belgian endocrine society

32th meeting of the
Belgian Endocrine Society

Friday 21 and Saturday 22 October 2022

# ABSTRACT BOOK

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# Program

(Mdeon visa: 22/V2/10413/008299 )

## Friday symposia 21-10-2022

14:00 Registration

14:30-15:45 Symposium 1 (sponsored by Ipsen)
Chair: Guy T'Sjoen (UGhent)
Part 1: Highlights of ECE

- Focus on neuroendocrine tumors
  Wouter de Herder (NL, Erasmus MC, Amsterdam)
- Focus on acromegaly
  Albert Beckers (ULiège)

Part 2: Innovation for patients with patients
Mrs S. Llahana (UK, University College of London)

Part 3: Discussion/Q&A

15:45-16:00 Pauze

16:00-17:15 Symposium 2 *(sponsored by Lilly)*
A new chapter on incretin development
The "RE-NAISSANCE" of GIP

- Welcome and introduction
  *André Scheen (chair, Belgium)*
- What we have learned in almost two decades of incretin treatment. *16:05-16:15*
  *André Scheen (chair, Belgium)*
- GIP and GLP-1, the twin-cretin hormones: Similarities and differences. *16:15-16:35*
  *Michael Nauck (Germany)*
- Novel dual incretin receptor agonists: surpassing standards in diabetes care. *16:35-16:55*
  *Christophe De Block (Belgium)*
- Panel discussion and audience key questions on data presented *16:55-17:10*
- Summary and consolidation
  André Scheen (chair, Belgium)

17:15-17:50 Coffee

17:50-18:00 Welcome
*B. Velkeniers (VUB), President*

18:00-20:00 Symposium: a message from three young emeriti
Chair : P. Petrossians (ULiège)

- How do they grow so tall ?
  Albert Beckers (ULiège)
- A 2022 update in the management of prolactinoma.
  Dominique Maiter (UCL)
- Immune checkpoint inhibitors and endocrine side effects.
  Brigitte Velkeniers (VUB)

20:00 Walking dinner

## Saturday meeting 22-10-2022

8:00-8:30 Registration

8:30-8:50 General assembly
*B. Velkeniers (VUB), President*

8:50-9:00 Introduction of BSED and EYES
Inge Van Boxelaer (BSED)
Jonathan Mertens (EYES)

9:00-9:30 Clinical grand-round: hypoparathyroidism
Introduced by O. Alexopoulou (UCL)

Brigitte Decallone (KUL) & Bernard Corvilain (ULB)

9:30-11:00 Part 1: Clnical and translational studies

Chair: L. Crenier (ULB)

- Exercise as a non-pharmacological intervention to protect pancreatic beta cells in patients with type 1 and type 2 diabetes.
  Alexandra Coomans de Brachène (ULB)
- A plasma miR-193b-365 signature predicts non-responsiveness to Lactococcus lactis-based antigen-specific immunotherapy in new-onset type 1 diabetes.
  Grabriele Sassi (KUL)
- The insulin sensitivity index derived from euglycemic clamps is correlated to liver fat content determined by magnetic resonance spectroscopy in type 1 diabetes .
  Jonathan Mertens (UA)
- Mortality-related risk factors of inpatients with diabetes and COVID-19: a multicentric retrospective study in Belgium
  Thomas Servais (UCL)

10:30-11:00 Part 2: Clinical case reports
*Chair: M. Bex (KUL)*

- Chromosome 22q11.2 deletion syndrome revealed by severe hypocalcemia and pulseless electric activity: Untangling a conglomerate of potential primary etiologic factors.
  Eric Balti (VITAZ St. Niklaas)
- Is this just vitiligo? Nelson is hiding.
  Maarten De Vis (VUB)

11:00-11:45 Coffee break, visit of the exhibits and poster view

11:45-12:00 Selected Belgian Publication Award (sponsored by Sandoz)
*Introduced by C. De Block (UA)*

*Margaretha Visser et al. The Lancet 2021; 397:2275-2283*
Comparing real-time and intermittently scanned continuous glucose monitoring in adults with type 1 diabetes (ALERTT1): a 6-month, prospective, multicentre, randomised controlled trial.
Presenting author: Margaretha Visser (KUL)

12:00-12:30 Belgian Endocrine Society Lecture Award (sponsored by Novo)
*Introduced by B. Velkeniers (VUB), President*

Determinants and consequences of sex steroid exposure in men.
Award winner: Bruno Lapauw (UGhent)

12:30-13:00 Invited lecture
*Chair: Patrick Petrossians (ULiège*)

Menopauze – 20 years after WHI (Women's Health Initiative).
Axelle Pintiaux (ULiège)

13:00-13:15 Award ceremony of the young investigators

13:15 Lunch

# Abstracts BES 2022

The abstracts will be published online, as a Volume of Endocrine Abstracts in html and as a downloadable PDF.

# TRANSLATIONAL STUDIES

# Exercise as a non-pharmacological intervention to protect pancreatic beta cells in patients with type 1 and type 2 diabetes

*Coomans de Brachène Alexandra[1], Scoubeau Corentin[2], Musuaya Anyïshai E.[1], Castela Angela[1], Carpentier Julie[2], Faoro Vitalie[3], Klass Malgorzata[2,4], Cnop Miriam[1,5], Eizirik Decio L.[1]*

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[5] Division of Endocrinology, Erasmus Hospital, Université Libre de Bruxelles, Brussels, Belgium.

**Aim of the work:** Diabetes is characterized by progressive loss of functional pancreatic beta cells. None of the therapeutic agents used to treat diabetes arrest this process and preventing beta cell loss remains a major unmet need. We have previously shown that serum from 8 young healthy males who exercised for 8 weeks protects human islets and human insulin-producing EndoC-βH1 cells from apoptosis induced by pro-inflammatory cytokines or the endoplasmic reticulum (ER) stressor thapsigargin. Whether this protective effect is influenced by sex, age, training modality, ancestry and diabetes is unknown.

**Methods**: We enrolled 82 individuals, male or female, nondiabetic or diabetic, from different origins, in different supervised training protocols for 8-12 weeks (including training at home during the COVID-19 pandemic). EndoC-βH1 cells were treated with “exercised” serum to ascertain cytoprotection from ER stress.

**Results**: The exercise interventions were effective and improved $VO_2$ peak values in both younger and older, non-obese and obese, non-diabetic and diabetic participants. Serum obtainedafter training conferred significant beta cell protection from severe ER stress-induced apoptosis.Cytoprotection was not affected by the type of exercise training or participant age, sex, BMI orancestry, and persisted for up to 2 months after the end of the training program. Serum from exercised patients with type 1 or type 2 diabetes was similarly protective.

**Conclusions**: These data uncover the unexpected potential to preserve beta cell health by exercise training, opening a new avenue to prevent or slow diabetes progression through humoral muscle-beta cell crosstalk.

# Function and composition of pancreatic islet cell implants in omentum of type 1 diabetes patients

*Van Hulle F[1], De Groot K[1], Hilbrands R[1,2], Van de Velde U[1,2], Suenens K[1], Stangé G[1], De Mesmaeker I[1,3], De Paep DL[1,3,4], Ling Z[1,3], Roep B[5], Gillard P[6], Pipeleers D[1], Keymeulen B[1,2], Jacobs-Tulleneers-Thevissen D[1,4]*

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**Introduction**
Intraportal (IP) islet cell transplants can restore metabolic control in type 1 diabetes patients, but limitations raise the need for establishing a functional beta cell mass (FBM) in a confined extrahepaticsite.

**Methods**
This study reports on function and composition of omental (OM) implants after placement of islet cellgrafts with similar beta cell mass as in our IP-protocol (2–5.10$^6$ beta cells/kg body weight)

**Results**
Four of seven C-peptide-negative recipients achieved low beta cell function (hyperglycemic clamp [HGC] 2–8 percent of controls) until laparoscopy, 2–6 months later, for OM-biopsy and concomitant IP-transplant with similar beta cell dose. This IP-transplant increased HGC-values to 15–40 percent.OM-biopsies reflected the composition of initial grafts, exhibiting varying proportions of endocrine- cell-enriched clusters with more beta than alpha cells and leucocyte pole, non-endocrine cytokeratin- positive clusters surrounded by leucocytes, and scaffold remnants with foreign body reaction. OM- implants on a polyglactin-thrombin-fibrinogen-scaffold presented larger endocrine clusters with infiltrating endothelial cells and corresponded to the higher HGC-values. No activation of cellular immunity to GAD/IA2 was measured post-OM-transplant.

**Conclusion**
Establishment of a metabolically adequate FBM in omentum may require a higher beta cell number in grafts but also elimination of their immunogenic non-endocrine components as well as local conditioning that favors endocrine cell engraftment and function.

# A plasma miR-193b-365 signature predicts non-responsiveness to *Lactococcus lactis*-based antigen-specific immunotherapy in new-onset type 1 diabetes

*Sassi Gabriele[1,*], Licata Giada[2,*], Ventriglia Giuliana[2], Ellis Darcy[1], Van Damme Niels[3], Saeys Yvan[3], Caluwaerts Silvia[4], Mori Alessia[2,3], Rottiers Pieter[5], Mathieu Chantal[1], Dotta Francesco[2,3], Gysemans Conny[1,**], Sebastiani Guido[2,**]*

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[*] shared first authorship; [**] shared senior authorship

**Background and aims:** Combining systemic immunomodulation with disease-relevant antigens could provide longer-term solutions for preventing and even reversing autoimmune type 1 diabetes (T1D). Our team established that a combination therapy (CT), composed of a short-course low-dose anti-CD3 treatment with oral delivery of genetically-modified *Lactococcus lactis* (*L. lactis*) bacteria secreting full proinsulin plus the anti- inflammatory cytokine IL-10 (LL-PINS+IL-10), was effective in reversing T1D in the non-obese diabetic (NOD) mouse model. Here, we aimed to identify robust peripheral biomarkers for prediction of CT response using circulating cell-free microRNAs (miRNAs). Furthermore, we exploited CITE-sequencing (CITE-seq), a multimodal phenotyping method that simultaneously measures RNA and cell surface proteins at single cell level to investigate the immune cell types as a possible source of the identified miRNA signature.

**Materials and methods:** New-onset diabetic NOD mice were injected intravenously with anti-CD3 (d0-5) andinoculated with LL-PINS+IL-10 for 6 weeks. TaqMan™ miRNA array followed by single-assay Q-PCR was performed on plasma samples taken from responder (R) and non-responder (NR) mice before CT initiation. CITE-seq was used to profile FACS-sorted circulating and pancreas-infiltrated CD45+ immune cells of new- onset diabetic NOD mice.

**Results:** Overall disease remission was 45% by the CT (n=110) compared to 0% in untreated controls (n=13; P<0.01) that remained hyperglycaemic. Using miRNA profiling, six miRNAs (miR-34a-5p, miR-125a-3p, miR- 193b-3p, miR-328, miR-365-3p, and miR-671-3p) were identified as differentially expressed in plasma of R andNR mice at therapy initiation. Of those, miR-193b-3p and miR-365-3p were selected to compose, together with age and glycaemia status at disease onset, a signature able to predict therapy non-responsiveness with 83% specificity and 89% sensitivity. Both miRNAs were highly abundant at disease onset in pancreas- infiltrating neutrophils and basophils. CITE-seq analysis revealed a pro-inflammatory and activated phenotype in pancreas-infiltrated neutrophils and basophils compared to those in the bloodstream.

**Conclusion:** The miR-193b-365 signature could serve as a novel circulating prognostic biomarker forprospective personalised *L. lactis*-based immunotherapy in human new-onset T1D.

# Understanding pathogenic mechanisms and identifying therapeutic avenues in MEHMO syndrome using patient's induced pluripotent stem cells.

*Shakeri Hadis[1], Akcay Izzet Mehmet[1], Bansal Mayank[1], Cai Ying[1], Vinci Chiara[1], Pachera Nathalie[1], Willems Hanne[1], Fantuzzi Federica[1], Tong Yue[1], Gasperikova Daniela[2], Cnop Miriam[1,3]*

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**Background and aims:** MEHMO is an X-linked syndrome comprising Mental retardation, Epilepsy, Hypogenitalism, Microcephaly and Obesity. It is caused by a damaging p.Ile465Serfs frameshift mutation in *EIF2S3* that encodes the  subunit of eukaryotic translation initiation factor 2 (eIF2), essential for protein synthesis and regulation of the integrated stress response. Patients with this *EIF2S3* mutation also have neonatal hypoglycemia, early onset insulin-dependent diabetes and hypopituitarism. Here we investigated pathogenic mechanisms and potential treatments using patient's induced pluripotent stem cell (iPSC)-derived pancreatic β-cells.

**Materials and methods:** Blood cells from a MEHMO patient with the p.Ile465Serfs mutation who developed diabetes at age 10 months were reprogrammed into iPSCs using the Yamanaka factors. iPSCs were differentiated *in vitro* into β-cells and exposed to endoplasmic reticulum (ER) stressors thapsigargin (1 µM) and brefeldin A (0.02 µg/ml). We investigated whether protection was conferred by exenatide (GLP-1 receptor agonist, 50 nM), forskolin (cAMP inducer, 10 µM) and ISRIB (integrated stress response inhibitor, 200 nM).

**Results:** Patient iPSCs showed morphological and developmental defects compared to control cells. Expression of crucial β-cell developmental markers NKX6.1 and PDX-1 was reduced during differentiation. The β-cell yield at the end of differentiation was lower compared to control cells (37□3% *vs* 62□2%, p<0.0001), as was insulin content (11□2 vs 19□8 ng insulin/µg protein, p<0.05, n=8). Basal β-cell death was not different (7□3% *vs* 8□4%, n=8-13), but the *EIF2S3* mutation sensitized the cells to thapsigargin (24□7% apoptosis *vs* 16□4%, p<0.05) and brefeldin (36□7% *vs* 26□6%, p<0.001). ISRIB improved cell morphology and NKX6.1 and PDX-1 expression along the differentiation but did not ameliorate β-cell survival. Exenatide and forskolin conferred protection against thapsigargin and brefeldin.

**Conclusions:** The diabetogenic *EIF2S3* frameshift mutation alters patient's iPSC-derived β-cell development and exacerbates ER stress-induced apoptosis. ISRIB rescues the developmental defect, and GLP-1 analogs protect from ER stress. This study points to an important role of *EIF2S3* in β-cells and identifies novel therapeutic avenues for MEHMO syndrome. The patient iPSCs provide a powerful disease-in-a-dish model and can be used to study other clinical characteristics of MEHMO syndrome.

# Identification of myokines potentially involved in the improvement of glucose homeostasis after bariatric surgery

*Orioli Laura[1,2], Canouil Mickaël[3,4], Sawadogo Kiswendsida[5], Ning Lijiao[3,4], Deldicque Louise[6], Lause Pascale[1], de Barsy Marie[2], Froguel Philippe[3,4,7], Loumaye Audrey[1,2], Deswysen Yannick[8], Navez Benoit[8], Bonnefond Amélie[3,4], Thissen Jean-Paul[1,2]*

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**Purpose:** Our study aims to identify myokines potentially involved in improved glucose homeostasis after bariatric surgery.

**Methods:** Obese patients were evaluated before and 3 months after bariatric surgery. Insulin resistance was assessed using the Homeostasis Model Assessment (HOMA) test. Muscle biopsies were taken from vastus lateralis. Genes encoding myokines involved in glucose homeostasis were identified using RNA-sequencing. Changes in myokines expression were confirmed by real-time quantitative PCR (RT-qPCR). Changes in myokines fractalkine and myostatin plasma levels were measured by ELISA. A linear regression analysis was used to predict changes in the HOMA test from changes in myokines expression or plasma levels.

**Results:** Insulin resistance was significantly improved (HOMA-IR, -53%, $p$<0.001). Up-regulated genes included *CX3CL1* (encoding fractalkine, +73%, $p$<0.001) and BDNF (encoding Brain-Derived Neurotrophic Factor, +30%, $p$=0.006) while *MSTN* (encoding myostatin) was down-regulated (-45%, $p$<0.001). Plasma levels of fractalkine and myostatin were, respectively, increased (+7%, $p$=0.001) and decreased (-32%, $p$<0.001). However, changes in insulin resistance were not correlated with changes in gene expression or plasmalevels of fractalkine or myostatin. In contrast, increased expression of *BDNF* was significantly associated with decreased insulin resistance (HOMA-IR, adjusted estimate -0.58 [-0.96; -0.19],$p$=0.004).

**Main conclusions:** Bariatric surgery is associated with both improved glucose homeostasis and changes in myokines involved in glucose homeostasis. Although gene expression and circulating levels of fractalkine and myostatin are changed by surgery, they do not correlate with changes in glucose homeostasis. In contrast, increased expression of *BDNF* is correlated with improved insulin resistance, suggesting its potential role in improved glucose homeostasis after bariatric surgery.

# NET proteome and bioenergetic profile of PMA- and ionomycin-stimulated neutrophils from people with established type 1 diabetes

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**Background and aims**: Type 1 diabetes (T1D) is a chronic autoimmune disease, characterized by T-cell mediated destruction of the pancreatic insulin-producing beta-cells. Neutrophils, cells of the innate immune system, have been shown to infiltrate the pancreas and undergo neutrophil extracellular trap (NET) formation (NETosis). However, little is known about the involvement of neutrophils and specifically the role of NETosis in the pathophysiology of the disease. Our aim was to study the NET proteome and bio-energetic profile of neutrophils from people with established T1D in response to stimuli such as phorbol 12-myristate 13-acetate (PMA) and ionomycin.

**Material and methods**: Peripheral blood neutrophils isolated from people with established T1D (14±7 years at T1D onset; 12±10 years disease duration; 133±37 mg/ml random glycaemia) and sex- and age-matched healthy controls (HC) were stimulated with PMA (100 nM) or ionomycin (20 µM) for 3 hours. The NETomes were studied by LC-MS/MS analysis, while metabolic changes during NETosis were explored by Seahorse extracellular flux analysis.

**Results**: Levels of PMA- and ionomycin-stimulated NETosis were comparable in HC and T1D neutrophils (PMA: 85% vs 90%; ionomycin: 63% vs 77% respectively), as well as plasma levels of NET markers. However, the NETome of T1D neutrophils was distinct from that of HC subjects in response to PMA and ionomycin. Upon quantification with Progenesis QI software, a total of 44 proteins were differentially expressed in the NETomes of HC and T1D subjects when stimulated with PMA. Ionomycin-induced NETomes contained 27 differentially expressed proteins (1% FDR, $P<0.05$). Reactome analysis revealed that the proteins enriched in HC NETomes in PMA- and ionomycin- stimulated conditions were involved in neutrophil degranulation and innate immunity (i.e., neutrophil elastase [ELANE], azurocidin [AZU1]). In both stimulated conditions, proteins enriched in T1D NETomes were involved in glucose metabolism, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK1), fructose-bisphosphate aldolase A (ALDOA), and UTP-glucose-1- phosphate uridylyltransferase (UGP2). Interestingly, metabolic profiling revealed

that the rate of extracellular acidification, an approximate measure for glycolysis, was similar between T1D and HC neutrophils, in response to both PMA and ionomycin. Lactate levels in cell supernatants of PMA- and ionomycin-stimulated neutrophils were also comparable in T1D and HC subjects. Despite a lack of response to ionomycin, PMA induced a comparable increase in mitochondrial respiration in T1D and HC subjects.

**Conclusion**: Our results showed that the T1D NET proteome was enriched in proteins involved in glucose and glycogen metabolism. Interestingly, T1D neutrophils did not have an aberrant bioenergetic profile as determined by Seahorse extracellular flux analysis compared to HC neutrophils. These results suggest that T1D neutrophils, when activated, may alter their NET proteome to avoid impaired glycolysis and dysfunctional NETosis.

# The impact of interferon-α on global gene expression in iPSC-derived β- and α-like cells

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**Aim**
IFNα is a key regulator of the initial dialogue between pancreatic β-cells and the immune system in type 1 diabetes (T1D). IFNα induces endoplasmic reticulum (ER) stress, insulitis and a massive HLA-ABC overexpression in human β-cells, three histological hallmarks of T1D. Against this background we investigated the global role of IFNα on iPSC-derived islet-like cells, used here to mimic islet cells in the early neonatal period when autoimmunity against β-cells starts in many patients.

**Methods**
Human iPSC were differentiated to islet-like cells following a 7 stage protocol and then treated with 2000 U/mL of IFNα for 24h (dose and timing selected based on time-course and dose-response studies). Bulk and single-cell (sc) RNA-seq were performed. In follow up experiments, dispersed islet-like cells were transfected with a siRNA control or a siRNA targeting *NLRC5* and then exposed or not to IFNα for 24h. Gene expression levels were measured by qPCR. NLRC5 protein expression was assessed by western blot. The HLA-ABC expression at the β-cell surface was measured by flow cytometry.

**Results**
At the end of the differentiation period (stage 7) there were around 50% insulin and 10% glucagon positive cells. Exposure to IFNα induced a predominance of upregulated genes (761 upregulated *vs* 302 downregulated) as indicated by the bulk RNA-seq data. The upregulated pathways identified were antigen processing and presentation, JAK-STAT signaling and antiviral responses. scRNA-seq of the iPSC-derived islet-like cells identified β- and α-like cells based on their characteristic gene expression. β-like cells exposed to IFNα had higher expression of the ER stress markers *CHOP* and *XBP1*, while α-like cells showed higher expression of the protective chaperone *BiP*, of the anti-apoptotic family member *BCL2L1* (*Bcl-XL*) and of the viral sensor *MDA5*. However, the expression of *HLA-ABC* was similar in α-like cells exposed to IFNα as compared to β-like cells, except for the protective *HLA- E*, which was higher in α-like cells. The transcriptional activator *NLRC5* was up-regulated after IFNα treatment in β- and α-like cells and silencing of *NLRC5* in iPSC-derived islet-like cells decreased HLA-ABC (gene expression and protein expression at the surface), as well as genes related with antigen presentation such as *TAP1* and *B2M*.

## Conclusions

IFNα induces a different response in β- and α-like cells. β-like cells have a more marked expression of ER stress- related genes while α-like cells have higher expression of anti-apoptotic genes, protective chaperones and antiviral mechanisms. These observations suggest that α-like cells can better endure viral infections and ER stress, which could improve their survival in the context to T1D when compared to β-like cells. On the other hand, IFNα induces similar upregulation of HLA-ABC on β- and α-like cells, downstream of the transcriptional regulator NLRC5.

# CLINICAL STUDIES

# Glycemic control in patients diagnosed with renal cell carcinoma. A case series.

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**Background:** Few cases have been described with a new-onset or worsening of a pre-existing diabetes mellitus in patients diagnosed with a renal cell carcinoma and amelioration of their diabetes following tumour resection.

**Methodology:** This is a retrospective study (2003-2021) including adult cases who were diagnosed with a renal cell carcinoma, who underwent tumour resection and whom glycemic control was monitored. HbA1c was measured at 3 time intervals; 18 months pre-operative, 6 months pre-operative and 6 months postoperative. Furthermore BMI, antidiabetic treatment and tumour characteristics were collected. The Fuhrman grading system (FGS), ranging from 1 to 4, is the most widely used pathological classification and predictor of renal cell carcinoma prognosis. A difference in HbA1c of 0.3% was considered clinically significant.

**Results:** In total, 12 cases (83% men) with a mean age of 66 ± 9 years were included. Two cases had metastases. Seven cases had a clear renal cell carcinoma, 4 a papillary renal cell carcinoma and 1 a chromophobe renal cell carcinoma. None of the cases had a new-onset diabetes. One case with a chromophobe renal cell carcinoma had type 1 diabetes and showed no worsening of the metabolic control despite having aggressive tumour characteristics (diameter 10 cm, FGS 3). Metabolic control stayed stable after nephrectomy, insulin doses were not reduced. Eight cases had type 2 diabetes. Five of this 8 cases had a worsening of glycemic control before diagnosis. All these cases had a clear cell carcinoma, besides 1 case with metastatic disease having a papillary renal cell carcinoma. This in contrast to the remaining 3 cases with type 2 diabetes without worsening of glycemic control having a papillary renal cell carcinoma. All the cases with a worsening of glycemic control pre-operatively showed a significant improvement after total tumour resection, as their BMI remained stable. In 1 case, insulin therapy was started before resection and could be reduced postoperatively. Three cases had no diabetes, however 1 case with a clear cell carcinoma revealed a clinically significant reduction of HbA1c postoperatively of whom BMI was unchanged. The FGS and tumour diameter were heterogeneously distributed between cases with or without deterioration of glycemic control.

**Conclusion:** We present the evolution of the glycemic control of 12 cases before and at diagnosis of renal cell carcinoma and after tumour resection. No case had a new-onset diabetes. The cases with a worsening and improvement of HbA1c pre- and postoperative, respectively, were all clear cell carcinoma compared to the remaining cases having a papillary or chromophobe renal cell carcinoma. Literature describes clear renal cell carcinoma tends to be more aggressive. In this case series these carcinoma were also associated with a worsening of glycemic control.

# The course and outcome of subacute thyroiditis: a retrospectiveanalysis and predictive model

*de Filette Jeroen[1], De Mey Lynn[2], De Rop Jonas[1], Unuane David[1], Barbé Kurt[3], Bravenboer Bert[1]*

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**Background:** Subacute thyroiditis (SAT) is a destructive thyroiditis of probable viral origin. Thyroid dysfunction evolves through a set of stages (hyperthyroidism - hypothyroidism - euthyroidism) and is usually temporary, although some patients develop permanent hypothyroidism. The risk factors for permanent hypothyroidism remain largely unclear.

**Methods:** A retrospective analysis of patients with SAT at the University Hospital of Brussels from 2001-2020 was performed. Firstly, a description of the patient characteristics, inflammatory and thyroid parameters is provided. Secondly, a predictive model for the requirement to initiate thyroid hormone therapy (THT) is developed using logistic regression analysis. The optimal model configuration was selected by forward sequential analysis maximizing the classification accuracy while the prediction performance was validated by leave-one-out cross-validation.

**Results:** A total of 35 patients were included, with a female predominance (4.8/1). Thyrotoxicosiswas detected in the majority of patients (91%), while hypothyroidism developed in 71% (of which27% were subclinical). THT was initiated in half of the patients (18/35). The discontinuation of THT was attempted in 10/18 patients and was successful in most of these (8/10). The logistic regression model selected the predictors 'age', 'season' and 'treatment regimen', and demonstrated a maximum accuracy of 86%, classifying 30 of 35 patients correctly in the outcomemeasure.

**Conclusion:** THT was initiated for hypothyroidism in half of the patients, which is more than previously reported, although discontinuation of replacement therapy was successful in most patients in whom attempts were made. A scoring model was developed to identify patients who may benefit from THT in clinical practice.

# The Insulin Sensitivity Index Derived from Euglycemic Clamps Is Correlated to Liver Fat Content Determined by Magnetic Resonance Spectroscopy In Type 1 Diabetes*

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**Background:** Insulin resistance is proposed as a causative factor of non-alcoholic fatty liver disease (NAFLD) in the general population. Reports on NAFLD in type 1 diabetes (T1D) are increasing , but whether insulin resistance is linked to NAFLD in this population needs elucidation. Furthermore, people with T1D are an excellent model to investigate insulin resistance in relation to NAFLD without confounding by autologous insulin production.

**Aims and Methods:** We aimed to assess whether insulin resistance is correlated with liver fat content (LFC) in adults with T1D without causes of secondary liver fat accumulation. A hyperinsulinemic-euglycemic clamp (HEC) was performed according to *DeFronzo et al.* LFC was measured by magnetic resonance spectroscopy (MRS) and calculated as the mean of three independent regions of interest. NAFLD was diagnosed by mean LFC > 6.0 % and NAFLD subjects were matched 1:1 to no-NAFLD controls with T1D based on age and sex.

**Results:** 20 subjects with an age of 48 ± 17 year, a BMI of 27.3 ± 4.3 kg/m$^2$, and a HbA1c of 7.3 ± 0.8 % were included. Mean M-index (HEC-derived index of insulin sensitivity) was 5.12 ± 3.03 mg/kg/min. Mean LFC was 15.6 ± 8.3 % (NAFLD) versus 2.8 ± 1.4 % (controls) , $p < 0.001$. Mean M-index differed significantly (2.88 ± 1.58 [NAFLD] vs. 6.74 ± 2.11 [controls] mg/kg/min, $p < 0.001$) . BMI was significantly higher in the NAFLD group (29.6 ± 3.6 vs. 25.1 ± 3.8 kg/m$^2$, $p = 0.014$) . There was a strong correlation between the M-index and LFC ($r = -0.85$, $p < 0.001$) . Linear regression showed that the M-index (B = -1.504, 95% CI: (-2.9 to -0.101) , $p = 0.037$) and BMI (B = 0.879, 95% CI: (0.047 to 1.711) , $p = 0.040$) but not age nor HbA1c where associated with LFC.

**Conclusion:** HEC-derived measures of insulin resistance are strongly correlated to LFC in people with T1D, independently from BMI. These data support the pivotal role of insulin resistance in NAFLD pathophysiology and as a therapeutic target for the treatment of NAFLD.

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# Diagnosis and management of patients with primary hyperaldosteronism; a single-centre experience.

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**Background**
Primary hyperaldosteronism (PA) is a prevalent, but underreported syndrome. Diagnostic proceduresand treatment options have been relatively constant since the development of the latest guideline of the Endocrine Society in 2016.

**Study objective**
To clinically and biochemically describe subjects with PA who underwent a salt infusion test (SIT) or anadrenal venous sampling (AVS) in a tertiary hospital since 2009 and provide an overview of their treatment.

**Results**
A total of 59 subjects with a mean age of 53 ± 13 were diagnosed with PA. 19% of patients only underwent a SIT and 46% underwent AVS after their SIT. In total 80% of patients underwent a AVS. Reasons for screening for PA were; therapy resistant hypertension (59%), hypertension with an incidental mass (7%) and hypertension with hypokalaemia (56%). One subject without hypertension had a positive screening for PA in the hormonal work-up of an adrenal incidentaloma. Echocardiogram showed left ventricular hypertrophy in 23 subjects (51%). Plasma aldosterone/ renin ratio (ARR) was above the threshold of 20 in 96% of subjects. With a mean plasma aldosterone (PA) of 349.4 pg/mL, 81% had a PA level above 150 pg/mL. A combination of both an elevated PA and ARR above the previous established threshold was seen in 77%. Of the patients who underwent a SIT, 15% had a PA between 100 - 150 µg/ml and 2 patients (5%) had a PA between 50 – 100 µg/mL. Of patients who received only a SIT, 75% had a positive SIT and except for 1 patient (11%), all were treated with medication. AVS was performed by the same experienced interventional radiologist. AVS showed unilateral aldosterone hypersecretion in 23 subjects and bilateral hypersecretion in 12 subjects. In 9 subjects the right adrenal vein was not reachable and in 3 subjects there were analytical problems. Of the 23 subjects with lateralization, 16 underwent a unilateral adrenalectomy of which 7 could stop allantihypertensive drugs. Of the 12 subjects with a non-diagnostic AVS, 6 underwent a unilateral adrenalectomy with the histological confirmation of an aldosterone producing adenoma and normal blood pressure was achieved in 3 of them.

**Conclusion**
Despite being a tertiary centre only 47 patients were referred since 2009 to undergo an AVS which suggests underdiagnosis of PA. Hypertension and hypokalaemia are not mandatory to screen for PA but were present in 63% and 56%, respectively. The number of patients with unilateral vs bilateral aldosterone hypersecretion was similar. The discrepancy between AVS and CT adrenals was 20%. Normal blood pressure was achieved in only 48% of patient who underwent a unilateral adrenalectomyafter confirmation of lateralization, but the number of antihypertensive medication could be reducedin 92% subjects from an average of 5 to 1 or 2 pills.

# Changes in serum androgen levels in transgender women withand without gonadectomy.

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**Introduction** Transgender women on gender-affirming hormone therapy (GAHT) with estrogens and anti-androgens have low serum androgen levels. These low levels may be associated with clinical symptoms such as depressed mood, reduced sexual desire and tiredness. Whether androgen levels change over the course of anti-androgen use and after gonadectomy, in a context of unchanged estrogen treatment, remains to be elucidated.

**Methods** This study is part of the European Network for the Investigation of Gender Incongruence (ENIGI) and aimed to describe androgen profiles in transgender women in the years after initiation of GAHT and after gonadectomy. Transgender women who initiated estrogens and cyproterone acetate (CPA) had regular follow-up at the Ghent University Hospital and the Amsterdam University Medical Center, (Location VUmc) at baseline, three months, twelve months, after two to four years or after gonadectomy. Levels of total testosterone (TT) and androstenedione (A4) were determined using liquid-chromatography tandem mass spectrometry (LC-MS/MS). Sex hormone binding globulin (SHBG) concentrations were obtained using immunoassay. Free testosterone (FT) was calculated according to Vermeulen. In Ghent, dehydroepiandrosterone (DHEA) and dehydroepiandrosteronesulfate (DHEAS) were additionally measured using LC-MS/MS and immunoassay respectively.

**Results** In total, 309 transgender women were included. At three months of GAHT, mean TT and FT decreased by 18.4 nmol/L [95% CI -19.24, -17.63] and 0.4 nmol/L [95% CI -0.41, -0.38], respectively compared to baseline and remained stable thereafter. SHBG increased upon initiation of GAHT (mean Δ= 19.3 nmol/L [95% CI 13.32, 25.30]) and continued to increase in the first year (mean Δ= 6.8 nmol/L [95% CI 2.54, 11.07]), remaining stable afterwards. DHEAS and DHEA decreased by 1.8 umol/l [95% CI -2.17, -1.42] and 6.52 nmol/L [95% CI -9.06, -3.97] respectively after one year of GAHT and did not change afterwards. A4 had decreased by 1.2 nmol/L [95% CI -1.37, -1.00] after three month and remained stable afterwards. No differences in TT, FT, DHEAS, DHEA, A4 between groups were observed between women on anti-androgens and after gonadectomy.

**Conclusion** In the first year after initiation of GAHT with estrogen and CPA, levels of TT, FT,

DHEAS, DHEA and A4 decrease while remaining stable thereafter. Androgen levels did not change further aftergonadectomy. Clinical correlates remain to be elucidated.

# TPO antibody status prior to first radioactive iodine therapy as a predictive parameter for hypothyroidism in Graves' disease

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**Objective** We investigated if a positive thyroid peroxidase antibody (TPO Ab) status before radioactive iodine (RAI) therapy in patients with Graves' hyperthyroidism is a predictive factor for developing hypothyroidism after RAI.

**Methods** We performed a retrospective study of patients with Graves' hyperthyroidism with known TPO Ab status, receiving a first administration of RAI. Patients from 4 thyroid outpatient centres in Belgium receiving a first RAI therapy between the years 2011 and 2019 were studied. Clinical, laboratory, imaging, and treatment data were recorded from medical charts. Hypothyroidism and cure (defined as combined hypo- and euthyroidism) were evaluated in period1 (≥2 and ≤9 months, closest to 6 months post RAI) and period 2 (>9 months and ≤ 24 months post RAI, closest to 12 months post RAI).

**Results** One hundred fifty-two patients were included of which 105 (69%) were TPO Ab positive.Compared to TPO Ab negative patients, TPO Ab positive patients were younger, had a larger thyroid gland, and had more previous episodes of hyperthyroidism. In period 1, 89% of the TPO Ab positive group developed hypothyroidism versus 72% in the TPO Ab negative group (p=0.007). In period 2, the observation was similar: 88% vs. 72% (p=0.019). In a multivariate logistic regression analysis, adjusting for age at diagnosis, fT4 at diagnosis, TSH-R Ab atdiagnosis, thyroid volume at diagnosis, ATD preceding RAI and RAI activity, the adjusted OR was 4.16 (95% CI: 1.0–18.83; P = 0.052) in period 1 and 4.78 (95% CI: 1.27–20.18; P = 0.024) in
period 2.

**Conclusion** To date, the role of the TPO Ab status in patients with Graves' hyperthyroidism has not been well studied as a predictive parameter for thyroid functional outcome after first administration of RAI. We show that TPO Ab-positive patients were more likely to develop early hypothyroidism after the first administration of RAI, regardless of previously established factors associated with cure or treatment failure after RAI. Future studies investigating pre-treatment parameters affecting the outcome after RAI in patients with Graves' disease should incorporate TPO Ab status as a variable.

# Relationship of Time-Varying Parameters of Glycemic Control and Glycation with Arterial Stiffness in Patients with Type 1 Diabetes.

*HbA1c remains valuable in a changing glycemic landscape.*

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**Aim:** To investigate the relationship of arterial stiffness with short- and long-term parameters of glycemic control and glycation in patients with type 1 diabetes.

**Methods**: Cross-sectional study at a tertiary care centre including 54 patients with type 1 diabetes free from known CVD. Arterial stiffness was assessed with carotid-femoral pulse wave velocity (cf-PWV). Current level and 10-years history of HbA1c was evaluated, and skin advancedglycation end-products (AGEs), urinary AGEs, and serum AGE-receptor (sRAGE) concentrations.Continuous glucose monitoring (CGM) for 7 days was used to determine time in range, time in hyper- and hypoglycemia, and glycemic variability parameters.

**Results:** Cf-PWV was associated with current HbA1c ($r_S$=+0.28), mean 10-years HbA1c ($r_S$=+0.36), skin AGEs ($r_S$=+0.40) and the skin AGEs-to-sRAGE ratio ($r_S$=+0.40), but not with urinary AGE or serum sRAGE concentrations; and not with any of the CGM-parameters. Multiplelinear regression for cf-PWV showed that the model with the best fit included age, type 1 diabetesduration, 24-hour mean arterial pressure and mean 10-years HbA1c (adjusted $R^2$= 0.645, p<0.001).

**Conclusion**: Long-term glycemic exposure and glycation as reflected by mean 10-years HbA1c and skin AGEs, respectively, are key predictors of arterial stiffness in patients with type 1 diabetes,while no relationship was found with any of the short-term CGM-parameters. Our findings stress the importance of early and sustained good glycemic control to prevent premature CVD in patientswith type 1 diabetes and suggest that HbA1c should continue to be used in the risk assessment fordiabetic complications.

**Key words**: Arterial Stiffness; Glycemic Control; Glycation; HbA1c; Type 1 Diabetes; ContinuousGlucose Monitoring; Time In Range.

# Modest changes in sex hormones during early and middle adulthood affect bone mass and size in healthy men. A prospective cohort study.

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Bone metabolism in men is in part determined by sex steroid exposure. This is especially clear during puberty and senescence but it remains to be established whether declines in sex steroid levels during young and middle adulthood associate with changes in bone mass and size. This study investigated changes in bone mineral content (BMC), areal and volumetric bone density (aBMD; vBMD) and bone size in relation to sex steroid levels in 999 young adult men (age 24-46 years) of whom 676 were re-evaluated after a mean period of 12 years. Sex hormone binding globulin (SHBG) levels were measured using immuno-assay, testosterone (T) and estradiol (E2) using LC-MS/MS, free fractions calculated (cFT; cFE2). Areal bone parameters and BMC were measured at the hip and lumbar spine using DXA. Radial and tibial vBMD and bone size were determined using pQCT. Linear mixed models were used for statistical analyses. With aging, we observed decreases in almost all bone mass and density indices whereas changes in bone geometry resulted in larger bones with thinner cortices. These changes in bone mass and size appeared related to sex steroid levels. Specifically, decreases in cFT (but not total T) levels were associated with larger decreases in lumbar spine BMC and especially with geometric changes in cortical bone at the tibia. Similarly, decreases in total E2 and cFE2 were associated with larger decreases in bone mass (all sites) and also with some geometric changes. Also increases in SHBG were independently associated with aging-related changes in bone mass andsize in these men. In summary, even small changes in T, E2 and SHBG levels during young and middle adulthood in healthy men are associated with changes in bone mass and size.

**Keywords**
sex steroids, aging, bone mass, bone geometry, evolution, healthy men

# Mortality-related risk factors of inpatients with diabetes and COVID-19: a Multicentric Retrospective Study in Belgium

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**Purpose**: We describe the characteristics and prognosis of inpatients with diabetes and coronavirus disease 2019 (COVID-19) in Belgium.

**Methods**: We conducted a multicentre retrospective study during the first wave of thepandemic, from March 1, 2020 to May 6, 2020. Data on admission of patients with diabetes and hospitalized due to confirmed COVID-19 were collected. COVID-19 diagnosis was basedon a positive polymerase chain reaction (PCR) test on nasopharyngeal swab and/or suggestive findings on computed tomography (CT) scan. Survivors were compared to non- survivors in order to identify prognostic risk factors for in-hospital death using multivariate analysis in both the total population and in the subgroup of patients admitted in the intensive care unit (ICU).

**Results**: The study included 375 patients. The median age was 73 [64-81] years and 93% had type 2 diabetes mellitus (T2DM). Median HbA1c was 7.1 [6.3-8] %. The prevalence of obesity was 49%. The mortality rate was 26.4% (99/375) in the total population and 40% (27/67) in ICU patients. Multivariate analysis identified older age (HR 1.049 [CI 1.03-1.07] per additionalyear of age, p <0.0001), male sex (HR 2.01 [1.31-3.07], p 0.0013) and C-reactive protein (CRP) elevation on admission (HR 1.00031 [1.0008-1.0054] for each 1 mg/L increase, p 0.0081) as independent risk factors for in-hospital death. Metformin (HR 0.51 [0.34-0.78], p 0.0018) and/or renin-angiotensin-aldosterone system (RAAS) blockers (HR 0.56 [0.36-0.86], p 0.0088) use before admission were independent protective factors.

**Main conclusions**: In-hospital mortality due to COVID-19 is high in patients with diabetes. We found in a well-phenotyped population that advanced age, male gender and elevated CRPon admission were independent risk factors for death in diabetic patients hospitalized due to COVID-19 in Belgium during the first wave of the pandemic. We also showed that metformin use before admission was associated with a significant reduction of COVID-19-related in-hospital mortality.

# Prolactinomas: our experience in Liège

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**Introduction**

The true prevalence of clinically relevant pituitary adenomas has been reevaluated at 1/1064 of the population (1). Among them, prolactinomas represent the majority with a prevalence of 1/2000.

They occur usually in females, aged 20–50 Y.O., and 80% are microadenomas. Nearly 5% of prolactinomas appear in a familial or genetic setting (MEN-1 or FIPA) (2). Cabergoline is proposed as the first line therapy and is usually efficient to normalize prolactin (PRL) levels, restore fertility and shrink tumor volume.

**Patients and methods**

We screened the patients followed for prolactinomas in our endocrinology department from 1980 to 2020. We looked for epidemiological and radiological data, prolactin (PRL) levels, treatments, a familial setting, cancer, associated endocrine problems and pregnancy follow-up.

**Results**

The study population consisted of 303 females (76%, median age: 34,5 Y.O.) and 97 males (24%, median age: 42,3 Y.O.). Tumors were mainly micro-adenomas in women and macroadenomas in men. Median tumor size was 7 mm in females and 18 mm in males. PRL levels were lower in females (95,4 ng/ml vs 461,5 ng/ml) and correlated with tumor size.

Main symptoms at diagnosis were amenorrhea and galactorrhea in women (80,1%). Men were complaining mainly of erectile dysfunction and/or loss of libido (44,3%) and headache (28,8%).

A familial/genetic form was present in 16 patients (4%). These patients had bigger tumors.

Overt or subclinical hypothyroidism occurred in 31,5% of our patients which is higher than the prevalence in comparable populations (3). Thyroid nodules were described in 22,5% of our patients. Breast cancer history was reported in 10 cases during follow up.

Surgery was used in 38% of our patients, mainly before the year 2000; thereafter Cabergoline became the almost exclusive treatment. Under this medication, PRL levels were normalized in 80,6% of cases and a significant decrease of tumor size (>50%) was noted in 67,2% of cases (4).

98 pregnancies occurred, 73 under cabergoline. The later was stopped at the discovery of pregnancy, but had to be restarted before delivery in 8 cases. No fetal complications were reported.

**Conclusions**

Prolactinomas are a frequent cause of infertility in young women. Cabergoline is now the first choice therapy due to its efficiency and tolerability. Our data show the presence of three different populations of patients, with different biological and radiological presentations: males, females and familial cases. Anamnesis should query for genetic forms due to the early onset and high prevalence of macroadenoma.

The prevalence of overt or subacute hypothyroidism raises the question whether patients with prolactinomas need to be systematically screened for thyroid function abnormalities.

Finally, we did not have complications during pregnancy, in line with other reports from the literature (5).

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# CASE REPORTS

# A rare cause of Cushing syndrome

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## Introduction

Diagnosis of Cushing Syndrome (CS) is challenging due to its various non-specific symptoms, and multiple endogenous and exogenous causes. The incidence of endogenous CS is rare and estimated at 2 to 3 cases per million inhabitants per year in Europe (1).

Primary bilateral macronodular adrenal hyperplasia (PBMAH) is an uncommon cause of endogenous ACTH-independent CS. It is a benign condition, characterized by the presence of bilateral macronodules (>1 cm), and autonomic hypersecretion of cortisol by the adrenal glands (2).

## Case

A 64-year-old woman consulted our endocrinology clinic for persistent complaints of fatigue, depressed mood and reduced muscle strength. Her medical history consisted of chronic obstructive pulmonary disease, arterial hypertension, and paroxysmal atrial fibrillation.

Physical examination showed a brittle skin with atrophy, and edema at the ankles. Proximal muscle wasting was present. The patient's face was round, highly hyperemic with dorsocervical fat accumulation representing a typical "moon face" with "facial plethora and buffalo hump" (Figure 1A).

24h urine collection showed an elevated urinary-free cortisol of 305.5 µg/24h (normal reference: <120 mcg/24h). Serum cortisol decreased insufficiently (30.6 µg/dL) after overnight 1 mg dexamethasone suppression (normal reference: <1.8mcg/dL). Morning blood analysis revealed a serum cortisol of 27.4 µg/dL (normal reference: 6,0 - 18,4 µg/dL), and a completely suppressed ACTH (<3.0 pg/mL) (normal reference: 7,20 - 63,30 pg/mL). Computed tomography (CT) and magnetic resonance imaging (MRI) of the adrenal glands showed bilateral hyperplastic adrenals sizing 58.9 by 29.7 mm on the right, and 55.6 by 42.6 mm on the left.

With informed consent of the patient, bilateral adrenalectomy was performed with postoperative substitution of glucocorticoids (hydrocortisone 20 mg/day divided over 3 doses) and mineralocorticoids (fludrocortisone 100 µg once daily).

Pathology report confirmed the diagnosis of PMBAH. At follow-up, a clear decrease of Cushing stigmata could be observed (Figure 1B). Genetic sequencing failed to reveal mutations in the ARMC5 gene.

## Discussion

PMBAH is a rare cause of endogenous CS. Its exact prevalence is not established as its manifestation varies from subclinical, to severe and progressive forms (3).

The pathophysiology of PBMAH is still under investigation. Although PBMAH is sporadic, ARMC5 germline mutations are described in up to 58% of patients, warranting pro-active analysis upon diagnosis (4).

Although bilateral adrenalectomy with lifelong gluco- and mineralocorticoid substitution remains the best treatment option, unilateral adrenalectomy may be considered in specific cases (2). Currently, no additive value is gained from adrenal vein sampling- based cortisol lateralization ratios in the guidance of unilateral adrenalectomy (5).

## Conclusion

In patients with suspicious signs such as atrophy of the skin with ecchymosis, red to purple-colored stretch marks, proximal muscle weakness and/or a plethoric/moon face, presence of CS should be considered. After confirmation of endogenous CS, differentiation between ACTH-dependent and ACTH-independent disease is essential.

PMBAH is a rare cause of endogenous ACTH-independent CS. Although bilateral adrenalectomy remains the best curative option, unilateral resection can be considered if significant differences in adrenal size are present.

## Figures

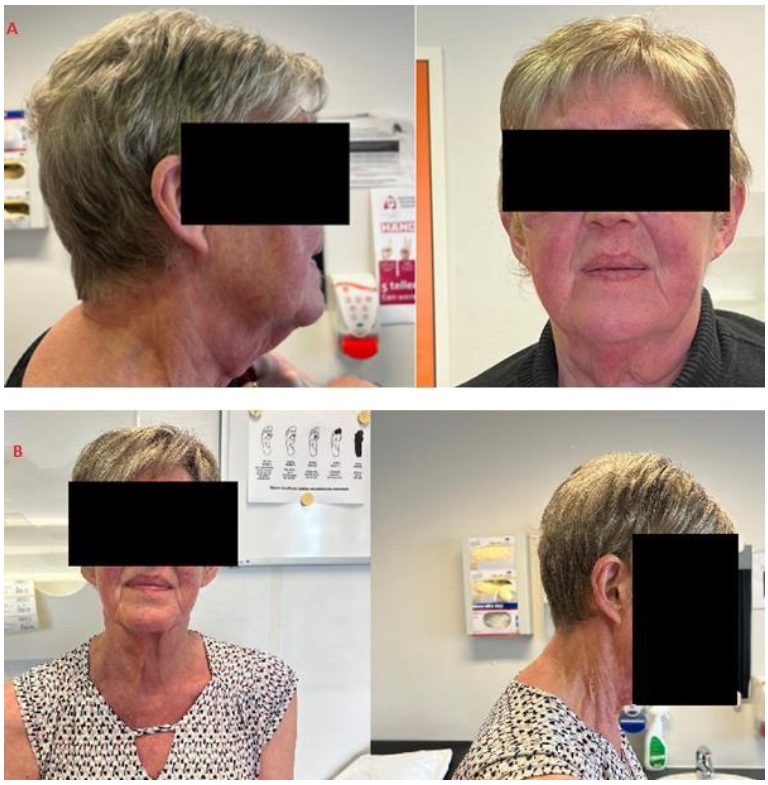


*Figure 1. Clinical examination at presentation revealed a plethoric face with dorsocervical and facial fat deposition (A).Postoperative evaluation after bilateral adrenalectomy showed a clear decrease of the Cushing stigmata (B).*

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# A profound hypocalcaemia following parathyroidectomy. A case report.

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**Background.**

Hungry bone syndrome is a relatively uncommon but serious complication in patients who underwent parathyroidectomy for primary hyperparathyroidism. The syndrome is described as a hypocalcaemia (corrected serum calcium < 2.1 mmol/L) lasting longer than four days after parathyroidectomy in the presence of a normal or elevated parathyroid hormone (PTH). Treatment is challenging and guidelines are based on clinical experience. To restore calcium levels high doses of calcium and active vitamin D are needed.

**Case Report.**

A 39-year-old woman with a medical history of kidney stones and osteoporosis was diagnosed with primary hyperparathyroidism. Corrected serum calcium was 2.72 mmol/L (2.15-2.50) in the presence of an elevated PTH (748 ng/L, 15-65). Further blood results showed a normal kidney function, a decreased phosphatase (0.51 mmol/L, 0.81-1.45), an elevated alkaline phosphatase (276 U/L, 38-126) and a decreased level of 25-hydroxyvitamin D (18.2 ng/mL, 30-100) for which substitution with D-cure was started. Guided by the results of localization studies a selective parathyroidectomy of the left (1.3x 0.7 cm) end right (1.8 x 1.0 cm) inferior parathyroid adenoma was performed together with a total thyroidectomy because of bilateral thyroid nodules. Peri-operatively PTH normalized to a level of 57 ng/mL (18.5-88). Postoperatively, 1.6 g elemental calcium supplementation (calcium carbonate 4 g) per day was started. Histology revealed both thyroid nodules and parathyroid adenoma were benign. At discharge, the patient had a low normal corrected calcium levels (2.13 mmol/L, 2.18-2.60) with a normal PTH level (72 ng/L). One and a half month postoperatively, she complained of paraesthesia and muscle cramps. Blood sample revealed a low corrected serum calcium of 1.61 mmol/L in combination with an unexpected elevated PTH of 210 ng/L. Other electrolytes (potassium, phosphate, magnesium) and thyroid function were normal. Calciuria was low (0.73 mmol/24h, 2.50–8.00). Patient was admitted to intensive care for close monitoring. She required 7.2 g elemental calcium (6 g calcium gluconate intravenous, 12 g calcium carbonate peroral) and 4 µg active vitamin D to correct hypocalcaemia and correct symptoms of tetany. After 6 days, intravenous calcium was stopped and the patient was further treated with an oral calcium supplement. To maintain low normal calcium levels a total daily dose of 9.6 g elemental calcium (24 g calcium carbonate) along with 4 µg active vitamin D was required. Serum calcium concentration levels finally normalized after 2 weeks of admission and the patient was discharged. Six months postoperative, the dose of elemental calcium is reduced to 1.4 g (calcium carbonate 3.5 g) and active vitamin D to 3 µg to maintain a low normal corrected calcium level (2.15 mmol/L). PTH is still elevated (170 ng/L) and alkaline phosphatase is normalized (98 U/L).

## Conclusion

We present a case of a severe hypocalcaemia due to a hungry bone syndrome after parathyroidectomy for primary hyperparathyroidism. Possible preoperative risk factors for the development of a hungry bone syndrome were the more than two-fold increased levels of PTH, increased alkaline phosphatase, depleted vitamin D status and osteoporosis (1). In this case report, the total daily dose of 9.2 g elemental calcium is within the range of 6-12 g to normalize calcium levels in HBS (1). Five months postoperative, the patient still requires calcium and active vitamin D supplements. There are no data about the mean duration of hungry bone syndrome, but can last up to 12 months postoperatively. To decrease the risk of a hungry bone syndrome in a hyperparathyroid patient with bone disease the use of preoperative bisphosphonates is suggested but there is a lack of high quality randomized studies (2).

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# A rare etiology of primary amenorrhea in a 16-year-old girl

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**Introduction**

Premature ovarian insufficiency (POI) is a rare cause of primary amenorrhea (1,2). We report a 16-year-old girl with normal secondary sexual characteristics, but no menses due to an autoimmune POI and associated with autoimmune gastritis. This is the first report of such constellation in an adolescent – both conditions separately already being rare in the pediatric population.

**Case presentation**

A 16-year-old girl was referred to our department with primary amenorrhea and hyperprolactinemia. She had breast development for 2 years. She had no other complaints, i.e. no galactorrhea, hirsutism, hot flushes or mood changes. No recent weight loss, excessive weight gain or monthly abdominal pains were noted. Her medical history was unremarkable and she took no drugs or food supplements. There was no family history of autoimmune or endocrine diseases, early menopause, or fragile X syndrome.

At physical examination, her weight was 50kg (Z score -0.84), height 171cm (Z score 0.67), and blood pressure 130/94mm Hg. She was at Tanner IV for breast and pubic hair development. Thyroid palpation was normal. Skin pigmentation and nails were normal. No dysmorphic features were noted.

Hormonal analysis one month before referral showed elevated prolactin (56.2µg/L) levels, high-normal FSH (23.3IU/L), normal LH (13.6IU/L) levels, normal estradiol (62.5ng/L), and normal thyroid function. Repeat laboratory investigations confirmed a lower but still elevated prolactin of 48.1µg/L (ref 3.71 - 23.12µg/L) with a normal monomeric recovery, and elevated morning cortisol (312µg/L), a high FSH (97.3IU/L), a high-normal LH (56.7IU/L) and SHBG (98nmol/L) with unmeasurable estradiol and AMH, suggesting an incipient POI. Genetic investigations including karyotype, CGH-microarray, and FISH of chromosome X were normal. FMR1 triplet repeat analysis showed a heterozygous intermediate expansion allele with 52 repeats. Screening for anti-ovarian, anti-adrenocortical, and anti-21-hydroxylase antibodies was repeatedly negative, but anti-TPO antibodies and anti-parietal cell antibodies consistently elevated. Abdominal ultrasound showed normal-sized ovaries without follicles and a uterus with an endometrial thickness of 5 mm. Pelvic MRI confirmed normal ovarian size and revealed only one follicle cyst in the right ovary (9 mm in diameter). By brain MRI a hypointense lesion (2.8mm in diameter) was found in the posterior part of the anterior pituitary. Spinal bone mineral density was normal (DEXA, Z-score of -0.2). Hematology tests showed a normal erythrocyte sedimentation rate and white blood cell count but revealed an iron deficiency anemia.

Serum gastrin was elevated while tissue transglutaminase IgA and fecal occult blood tests were negative. Endoscopic-histologic evaluation confirmed the presence of atrophic gastritis.

## Discussion

We report on an adolescent girl with autoimmune POI associated with autoimmune gastritis. The initial transient ovarian failure during immune attacks complicate the diagnosis of autoimmune POI and in this case probably explains the absence of hot flashes, the initially normal estradiol levels, and the normal bone mineralization (3). Furthermore, diagnosing autoimmune POI is also difficult due to low sensitivity and specificity of anti-ovarian antibody testing - frequently false negative as seen in our patient (3). In this girl, the diagnosis was further complicated by elevated prolactin and cortisol levels, which were likely related to stress or transient FSH induced hyperestrogenism. The diagnosis of atrophic gastritis and thyroid autoimmunity and the exclusion of other diagnoses allowed us to diagnose autoimmune POI. Diagnosing autoimmune forms of POI is important in view of the incipient risk of autoimmune adrenal insufficiency and need for follow-up (4).

## Conclusion

Autoimmune POI can present with primary amenorrhea in adolescent women. Thorough immune evaluation is needed to diagnose autoimmune POI when anti-ovarian antibodies are negative. Full autoimmune screening, including not only anti-adrenocortical and anti-thyroid antibodies but also anti-parietal cell antibodies should be done in unexplained POI, especially when iron deficiency is present.

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# Chromosome 22q11.2 deletion syndrome revealed by severe hypocalcemia and pulseless electric activity: Untangling a conglomerate of potential primary etiologic factors

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**Background**: chromosome 22q11.2 deletion syndrome has been reported to occur in about 1 per 347 to 992 fetuses.[1] Patients present with velocardiofacial syndrome (VCFS) or DiGeorge syndrome (DS). Hypocalcemia has been reported to occur in up to 80.4% of cases.[2]

**Case report:** a 28-year-old man, known with autism spectrum disorder (ASD), presented at the emergency department (ED) for muscle cramps. Medical history included chronic myeloid leukemia treated with tyrosine kinase inhibitor (TKI) the last 10 years, gastric bypass and recurrent fractures due to osteoporosis managed by alendronate. Four months prior to the admission, alendronate was switched to zoledronate because of digestive adverse effects. Under zoledronate, there was exacerbation of the muscle cramps.

At the ED, temperature was 36.5°c, respiratory rate 22 cycles/min, heart rate 110 beats/min and upper limbs spasms (Trousseau sign) hindered blood pressure measurement. Shortly after arrival at the ED, the patient suddenly developed pulseless electric activity, requiring advanced cardiopulmonary resuscitation including endotracheal intubation followed by admission to the intensive care unit.

Investigations revealed a long QTc at 513 msec, severe hypocalcemia (Ca 1.32 mmol/L [range 2.12- 2.60], ionized Ca 0.66 mmol/L [range 1.17-1.33]), normal magnesium (0.68 mmol/L [0.74-0.99]), hypophosphatemia (phosphor 0.49 mmol/L [0.78-1.42]), normal vitamin D 53 ng/L and inadequate low parathyroid hormone (PTH) level (38 ng/L [range 7-70]).

Management included parenteral magnesium, calcium and oral calcitriol. Amiodarone was temporally administered for sustained ventricular tachycardia. TKI and biphosphonate were interrupted.

The medical records showed low calcium level immediately after initiation of TKI ruling out gastric bypass and biphosphonate as primary etiologic factors. Genetic testing was requested based on low calcium level, inadequately low PTH and atypical facial features (asymmetric crying facies, malar flatness and hooded eyelid). This identified deletion of chromosome 22q11.2 spanning the region of catechol-O-methyltransferase *(COMT)* and T-box transcription factor 1 *(TBX1)* genes.[3] Upon discharge, oral substitution of calcium, magnesium and calcitriol was continued.

**Discussion:** severe hypocalcemia can represent a life-threatening condition. Etiologies of hypocalcemia are divided into PTH and non-PTH mediated causes.[4] In our patient this could be due to TKI, malabsorption following gastric bypass, biphosphonate initiation and, besides his psychiatric past medical history, the patient's phenotype suggested a congenital

disorder. Identification of chromosome22q11.2 deletion confirmed a constitutional defect in calcium metabolism which might have been sequentially exacerbated by initiation of TKI, gastric bypass and biphosphonate. Hypocalcemia in chromosome 22q11.2 deletion syndrome results from hypoparathyroidism and occurs not only in the neonatal period, but also in adulthood.[2] Other clinical characteristics encompass cardiac defects, abnormal facies, thymic hypoplasia, cleft palate (and hypocalcemia) defined by the acronym CATCH-22. The disease spectrum is heterogeneous. In our patient, structural cardiac defects and immune deficiency have been excluded. While ascertainment of *TBX1* deletion relates to the clinical presentation encompassing hypoparathyroidism that of *COMT* gene could be associated with autism spectrum disorder.[3,5] Indeed, *TBX1* gene has been reported to influence cell proliferation and differentiation as well as signaling pathways involving fibroblast growth factor, retinoic acid, bone morphogenic protein among others. Deletion of one copy of *TBX1* impacts on the development of pharyngeal arch artery, leading to at least one of the common presentations of VCFS/DS. COMT enzyme degrades catecholamines, entailing dopamine. Genetic alteration involving *COMT* gene results in dopamine excess and has been linked to psychiatric defects including ASD and psychosis.

**Conclusion:** this case highlights the diagnostic complexity of hypocalcemia in a setting of multiple potential etiologic factors. It raises the call to keep in mind that chromosome 22q11.2 deletion syndromeis not that uncommon and can be revealed in adulthood. Specific phenotype, specifically PTH dependent low calcium level and facial dysmorphia, should prompt clinicians to order genetic testing independentlyof the patient's age.

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# Rapid Growing Thyroid Nodule: The Good One In The Bad Clothes.

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**Background**: Primary thyroid lymphoma (PTL) is a rare thyroid tumor that accounts for only 5% of thyroid malignancies, but is associated with higher mortality than differentiated thyroid cancer. The major risk factor for thyroid lymphoma is the presence of Hashimoto's thyroiditis (HT) with an estimated 60-fold increased risk. The lymphocytic infiltrate in HT appears to develop into lymphoma in a minority of patients. The differential diagnosis between Hashimoto's thyroiditis and thyroid lymphoma can be difficult.

**Case report:** We report a 34-year-old man who was referred to the endocrinologist for a large, rapidly growing thyroid nodule. The patient had not noticed the nodule until 2 months earlier. He had no known thyroid disease. Blood analysis showed normal thyroid hormone levels with positive antithyroid autoantibodies (TSH 1.97 mUI/L; free T4 14 pmol/L; aTg 437 mUI/L; aTPO 283 mUI/L; and TRAb 1.84 mUI/L). Ultrasound examination (US) showed a large solitary hypoechogenic thyroid nodule of 53 mm in the right lobe, EU-TIRADS V. Scintigraphy confirmed the large nodule in the right lobe with low iodine uptake. Fine needle aspiration cytology was performed and showed the prevalence of lymphoid population with abundant presence of $CD20^+$ lymphocytes by immuno-histochemistry (IHC). The diagnosis of lymphoproliferative disease could not be ruled out. To obtain a definite diagnosis, an anatomopathological examination with flow cytometry was necessary. The patient underwent a right hemithyroidectomy.

The postoperative anatomopathological examination showed a lymphocytic infiltrate with the formation of germinal centers, with $CD20^+$ and $CD10^+$ lymphocytes, and expression for both chain kappa and lambda by plasma cells. There was no t(14; 18) chromosome translocation on BCL2 FISH, making the diagnosis of lymphoma less likely. Following the results of the pathological examination and IHC, the diagnosis of a thyroid nodule in the context of Hashimoto's thyroiditis was made. Given the diagnostic complexity, a $^{18}$F-FDG PET-CT scan was also performed which showed no signs of malignancy or extrathyroidal disease.

**Discussion:** the reported case describes a difficult diagnosis between Hashimoto's thyroiditis and thyroid lymphoma in a patient presenting with a single large thyroid nodule. There is a correlation between the presence of Hashimoto's thyroiditis and the occurrence of thyroid lymphoma (25-75 % of patients with PTL also have a diagnosis of Hashimoto). The association between HT and PTL seems to originate from the development and alteration of intrathyroidal lymphoid tissue in HT. Cytological examination (FNAC) with IHC is often sufficient for definitive diagnosis, but in rare cases, histology is necessary for a definite diagnosis (as in our patient). The two pathologies share similarities on FNAC with IHC. The cellular infiltrate of PTL is characterized by $CD20^+$ and $CD19^+$ lymph nodes and the presence of K/L monoclonality (usually between 3-4), whereas the cellular infiltrate of the nodular form of Hashimoto's disease "lymph node-like pattern" is characterized by a $CD19^+$ prevalence and also by a variable K/Lambda monoclonality (usually between 0. 5 to 3.0). The diagnostic

difficulty in ourpatient was due to the presentation with a large and rapidly growing nodule, with FNAC showing a large $CD20^{+}$ lymphocyte population, for which a definite diagnosis could only be made with histology and other diagnostic tests such as PET-CT to exclude extra-thyroid involvement.

**Conclusions**: We present the case of a 34-year-old man with a large, rapidly growing thyroid nodule. Faced with a large and fast-growing nodule, one immediately thinks of a possible malignancy such as PTL or anaplastic thyroid cancer, but a similar clinical presentation can alsobe observed in the presence of Hashimoto's thyroiditis. In these cases, only a biopsy can confirm or deny the diagnosis of malignancy.

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# Is this just vitiligo? Nelson is hiding

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**Introduction**
Autoimmune disease is a rare event occurring after remission of Cushing's syndrome. We report on the appearance of new onset vitiligo in a patient treated for Cushing disease due to a an invasive pituitary adenoma, after bilateral adrenalectomy.

**Case report**
A 64-year-old man presented to our endocrinology clinic with progressive lumbar pain that had developed two months earlier. He had undergone bilateral adrenalectomy for Cushing's syndrome (CS)four years ago and transsphenoidal resection for Nelson tumor two years later.

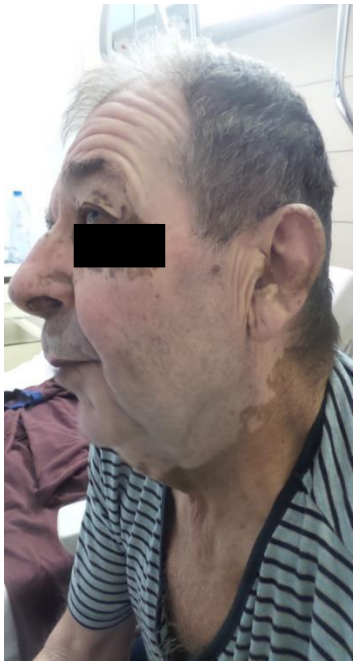
On physical examination, the patient had remarkable hyperpigmentation due to ACTH hypersecretion periorbital, peri-auricular and in the lower-neck region (**figure 1**). These findings were evident in his case due to the extensive facial vitiligo. Findings on bone scintigraphy were suggestive for metastatic lesions (**figure 2**) and CT-guided bone biopsy confirmed our suspicion of bone-invasive pituitary carcinoma. The patient was referred to the oncology department as a candidate for immunotherapy but opted for palliative care because of his weakened general condition.

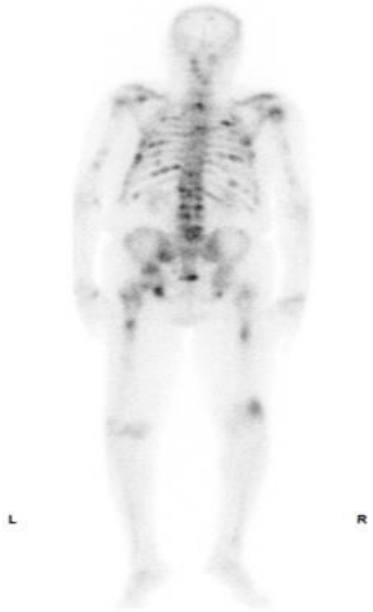
**Discussion**
This case highlights the fact that hypercortisolism induces a state of immunosuppression. Aftertreatment and normalization of cortisol hypersecretion in Cushing's syndrome, rebound immunity mayresult in overt autoimmune diseases, in casu vitiligo.

In a study by *de Mota et al*. it was reported that 8 out of 78 (10.3 %) adult patients with endogenous CS presented with a new autoimmune or allergic disease after treatment [1]. Other studies have reported an increased incidence of autoimmune thyroid disease in patients after treatment of CS [2, 3]. There have been descriptions of other forms of autoimmunity after cure of CS, mainly in case reports, such as rheumatoid arthritis, celiac disease, and systemic lupus erythematosus [2, 4-5].

This immunological phenomenon has been described in both ACTH-dependent and -independent cases but a lot of questions remain open to discussion. What is the underlying pathophysiological mechanism? Would autoimmunity be there if CS had not occurred? Is autoimmunity a transient phenomenon in these patients?

**Conclusion**
We present the case of a 64-year-old man with rapidly developing vitiligo, after bilateral adrenalectomy for an invasive pituitary ACTH secreting adenoma. The diagnosis of a metastasized pituitary carcinoma was confirmed .Patients with CS are considered immune-depressed. After treatment of CS, patients are at risk for developing autoimmune diseases. Early recognition of these autoimmune conditions and appropriate treatment are warranted.



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