6. Kash SF, Condie BG, Baekkeskov S (1999) Glutamate decarboxylase and GABA in pancreatic islets: lessons from knock-out mice. Horm Metab Res 31:340–344
7. Trembleau S, Penna G, Gregori S et al. (2000) Early Th1 response in unprimed nonobese diabetic mice to the tyrosine phosphatase-like insulinoma-associated protein 2, an autoantigen in type 1 diabetes. J Immunol 165:6748–6755
8. Bonifacio E, Atkinson M, Eisenbarth G et al. (2001) International Workshop on Lessons From Animal Models for Human Type 1 Diabetes: identification of insulin but not glutamic acid decarboxylase or IA-2 as specific autoantigens of humoral autoimmunity in nonobese diabetic mice. Diabetes 50:2451–2458
9. Notkins AL (2002) Immunologic and genetic factors in type 1 diabetes. J Biol Chem 277:43545–43548

Abner L. Notkins MD (✉), National Institutes of Health, Building 30, Room 121, 30 Convent Drive, MSC 4322, Bethesda, MD 20892-4322 USA
E-mail: anotkins@mail.nih.gov

Observation

Abnormal ghrelin secretion in new onset childhood Type 1 diabetes

To the Editor: Ghrelin is a new circulating peptide hormone produced mainly by the stomach [1] and involved in the regulation of feeding behaviour and energy homeostasis [2]. The concentrations of ghrelin are low in obese subjects [3, 4] and high in anorexic subjects [5]. In normal subjects the ghrelin secretion is stimulated by fasting and reduced by feeding [6] and by oral glucose load [4]. The mechanism of hormone release from the A-like ghrelin-producing cells [7] of the gastric mucosa is not yet known.

We examined ghrelin concentrations in 22 children; 11 boys and 11 girls, age 16±3.6 years (mean±SD), BMI 18.0±5.2 kg/m^2 (mean±SD) with a new onset of Type 1 diabetes. Blood samples were obtained at diagnosis before and after 10 days of insulin treatment, blood glucose 24.6±8.7 and 6.6±3.0 mmol/l, respectively, and in conjunction with meal tests, carried out in 15 and 20 of the cases at 3 and 9 months after the start of insulin therapy, respectively. At meal tests the patients were fasting over night and had received no insulin in the morning. Then they had a standardised breakfast (20% of their daily energy intake, containing 33% fat, 50% carbohydrates and 17% proteins) and blood was drawn at time zero and after 30, 60, 90, 120, and 150 min. Ghrelin was measured in the samples taken just before the test and when C-peptide reached its maximum. Ghrelin was determined in serum using a radioimmunoassay (Phoenix Pharmaceuticals, Belmont, Calif., USA), which uses ^{125}I-labelled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the full-length, octanoylated human ghrelin. Intra-assay and inter-assay coefficients of variance were 5.3% and 13.6%, respectively. Statistical significance was evaluated by Mann-Whitney U test and Wilcoxon signed rank test. The study was approved by the Research Ethics Committee at the Faculty of Health Sciences, Linköping.

Ghrelin concentrations at diagnosis prior to insulin treatment and after 10 days were 48.9 ±24.6 and 74.3±61.5 pmol/l (mean±SD), respectively (p=0.007). Blood glucose and C-peptide values increased during the meal tests (Fig. 1), whereas no changes in serum ghrelin were observed; the ghrelin values before and after test meals were 61.9±43.9 and 59.1±32.2 pmol/l at 3 months, and 93.4±72.4 and 81.9±55.5 pmol/l at 9 months, respectively. In a group of ten healthy children, five boys and five girls with a mean age of 12 years, the fasting serum ghrelin concentration was 108.5±32.5 pmol/l, (p=0.001), compared with the ghrelin values of the patients prior to insulin treatment.

To our knowledge, there are no previous reports on circulating ghrelin concentrations in patients with Type 1 diabetes. Patients with Type 2 diabetes were included in a previous study [6] which examined ghrelin concentrations in lean, normal weight and obese non-diabetic subjects and included observations on 42 subjects with Type 2 diabetes, 8 of whom were treated with diet alone, 22 with oral hypoglycaemic agents and 8 with insulin. Fasting plasma ghrelin concentration was negatively correlated with BMI in both subjects with Type 2 diabetes and those without. The plasma ghrelin concentrations of normal subjects decreased significantly after oral glucose administration, and a similar response was observed in patients with Type 2 diabetes after a meal tolerance test, reaching a nadir of 69% of the basal level after the meal. Similarly, a suppressive effect of a mixed liquid meal or oral glucose on serum ghrelin values in healthy human subjects was noted [8]. In contrast, we found that the children with Type 1 diabetes did not respond to meal tests with suppression of ghrelin values. One could speculate that ghrelin concentrations are low at di-

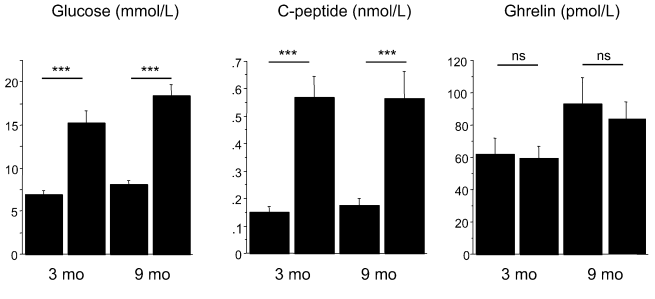

Fig. 1. Response to meal tests, carried out at 3 (n=15) and 9 (n=20) months after starting insulin treatment in childhood Type 1 diabetes. Results (mean ± SEM) from pre- and post-meal blood samples at each time point; ***p<0.0001; NS, non-significant

DOI 10.1007/s00125-003-1258-6
Received: 22 August 2003 / Revised: 29 September 2003
Published online: 12 November 2003

agnosis before the first insulin injection because of the insulinopaenia, high blood glucose load and deranged lipid metabolism, and then increases at later follow up with more normal blood glucose and metabolic control.

In conclusion, childhood Type 1 diabetes seems to be associated with abnormalities in ghrelin secretion. The circulating concentrations are low prior to insulin treatment and responses to meal tests are absent. As ghrelin is involved in the regulation of feeding behaviour and energy homeostasis, abnormalities in ghrelin secretion may play a role for the metabolic balance in diabetic children.

C. Holdstock[1], J. Ludvigsson[2], F. A. Karlsson[1]
[1] Department of Medical Sciences, Section of Medicine, University Hospital, Uppsala, Sweden
[2] Division of Pediatrics, Department of Molecular and Clinical Medicine, Linköping University, Linköping, Sweden

References

1. Kojima M, Hosoda H, Matsuo H, Kangawa K (2001) Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 12:118–122
2. Casanueva FF, Dieguez C (2002) Ghrelin: the link connecting growth with metabolism and energy homeostasis. Rev Endocr Metab Disord 3:325–338
3. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML (2001) Circulating ghrelin levels are decreased in human obesity. Diabetes 50:707–709
4. Shiiya T, Nakazato M, Mizuta M et al. (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 87:240–244
5. Otto B, Cuntz U, Fruehauf E et al. (2001) Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur J Endocrinol 145:679–683
6. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719
7. Dornonville de la Cour C, Bjorkqvist M et al. (2001) A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. Regul Pept 99:141–150
8. Caixas A, Bashore C, Nash W, Pi-Sunyer F, Laferrere B (2002) Insulin, unlike food intake, does not suppress ghrelin in human subjects. J Clin Endocrinol Metab 87:1902–1906

F. A. Karlsson (✉), Department of Medical Sciences, Section of Medicine, University Hospital, 751 85 Uppsala, Sweden
E-mail: anders.karlsson@medsci.uu.se

Observation

CD14 triggers autoimmune Type 1 diabetes in the NOD mouse

To the Editor: Type 1 diabetes is an autoimmune disease caused by selective destruction of the insulin-producing beta cells. Both, genetic predisposition and non-genetic, environmental factors, are believed to be involved in inducing Type 1 diabetes [1]. Several mechanisms by which environmental factors, such as pathogenic agents, might be involved in the development of autoimmune diseases have been proposed. One mechanism, "molecular mimicry"—has become popular in explaining the loss of tolerance in autoimmune diseases. In this hypothesis an immune response mounted against a foreign determinant cross-reacts with a host determinant, leading to inflammation and autoimmune destruction of the host tissue or organ. Similarities in sequence, structure or epitope-sharing between pathogens and host antigens have all been put forward as mechanisms which might drive this phenomenon [2, 3]. An association of infection with a particular pathogen and the induction of autoimmunity have been shown for reactive arthritis (RA), Guillain-Barre syndrome (GBS) and multiple sclerosis (MS) [4, 5, 6, 7]. Bacteria as potent immunogens express many factors that can act as immune stimulants. A multifunctional receptor CD14, interacts with several cell-wall components of gram-positive and gram-negative bacteria including lipopolysaccharide (LPS) and peptidoglycan. Recent studies suggest that CD14 is not only able to act as an immune receptor for "non-self" components such as LPS but it is also able to interact with "self" components (apoptotic cells) [8]. Since CD14 plays a key role in the regulation of the inflammatory cascade and since it also interacts with "self" components, perhaps there is an involvement of CD14 in the development of Type 1 diabetes in the non-obese diabetic (NOD) mouse model. To this end we have used classic genetic procedures to generate a NOD congenic line carrying a targeted deletion of the *CD14* gene.

Our experiments where carried out in accordance with the rules for animal care of the Ministry of Nutrition, Agriculture and Forestry of the German government and were approved by the Institution's animal care and use committee. *CD14*-deficient male mice (backcrossed into the C57BL/6J, a gift from Dr. D. Golenbock (Boston Medical and Boston University School of Medicine, Boston, Mass., USA) were crossed with NOD female mice (M&B A/S, Denmark) to produce F1 hybrids which were backcrossed onto NOD background. In each backcross generation mice which were heterozygous for *CD14* deficiency (*CD14*+/–) were selected using primers for *CD14* (F: 5′-CCAAGTTTTAGC GCTGCGTAAC-3′; R: 5′-GCCA-GCCAAGGATACATAGCC-3′) and for the *neo*-gene of *CD14*–/– (F: 5′-GTCAAGACCGACCTGTCCGG-3′;R: 5′-TC-

DOI 10.1007/s00125-003-1251-0
Received: 11 August 2003 / Revised: 26 September 2003
Published online: 12 November 2003