissues, but there is no direct evidence of their physiological importance. Unlike the suprachiasmatic nucleus clock that is set by light and drives rest-activity and fasting-feeding cycles, peripheral clocks are set by daily feeding, suggesting that at least some contribute metabolic regulation.

Results: The liver plays a well-known role in glucose homeostasis, and the authors report here that mice with a liver-specific deletion of *Bmal1*, an essential clock component, exhibited hypoglycemia restricted to the fasting phase of the daily feeding cycle, exaggerated glucose clearance, and loss of rhythmic expression of hepatic glucose regulatory genes.

Conclusions: The liver clock is important for buffering circulating glucose in a time-of-day-dependent manner. The findings suggest that the liver clock contributes to homeostasis by driving a daily rhythm of hepatic glucose export that counterbalances the daily cycle of glucose ingestion resulting from the fasting-feeding cycle.

That hypothalamic neurons have a biological clock is well established. The current study provides persuasive evidence for a liver clock that counterbalances the fluctuating sugar supply provoked by hypothalamus-driven feeding–fasting cycles. The dependence of oscillating *Glut2* transcription on hepatocyte-derived *Bmal1* suggests that circadian glucose export is indeed driven by local liver clocks. The liver circadian clock, entrained by the feeding cycle, has daily cycles of metabolic activity that ensure a steady supply of energy for the organism.

New concepts: always look back at old things

Brain-derived neurotrophic factor and obesity in the WAGR syndrome

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N Engl J Med 2008;359:918–927

Background: Brain-derived neurotrophic factor (BDNF) has been found to be important in energy homeostasis in animal models, but little is known about its role in energy balance in humans. Heterozygous, variably sized, contiguous gene deletions causing haploinsufficiency of the *WT1* and *PAX6* genes on chromosome 11p13, approximately 4 Mb centromeric to BDNF (11p14.1), result in the Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome. Hyperphagia and obesity are observed in a subgroup of patients with the WAGR syndrome. The authors hypothesized that the subphenotype of obesity in the WAGR syndrome is attributable to deletions that induce haploinsufficiency of BDNF.

Methods: The relationship between genotype and body mass index (BMI) in 33 patients with the WAGR syndrome who were recruited through the International WAGR Syndrome Association was studied. The extent of each deletion was determined with the use of oligonucleotide comparative genomic hybridization.

Results: Deletions of chromosome 11p in the patients studied ranged from 1.0 to 26.5 Mb; 58% of the patients had heterozygous BDNF deletions. These patients had significantly higher BMI z scores throughout childhood than did patients with intact BDNF (mean [$\pm$ SD] z score at 8–10 years of age, 2.08 ± 0.45 in patients with heterozygous BDNF deletions vs. 0.88 ± 1.28 in patients without BDNF deletions; $p = 0.03$). By 10 years of age, 100% of the patients with heterozygous BDNF deletions (95% confidence interval [CI] 77–100) were obese (BMI ≥ 95 th percentile for age and sex) as compared with 20% of persons without BDNF deletions (95% CI 3–56; $p < 0.001$). The critical region for childhood-onset obesity in the WAGR syndrome was located within 80 kb of exon 1 of BDNF. Serum BDNF concentrations were approximately 50% lower among the patients with heterozygous BDNF deletions ($p = 0.001$).

Conclusions: Among persons with the WAGR syndrome, BDNF haploinsufficiency is associated with lower levels of serum BDNF and with childhood-onset obesity; thus, BDNF may be important for energy homeostasis in humans.

This paper started with a clinical observation that some of the patients with WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation) have hyperphagia and obesity.

Rather than dismissing the observation, the authors collected a series of patients with this rare disease, mapped the chromosome 11p13 deletion, and found that obese patients have a contiguous gene syndrome and a deletion of the BDNF (brain-derived neurotrophic factor). Functionally those with a heterozygous deletion of the BDNF gene had lower levels of circulating BDNF. The BDNF locus has been identified as being involved in the susceptibility to obesity by GWAS studies and is involved in the hypothalamus melanocortin pathway [2]. In addition, BDNF can be experimentally manipulated and modulate food intake in experimental obesity [3]. Leptin and the like have disappointed us; BDNF holds new promise in the development of pharmaceutical approaches to obesity. Beyond its result, this paper shows that careful clinical observations can lead to important findings with impacts that go well beyond the context of the initial observation.

Concepts revised: insulin resistance look at the fat

Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis

Sample RK, Sleight A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, Vottero A, Kanabar D, Charlton-Menys V, Durrington P, Soos MA, Carpenter TA, Lomas DJ, Cochran EK, Gordon P, O'Rahilly S, Savage DB
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Background: Metabolic dyslipidemia is characterized by high circulating triglyceride (TG) and low HDL cholesterol levels and is frequently accompanied by hepatic steatosis. Increased hepatic lipogenesis contributes to both these problems. Because insulin fails to suppress gluconeogenesis but continues to stimulate lipogenesis in both obese and lipodystrophic insulin-resistant mice, it has been proposed that a selective postreceptor defect in hepatic insulin action is central to the pathogenesis of fatty liver and hypertriglyceridemia in these mice.

Methods and Results: Humans with generalized insulin resistance caused by either mutations in the insulin receptor gene or inhibitory antibodies specific for the insulin receptor uniformly exhibited low serum TG and normal HDL cholesterol levels. This was due at least in part to surprisingly low rates of de novo lipogenesis and was associated with low liver fat content and the production of TG-depleted VLDL cholesterol particles. In contrast, humans with a selective postreceptor defect in AKT2 manifest increased lipogenesis, elevated liver fat content, TG-enriched VLDL, hypertriglyceridemia, and low HDL cholesterol levels. People with lipodystrophy, a disorder characterized by particularly severe insulin resistance and dyslipidemia, demonstrated similar abnormalities.

Conclusions: These data from humans with molecularly characterized forms of insulin resistance suggest that partial postreceptor hepatic insulin resistance is a key element in the development of metabolic dyslipidemia and hepatic steatosis.

We are used to thinking of insulin resistance as a complex picture linking deranged insulin-mediated glucose metabolism, abnormal lipid partitioning and elevated lipid levels. However the interrelationship between glucose and lipid abnormalities remains incompletely understood. Rather than using fancy knockout mouse models, these authors elected to study patients with molecularly characterized defects leading to insulin resistance. They confirm previous findings that patients with insulin receptor mutations or antibodies have high glucose but normal lipid levels whereas those affected with postreceptor defects have both. This indicates that alterations in the liver insulin signal itself are not sufficient to generate dyslipidemia, and that insulin action on liver lipid metabolism results from postreceptor modification. The interplay of glucose and lipids in type 2 diabetes is not new and was first proposed in a famous editorial by McGarry, 'What if Minkowski had been ageusic? An alternative angle on diabetes' [4]. This important aspect of pathophysiology is further discussed in a nice recent review by Brown and Goldstein [5] and will need to be sorted in order to better hierarchies the therapeutic approaches to insulin resistance.

Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study

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Lancet 2009;373:2027–2033

Background: The incidence of type 1 diabetes in children younger than 15 years is increasing. Prediction of future incidence of this disease will enable adequate fund allocation for delivery of care to be planned. The authors aimed to establish 15-year incidence trends for childhood type 1 diabetes in European centers, and thereby predict the future burden of childhood diabetes in Europe.

Methods: 20 population-based EURODIAB registers in 17 countries registered 29,311 new cases of type 1 diabetes, diagnosed in children before their 15th birthday during a 15-year period, 1989–2003. Age-specific log linear rates of increase were estimated in 5 geographical regions, and used in conjunction with published incidence rates and population projections to predict numbers of new cases throughout Europe in 2005, 2010, 2015, and 2020.

Results: Ascertainment was better than 90% in most registers. All but 2 registers showed significant yearly increases in incidence, ranging from 0.6 to 9.3%. The overall annual increase was 3.9% (95% CI 3.6–4.2), and the increases in the age groups 0–4, 5–9, and 10–14 years were 5.4 (4.8–6.1), 4.3 (3.8–4.8), and 2.9% (2.5–3.3), respectively. The number of new cases in Europe in 2005 is estimated as 15,000, divided between the 0–4, 5–9, and 10–14 year age groups in the ratio 24, 35, and 41%, respectively. In 2020, the predicted number of new cases is 24,400, with a doubling in numbers in children younger than 5 years and a more even distribution across age-groups than at present (29, 37, and 34%, respectively). Prevalence under age 15 years is predicted to rise from 94,000 in 2005, to 160,000 in 2020.

Conclusion: If present trends continue, doubling of new cases of type 1 diabetes in European children younger than 5 years is predicted between 2005 and 2020, and prevalent cases younger than 15 years will rise by 70%. Adequate healthcare resources to meet these children's needs should be made available.

Epidemiology is often considered a 'soft' science as opposed to 'hard' laboratory work. However, it is essential not only to provide precise estimates of the number of patients affected with a disease but also to allow an evaluation of the basic concepts in light of real life data. In this study, the Eurodiab group evaluated the incidence of type 1 diabetes and its evolution over time in 17 countries of Western Europe (Spain, Luxembourg, Belgium, 2 German centers), Central Europe (Czech Republic, Austria, and Slovenia) and East Europe (Lithuania, Poland, Slovakia, Hungary, Romania). The findings confirm the marked increase in diabetes incidence, striking particularly the younger kids. Cumulatively, the data extrapolated predict about twice as many new cases annually in 2020. Together with the decreased age at onset, the number of prevalent cases is predicted to rise by more than 50% in 2020. These findings confirm previous publications [6] and importantly show that earlier predictions are true, and even exceeded. The paper has a public health perspective and its main message is towards the organization of healthcare for children with type 1 diabetes. One other aspect is the importance of basic research towards finding the genes for type 1 diabetes. Indeed, type 1 diabetes research has been a paradigm for all types of gene association studies and the last GWAS analysis by the Type 1 Diabetes Genetics Consortium, using approximately 20,000 patients and 20,000 controls revealed more than 40 loci spread throughout the genome, most of them with minuscule effect sizes [7]. It may be time to concentrate our efforts on the environmental and epigenetic modifications that have induced such a rapid change in the disease incidence?

Innate immunity and intestinal microbiota in the development of type 1 diabetes

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Background: Type 1 diabetes (T1D) is a debilitating autoimmune disease that results from T-cell-mediated destruction of insulin-producing β -cells. Its incidence has increased during the past several decades in developed countries, suggesting that changes in the environment (including the human microbial environment) may influence disease pathogenesis. The incidence of spontaneous T1D in non-obese diabetic (NOD) mice can be affected by the microbial environment in the animal housing facility or by exposure to microbial stimuli, such as injection with mycobacteria or various microbial products.

Methods: Manipulation of NOD mice that are deficient in the MyD88 protein, a key element of innate immunity with various microbiota.

Results: Specific pathogen-free NOD mice lacking MyD88 protein (an adaptor for multiple innate immune receptors that recognize microbial stimuli) do not develop T1D. The effect is dependent on commensal microbes because germ-free MyD88-negative NOD mice develop robust diabetes, whereas colonization of these germ-free MyD88-negative NOD mice with a defined microbial consortium (representing bacterial phyla normally present in human gut) attenuates T1D. We also find that MyD88 deficiency changes the composition of the distal gut microbiota, and that exposure to the microbiota of specific pathogen-free MyD88-negative NOD donors attenuates T1D in germ-free NOD recipients.

Conclusions: Interaction of the intestinal microbes with the innate immune system is a critical epigenetic factor modifying T1D predisposition.

Last year, we learnt that the gut microbiome could play an important role in the pathogenesis of obesity both in mice and humans [8, 9]. The major focus of type 1 diabetes immunology research has been to identify the partners of cognate immunity, i.e. an antigen-specific immune cell with a tissue-specific antigen of antigen-derived peptide. Although some have identified a role of α -interferons, non-specifically induced by viruses or by inflammation, it must be remembered that the first step of immune activation is a non-cognate interaction of a class of 'foreign' determinants with the immune compartment called 'innate immunity'. In addition, it has been known for a long time that NOD mice raised in a clean environment had a much higher incidence of diabetes than mice raised in the presence of weak pathogens, such as gut parasites. Wen et al. manipulated the mouse microbiome to evaluate the impact of the intestinal microbes on the pathogenesis of diabetes in the NOD mouse. Their main findings are that mice that are incapable of sensing the microbiota through Myd88 are protected from diabetes and are colonized by an altered microbiota that can transfer protection against the disease. Although the exact mechanisms involved have not yet been worked out completely, this approach opens new avenues in diabetes research, and we could end up inoculating children at risk of diabetes with anti-diabetic bacteria earlier than we think.

Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes

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Background: The risk determinants of type 1 diabetes, initiators of autoimmune response, mechanisms regulating progress toward β -cell failure, and factors determining time of presentation of clinical diabetes are poorly understood.

Methods: The authors investigated changes in the serum metabolome prospectively in children who later progressed to type 1 diabetes. Serum metabolite profiles were compared between sample series drawn from 56 children who progressed to type 1 diabetes and 73 controls who remained nondiabetic and permanently autoantibody negative.

Results: Individuals who developed diabetes had reduced serum levels of succinic acid and phosphatidylcholine (PC) at birth, reduced levels of triglycerides and antioxidant ether phospholipids throughout the follow-up, and increased levels of proinflammatory lysoPCs several months before seroconversion to autoantibody positivity. The lipid changes were not attributable to HLA-associated genetic risk. The appearance of insulin and glutamic acid decarboxylase autoantibodies was preceded by diminished ketoleucine and elevated glutamic acid. The metabolic profile was partially normalized after the seroconversion.

Conclusion: Autoimmunity may be a relatively late response to the early metabolic disturbances. Recognition of these pre-autoimmune alterations may aid in studies of disease pathogenesis and may open a time window for novel type 1 diabetes prevention strategies.

When we fail to understand the course of events, we postulate working hypotheses. The current dogma is that immune abnormalities precede metabolic abnormalities in type 1 diabetes, and that environmental or other triggers initiate the autoimmune process. Here, the Finnish group used a large collection of serum samples of children followed from birth to study their metabolome and try to detect early metabolic alterations prior to the appearance of diabetes-associated antibodies. Differences were found between progressors and non-progressors in terms of lipid levels (triglycerides, phosphatidylcholine, ceramides) and amino acid levels. The intriguing results suggest that the appearance of GAD- and insulin-specific autoantibodies may be a relatively late response to earlier metabolic disturbances. Glutamate, the amino acid ligand of specific brain and β -cell receptors and transporters, was much elevated in children who went on to develop diabetes. The Chinese restaurant syndrome-related monosodium leads, among others, to higher insulin concentrations. Theoretically, it is possible that a glutamate load in β cells increases the activity of GAD65, one of the major β -cell antigens. Of note, previous epidemiological data had linked the intake of ω -3 polyunsaturated fatty acid to the risk of type 1 diabetes [10]. Although no clear picture emerges from the study and no working hypothesis seems to be formulated at this time, it is clear that similar approaches will help explore new avenues in one of the major medical mysteries that has resisted the sophisticated and expensive GWAS approaches: susceptibility to autoimmune diseases and type 1 diabetes [11].

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