

This meeting is kindly sponsored by the Dutch Endocrine Society (NVE) and the European Society of Endocrinology.



JNVE meeting 2016

Table of contents

Introduction	4
Scientific Program	
Thursday October 27 th 2016	5
Friday October 28 th 2016	8
General Information	13
Contact information	13
Venue information and maps	14
Announcement DEM conference 2017	15
Abstracts	
Thursday 27th October 2016	
Delegate session 1	16
Delegate session 2	22
Friday 28th October 2016	
Delegate session 3	26
Delegate session 4	30
Delegate session 5	35
Delegate session 6	40
Contact information participants	44

Introduction

Dear colleagues,

Welcome at the third meeting of the Jonge Nederlandse Vereniging voor Endocrinologie (JNVE). We, as JNVE-board, hope to provide you an inspiring and interactive meeting with lectures of well-known national invited speakers, a talented international speaker from Israel and presentations of 29 young endocrine researchers and clinicians from all over the Netherlands. We also welcome a colleague from Germany who has come to share some of her research findings with us.

The JNVE was founded in March 2014 by eight young endocrine researchers and clinicians and is part of the Dutch Endocrine Society (Nederlandse Vereniging voor Endocrinologie (NVE)). We aim to provide a highly interactive platform for those that are at an early stage in their career related to the field of endocrinology. This includes everyone with a general interest in endocrinology including students, researchers (PhD students and postdocs) and medical doctors in training for internist, pediatrician, obstetrician as well as clinical chemists. The JNVE meeting is aiming to promote interaction and communication between young basic researchers and clinicians and to provide many of you the opportunity to discuss their recent research data in an interactive and open atmosphere.

The JNVE is part of a group of similar initiatives for young endocrine professionals all over Europe. The very beginning of the initiative to unite young endocrinologists lies back in the 1990s, when the German Young and Active Research in Endocrinology (YARE) group was founded by Wiebke Arlt. She and her colleagues aimed to unite young endocrinologists of all kind and provide them a chance to interact with young colleagues and to present their work at an annual conference. In the subsequent years several other initiatives of young endocrine professionals have emerged all over Europe: YARE in Germany, ENGIOI in Italy, Klub 30 in Poland, FYEN in Denmark, the Young Endocrinologists in the United Kingdom and now the JNVE in the Netherlands. At this JNVE meeting one member of our German counterpart at YARE will present her work.

These national initiatives are united in the European Young Endocrine Scientists (EYES) as a part of the European Society of Endocrinology (ESE), which is a vibrant group of young endocrine researchers and clinicians from all over Europe. EYES has its own symposium at the annual European Congress on Endocrinology as well as an own meeting every year.

We are happy that as many of you are willing to participate in this 2016 JNVE meeting and hope that you will enjoy being part of the vibrant world of young endocrine professionals. Hopefully many of you will stay with us for a couple of years, until we have to admit that we are all getting too old for the JNVE and a new generation of young endocrinologists will take over the JNVE torch.

The JNVE-board

Mariëtte Boon (LUMC, Leiden), *Chair*

Michiel Nijhoff (LUMC, Leiden), *Secretary*

Anouk van Berkel (Radboud UMC, Nijmegen), *Treasurer*

Lonneke Bähler (AMC, Amsterdam)

Anneke van den Beukel (Erasmus MC, Rotterdam)

Thamara Osinga (UMCG, Groningen)

Angela Sarabdjitsingh (UMC Utrecht, Utrecht)

Dirk van Moorsel (MUMC, Maastricht)

Mariska Vlot (VUMC, Amsterdam)

Program JNVE meeting 2016

Day 1: Thursday 27th October 2016

12.00 **Registration and check-in Holiday Inn Hotel Leiden**

12.00 **Lunch at 'Garden Restaurant' Holiday Inn Hotel Leiden**

Lectures at 'Amsterdam' venue at Holiday Inn Leiden

12.45 **Opening and Introduction**

Mariëtte Boon (Chair JNVE)

13.00 **Invited lecture 1: Disturbances in HPA axis in young delinquents**

Dr. Lucreas Nauta (*VUMC, Amsterdam*)

Chairs: Mariska Vlot and Thamara Osinga

13.45 **Delegate session 1: Steroid hormones**

Chairs: Mariska Vlot and Dirk van Moorsel

14.45 **Update BijnierNET**

Johan Beun

Chairs: Thamara Osinga and Dirk van Moorsel

15.10 **Tea and coffee**

15.30 **Invited lecture 2: Klotho and FGF23: regulation and clinical utility**

Dr. Marc Vervloet (*VUMC, Amsterdam*)

Chairs: Mariska Vlot and Michiel Nijhoff

16.15 **Delegate Session 2: Lipid & bone metabolism**

Chairs: Mariska Vlot en Mariëtte Boon

17.00 **Invited lecture 3: Microbiome time**

Drs. Christoph Thaiss (*Weizmann Institute of Science, Tel Aviv, Israel*)

Chairs: Dirk van Moorsel and Lonneke Bahler

17.45 **Drinks at 'Ocean Bar' Holiday Inn Hotel Leiden**

19.00 **Diner at 'Garden Restaurant' Holiday Inn Hotel Leiden**

21.00 **Social evening program with Pub Quiz and Halloween party at 'Alkmaar/Haarlem' venue**

Delegate Session 1:

Steroid hormones

Chairs: Mariska Vlot and Dirk van Moorsel

- 1. The effect of loss of anti-Müllerian Hormone signaling on metabolism**
Magreet Vonk Noordegraaf et al. (*Erasmus MC, Rotterdam*)
- 2. Effects of intranasal insulin application on Blood Oxygen Level Dependent (BOLD) responses to glucose ingestion in the hypothalamus and ventral tegmental area**
Abimbola Akintola et al. (*LUMC, Leiden*)
- 3. Target gene selectivity of mineralocorticoid over glucocorticoid receptor in the brain**
Lisa van Weert et al. (*LUMC, Leiden*)
- 4. Plasma acylated and unacylated ghrelin are suppressed in acromegaly during combination therapy of somatostatin analogues and pegvisomant, but not in patients using pegvisomant monotherapy or during active acromegaly**
A Muhammad et al. (*Erasmus MC, Rotterdam*)
- 5. Co stimulation with IGF-1 and TLR ligands induces a pro-inflammatory response in Peripheral Blood Mononuclear Cells (PBMCs) via activation of the MAPK pathway**
Thalijn Wolters et al. (*Radboud UMC, Nijmegen*)
- 6. Sex difference in thermal preference in adult mice is not affected by gonadectomy**
Kasiphak Kaikaew et al. (*Erasmus MC, Rotterdam*)

Delegate Session 2:

Lipid and bone metabolism

Chairs: Mariska Vlot and Mariëtte Boon

7. Quantitative bone SPECT/CT clinically applied in patients with sternocostoclavicular hyperostosis

Pieter Valkema et al. (*LUMC, Leiden*)

8. Various calibration procedures result in optimal standardization of routinely used 25(OH)D I D-LC-MS/MS methods

Niek Dirks et al. (*VUMC, Amsterdam*)

9. Fluxomic characterization of metabolic reprogramming in activated brown adipose tissue

Ntsiki Held et al. (*AMC, Amsterdam*)

10. The effect of exenatide on brown adipose tissue and energy expenditure in healthy young men

Laura Janssen et al. (*LUMC, Leiden*)

Program JNVE meeting 2016

Day 2: Friday 28th October 2016

Lectures at 'Amsterdam' venue at Holiday Inn Leiden

- 8.30 **Invited lecture 4: Late effects of thyroid cancer in adults and children**
Prof. dr. Thera Links (*UMC, Groningen*)
Chairs: Tamara Osinga and Michiel Nijhoff
- 9.15 **Delegate Session 3: (Para)Thyroid metabolism**
Chairs: Michiel Nijhoff and Anouk van Berkel
- 9.55 **Coffee and tea**
- 10.15 **Invited lecture 5: The quest for the elixir of life: hormones and the pharmaceutical enterprise (1881-2016)**
Prof. dr. Toine Pieters (*VUMC/UMCU*)
Chairs: Mariska Vlot and Lonneke Bahler
- 11.00 **Delegate Session 4: (Neuro)endocrine tumors**
Chairs: Tamara Osinga and Michiel Nijhoff
- 11.50 **Lunch at 'Garden Restaurant' at Holiday Inn Leiden**
- 12.50 **Invited lecture 5: Childhood obesity**
Dr. Erica van den Akker (*Erasmus MC, Rotterdam*)
Chair: Dirk van Moorsel and Mariëtte Boon
- 13.35 **Delegate Session 6: Obesity and metabolism**
Chairs: Dirk van Moorsel and Mariëtte Boon
- 14.35 **Coffee and tea**
- 14.55 **Delegate session 7: Diabetes**
Chairs: Lonneke Bahler and Michiel Nijhoff
- 15.35 **Evaluation, JNVE award and farewell**

Delegate Session 3:

(Para)Thyroid metabolism

Chairs: Michiel Nijhoff and Anouk van Berkel

12. Effect of Pre-analysis Conditions and Performance of Different Plasma n-oxPTH Assays

Stan Ursem et al. (*VUMC, Amsterdam*)

13. Thyroid signaling, insulin resistance and type 2 diabetes mellitus: a Mendelian randomization study

Maxime Bos et al. (*LUMC, Leiden*)

14. A novel L341V mutation of the thyroid hormone (TH) receptor β gene in a girl with resistance to TH

Karn Wejaphikul et al. (*Erasmus MC, Rotterdam*)

15. Mutations in TBL1X are associated with central hypothyroidism

Charlotte Heinen et al. (*AMC, Amsterdam*)

Delegate Session 4:

(Neuro)endocrine tumors

Chairs: Tamara Osinga and Michiel Nijhoff

16. Incidence and prognostic value of serotonin-secretion in pancreatic neuroendocrine tumors

Wouter Zandee et al. (*EMC, Rotterdam*)

17. Toward the Understanding and Treatment of Pheochromocytoma

Marjolein ter Laak et al. (*Radboud UMC, Nijmegen*)

18. Genotype-dependent brown adipose tissue activation in patients with PGL

Anouk van Berkel et al. (*Radboud UMC, Nijmegen*)

19. Hypoxia-inducible factor 2a mutation-related paragangliomas classify as discrete pseudohypoxic sub-cluster

Stephanie Fliedner et al. (*University Medical Center Schleswig-Holstein, Lübeck, Germany*)

Delegate Session 5:

Obesity and metabolism

Chairs: Dirk van Moorsel and Mariëtte Boon

20. McTOM: Metformin and Core body temperature in lean and obese males

Lonneke Bahler et al. (*AMC, Amsterdam*)

21. G Protein-coupled receptor 120 signaling activates brown adipocytes

Maaïke Schilperoort et al. (*LUMC, Leiden*)

22. Insulin resistance in overweight and obese adolescents is associated with gender, puberty stage, BMI z-score and fat mass - a PREVIEW study

Elke Dorenbos et al. (*MUMC, Maastricht*)

23. Evaluating the role of the E3-ubiquitin ligase IDOL in diet-induced diabetes

Nienke van Loon et al. (*AMC, Amsterdam*)

24. How obesity may predispose for anxiety

Lisa Koorneef et al. (*LUMC, Leiden*)

25. Markers for inflammation and endothelial dysfunction are associated with deteriorated augmentation indices in overweight, obese and morbidly obese boys

Kylie Karnebeek et al. (*MUMC, Maastricht*)

Delegate Session 6:

Diabetes

Chairs: Lonneke Bahler and Michiel Nijhoff

26. A-Kinase Anchoring Proteins in the Protection of Pancreatic Beta-cells from Cytokines

Rianne Ellenbroek et al. (*LUMC, Leiden*)

27. Fasting relative proinsulin secretion as a marker for β -cell secretory strain and β -cell function in patients with type 1 diabetes

Bas Uitbeijerse et al. (*LUMC, Leiden*)

28. High-intensity interval exercise reduces awareness of hypoglycemia in patients with type 1 diabetes

Hanne Rooijackers et al. (*Radboud UMC, Nijmegen*)

29. The diurnal rhythm of adipose tissue gene expression is reduced in patients with type 2 diabetes

Dirk-Jan Stenvers et al. (*AMC, Amsterdam*)

Contact information:

JNVE-website: <http://www.nve.nl/de-jnve>

JVNE-mail: jnve@nve.nl

Organizing Committee

Name	Affiliation	E-mail address
Mariëtte Boon	LUMC	m.r.boon@lumc.nl
Anneke van den Beukel	Erasmus MC	j.vandenbeukel@erasmusmc.nl
Anouk van Berkel	UMC st. Radboud	anouk.van.berkel@radboudumc.nl
Michiel Nijhoff	LUMC	m.f.nijhoff@lumc.nl
Dirk van Moorsel	MUMC	d.vanmoorsel@maastrichtuniveristy.nl
Angela Sarabdjitsingh	UMCU	r.a.sarabdjitsingh@umcutrecht.nl
Mariska Vlot	VUmc	m.vlot@vumc.nl
Lonneke Bähler	AMC	l.bahler@amc.uva.nl
Thamara Osinga	UMCG	t.osinga@umcg.nl

If there are any questions during the conference please contact:

Mariëtte Boon (Phone: 06-48126425) or Michiel Nijhoff (Phone: 06-15568267).



Venue and hotel accommodation:

Holiday Inn Leiden

Haagse Schouwweg 10

2332 KG LEIDEN

Phone number: 071-5355555

Website: www.holidayinnleiden.com



ROUTE

Car and parking

The Holiday Inn Hotel is located at the border of the city center of Leiden, at the N206 and near the A44 (exit 8). The hotel has its own parking and this can be freely used for guests.

Public transport

From Leiden Central Station, you can take bus 43 in the direction of The Hague and take the halt 'Holiday Inn' (about 10 min drive).

Dutch Endocrine Meeting

February 10-11, 2017



NVE
NEDERLANDSE VERENIGING
VOOR ENDOCRINOLOGIE

NH Conference Centre Leeuwenhorst
Noordwijkerhout, the Netherlands

1. The effect of loss of anti-Müllerian hormone signaling on metabolism

Margreet A. Vonk Noordegraaf, E. Leonie A. F. van Houten, Anke McLuskey-Dankbar, Piet Kramer, Axel P.N. Themmen, Jenny A. Visser

Erasmus Medisch Centrum, Rotterdam

Introduction: The gonadal-specific anti-Müllerian hormone (AMH) is known for its role in male sexual development and plays a role in the regulation of female folliculogenesis. AMH signals through the AMH type II receptor (AMHR2), expressed by the gonads. However, recent studies have shown that AMH may also affect the hypothalamus-pituitary-gonadal (HPG) axis by influencing GnRH neuron firing, possibly by binding to the AMHR2 present in specific neurons in the brain. Previously, we have shown that AMHR2 is not expressed in tissues beyond the HPG axis.

Loss of AMH signaling results in infertility in male mice, mainly due to a blockade of sperm transfer as a result of the persistence of the müllerian duct. Female mice deficient in AMH signaling display enhanced recruitment of primordial follicles resulting in an increased number of growing follicles. The female mice have a normal ovulation rate and a normal estrous cycle, which allows us to study the effect of the altered ovarian follicular composition independent from cycle irregularities.

To determine the effect on the altered gonadal function on metabolic parameters, we studied the metabolic phenotype of male and female mice lacking AMH signaling.

Methods: Male and female AMH knockout mice (AMHKO), AMH type II receptor knockout mice (AMHR2KO) and wild type littermates were analyzed at 2, 5 and 8 months of age. An intraperitoneal glucose tolerance test (IPGTT) was performed. Gonads and several adipose tissue depots were collected for mRNA and protein expression analysis and for morphological analysis. Furthermore, serum was collected for hormone and adipokine measurements.

Results: Female mice lacking AMH signaling show an improved glucose tolerance ($P < 0.002$) compared to WT littermates upon ageing. In addition, female mice had lower weights of adipose tissue depots, smaller adipocytes ($P < 0.001$) and lower serum leptin levels ($P < 0.01$) compared to age-matched controls. Furthermore, absence of AMH signaling in female mice resulted in more active BAT and increased browning of WAT. Besides, serum sex steroid levels were normal in these mice.

In contrast, absence of AMH in male mice did not result in a different metabolic phenotype, illustrated by the normal glucose tolerance and adipose tissue weight and morphology. However, at 5 months of age, male AMHR2KO mice showed an impaired glucose tolerance compared to WT mice ($P = 0.007$), which persisted at 8 months of age ($P = 0.001$). In addition, male AMHR2KO mice show a reduced browning of the inguinal WAT depot compared to WT mice.

Conclusions: Male and female mice lacking AMH signaling show an opposite metabolic phenotype, illustrated by a protection of female mice against the age-related decline in metabolism, whereas male mice lacking AMHR2 show a worsened metabolic phenotype upon ageing. Because the AMHR2 is not expressed in metabolic tissues, the observed metabolic phenotype in females most likely is the result of an altered ovarian secretory profile, which is currently studied further. In conclusion, our results suggest that changes in gonadal function have metabolic consequences that are independent from sex steroid hormones and sex-dependent.

2. kintoEffects of intranasal insulin application on Blood Oxygen Level Dependent (BOLD) responses to glucose ingestion in the hypothalamus and ventral tegmental area

Anna M. van Opstal^{1*}, Abimbola A. Akintola^{2*}, Marjan van der Elst², Rudi G. Westendorp^{2,3}, Hanno Pijl⁴, Diana van Heemst², Jeroen van der Grond¹

1. Department of Radiology, Leiden University Medical Centre, Leiden, the Netherlands
2. Department of Internal Medicine, Section Gerontology and Geriatrics, Leiden University Medical Centre, Leiden, the Netherlands
3. Department of Public Health, University of Copenhagen, Copenhagen, Denmark
4. Department of Internal Medicine, Section Endocrinology, Leiden University Medical Centre, Leiden, the Netherlands

*Equal contribution of authors

The brain, along with peripheral glucose- regulatory systems regulates glucose homeostasis. Key brain areas that have been implicated in responses to metabolic cues comprise the hypothalamus, via its nuclei and glucose sensitive neurons, and the ventral tegmental area (VTA) via the reward system. To study the effects of intranasal insulin application on neuronal activity in both the hypothalamus and ventral tegmental area after glucose ingestion in young healthy volunteers, we performed a randomized, placebo-controlled cross- over trial and selectively applied 40 IU insulin or placebo via the nose using a customized nasal atomizer. Hypothalamic blood oxygen level dependent (BOLD) signals were repeatedly measured before and over 30 minutes after ingestion of 75g glucose drink or water. In both the hypothalamus and the VTA, BOLD signals tended to decrease after ingestion of glucose as compared to water. Compared to placebo, the overall decrease in hypothalamic BOLD response after glucose ingestion as compared to water ingestion was stronger after intranasal insulin application ($p < 0.001$) as were the decreases at 18-24 minutes ($p < 0.001$) and 24-30 minutes ($p < 0.001$) after the glucose drink. Similarly, compared to placebo, the overall decrease in BOLD response in the VTA after glucose ingestion as compared to water ingestion was stronger after intranasal insulin application ($p < 0.001$) as were decreases at 18-24 minutes ($p < 0.001$) and 24-30 minutes ($p = 0.019$) after the glucose drink. Thus, decreases in fMRI BOLD responses in both the hypothalamus and the VTA after glucose ingestion as compared to water ingestion, were augmented after intranasal application of insulin. Further research is needed to investigate the mechanisms through which the augmentation of the signal suppression occurs, whether this occurs in other brain areas that play a role in fuel metabolism as well, and what its functional consequences are.

3. Target gene selectivity of mineralocorticoid over glucocorticoid receptor in the brain

L.T.C.M. van Weert^{1,3,4}, J.C. Buurstedde¹, I.M. Mol¹, R.A. Sarabdjitsingh², O.C. Meijer¹

1. Department of Medicine, Division of Endocrinology, Leiden University Medical Center, The Netherlands
2. Department of Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center Utrecht, The Netherlands
3. Department of Cognitive Neuroscience, Radboud University Medical Center and
4. Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands

In the limbic brain, mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) both function as receptors for glucocorticoids. MR and GR mediate distinct effects on cellular physiology via transcriptional mechanisms, but how and to what extent target gene specificity is achieved has remained enigmatic.

We have detected DNA binding sites of MR and GR in the hippocampus of rats injected IP with corticosterone (3 mg/kg), using chromatin immunoprecipitation followed by sequencing (ChIP-Seq). Next, we identified the extent of MR/GR binding selectivity and underlying mechanisms. This revealed 475 overlapping, and 918 MR- and 1450 GR-specific binding sites. *De novo* motif analysis resulted in a similar binding motif for both proteins at 100% of the target loci, which matched the known glucocorticoid response element (GRE). An additional motif was found, that co-occurred near all MR-specific binding sites, but was absent for GR-specific and MR-GR overlapping sites. We previously showed that NeuroD factors could bind this additional motif and coactivate transcriptional activity of MR/GR *in vitro*.

In order to further explore the functional effects of MR/GR DNA binding, i.e. transcription regulation, binding sites were associated to their nearest gene. This resulted in lists of MR-specific, MR-GR overlapping and GR-specific putative target genes. Gene expression levels, for a subset of each category, were measured in forebrain specific MR knockout mice (fbMRKO) sacrificed at the time of their endogenous corticosterone peak. The MR-associated genes were hypothesized to have altered expression levels in the fbMRKO compared to wild type mice.

Besides a slight decrease in one of the GR-associated genes, reductions were observed in the expression levels of several MR-GR overlapping and MR-specific putative targets. The most robust effect amongst the MR-associated genes was found in the *Jun dimerization protein 2 (Jdp2)* mRNA levels, which were reduced by 50% in the fbMRKO compared to wild type animals. In conclusion, MR-selective DNA binding can reveal functional regulation of genes associated with bound receptors, and further elucidates distinct MR-specific effector pathways.

4. Plasma acylated and unacylated ghrelin are suppressed in acromegaly during combination therapy of somatostatin analogues and pegvisomant, but not in patients using pegvisomant monotherapy or during active acromegaly

A. Muhammad, M. Huisman, P.J. Delhanty, A.J. van der Lely, S.J. Neggers

Erasmus Medisch Centrum, Rotterdam

Background: Data on plasma ghrelin levels in acromegaly is limited. Most studies reporting ghrelin levels have included a small number of patients and measured only total ghrelin with a radioimmunoassay using polyclonal antibodies. Also the effect of medical treatment on ghrelin levels in acromegaly is conflicting.

Objective: To compare fasting plasma Acylated Ghrelin (AG) and Unacylated Ghrelin (UAG) levels in acromegaly patients on combination therapy of long-acting somatostatin analogues and the growth hormone receptor antagonist, pegvisomant (N=60), to patients controlled with pegvisomant monotherapy (N=4), and to medically naïve patients with active acromegaly (N=5). To study the relationship *between* plasma AG, UAG and AG/UAG ratios versus biochemical and clinical parameters [age at diagnosis, sex, HbA1c, serum IGF-1, body fat mass and pegvisomant dose]

Methods: Fasting venous blood samples (N=69) were collected and directly stabilized with 4-(2-Aminoethyl) benzenesulphonyl fluoride hydrochloride after withdrawal. Plasma AG and UAG levels were determined by ELISA using a monoclonal antibody.

Results: Plasma AG and UAG levels were significantly lower in acromegaly patients on combination treatment (median AG: 8.5, pg/ml IQR: 2.9-21.1) (median UAG: 26.9 pg/ml, IQR: 11.2-42.1) compared to patients using pegvisomant alone (median AG: 60.5, pg/ml IQR: 58.8-77.4) (median UAG: 153.7 pg/ml, IQR: 127.3-196.0) and medically naïve acromegaly patients (median AG: 24.0, pg/ml IQR: 12.6-49.7) (median UAG: 56.3 pg/ml, IQR: 43.4-61.5). However, the AG/UAG ratio in all groups was similar. No significant association was observed between plasma AG, UAG and AG/UAG ratios versus diabetes, body fat mass and IGF-I levels.

Conclusions: Somatostatin analogues suppress plasma acylated and unacylated ghrelin in acromegaly, and this seems to be independent of co-treatment with pegvisomant and baseline IGF-I levels.

5. Co stimulation with IGF-1 and TLR ligands induces a pro-inflammatory response in Peripheral Blood Mononuclear Cells (PBMCs) via activation of the MAPK pathway

Thalijn L.C. Wolters¹; Mihai G. Netea²; Ad R.M.M. Hermus¹, Jan W.A. Smit¹, Romana T. Netea-Maier¹

1. Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Center, Nijmegen

2. Department of Internal Medicine, Division of Experimental Internal Medicine, Radboud University Medical Center, Nijmegen

Introduction: Patients with acromegaly display an increased risk to develop cardiovascular disease (CVD). Recent data demonstrate a crucial role of activation of the innate immune system in the development of CVD. In addition, a novel regulatory role of IGF-1 in the development of subclinical inflammation through the pro-inflammatory activation of peripheral monocytes has been suggested. Therefore, we hypothesize that Growth Hormone (GH) and/or Insulin-like Growth Factor 1 (IGF-1) excess induce a pro-inflammatory state via circulating immune cells, which contributes to an increased CVD risk.

Aim of the study: to assess the effect of GH/IGF-1 on cytokine production induced by various TLR ligands.

Methods: PBMCs (peripheral blood mononuclear cells) were obtained from buffy coats from healthy volunteers and were stimulated with Toll-like receptor (TLR) ligands (LPS, Pam3Cys, C. Albicans) and different concentrations of GH & IGF-1. Levels of pro-inflammatory (TNF- α , IL-6, IL-1 β , IFN- γ , IL-17, IL-22) and anti-inflammatory (IL-10) cytokines were measured. The underlying signaling pathways were investigated by targeted inhibition of PI3K-mTOR and MAPK pathways which are known downstream targets of the IGF-1 receptor.

Results: Direct stimulation of PBMCs with various concentrations of GH and IGF-1 alone did not influence inflammatory cytokine production. Neither did GH affect TLR-induced cytokine production. In contrast, co-stimulation with IGF-1 increased the LPS-induced and P3C-induced IL-6 production (P= 0.004 resp. P=0.037), LPS-induced TNF- α production (P= 0.016), but also Candida-induced Th1-derived IFN- γ production (P= 0.002) and TLR-induced Th2-derived anti-inflammatory IL-10 production (P=0.01). These effects were dose-dependent. In contrast, IGF-1 had no effects on IL-1 β and Th17-derived IL-17 & IL-22 production. Blocking the PI3K-mTOR pathway by targeted inhibition with Rapamycin and Wortmannin did not result in a reduction of TNF- α or IL-6 production in PBMCs stimulated with both IGF-1 and LPS, compared to stimulation with LPS alone. However, treatment with the MEK-inhibitor U0126 resulted in significantly reduced production of TNF- α and IL-6 (P=0.016 resp. P=0.008) in PBMCs stimulated with IGF-1 and LPS; in addition, we observed reduced expression of phosphorylated ERK.

Conclusion: IGF-1, but not GH, has important pro-inflammatory effects on specific cytokines, a process in which the MAPK pathway is involved. This may represent one of the mechanisms involved in the pathogenesis of atherosclerosis in patients with acromegaly. The observed increased IL-10 production could have counteracted some of the effects of the pro-inflammatory cytokines. Further studies of these mechanisms are needed in order to elucidate the role of inflammation in the pathogenesis of CVD in patients with acromegaly.

6. Sex difference in thermal preference in adult mice is not affected by gonadectomy

Kasiphak Kaikaew^{1,2}, Jacobie Steenbergen¹, Axel P.N. Themmen¹, Jenny A. Visser¹, Aldo Grefhorst¹

1. Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

2. Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Introduction: Warm-blooded animals prefer specific environmental temperatures within their species-dependent thermoneutral (TMN) zone at which their metabolic rate is at its lowest. At temperatures below TMN, animals increase their metabolic rate to generate heat while temperatures above TMN induces metabolic changes such as sweating. Research has shown that female mice prefer slightly higher temperatures than male mice. We questioned whether this difference in TMN between male and female mice depends on the presence of gonads.

Methods: Thirty-two 8-week-old C57Bl/6J mice were individually housed in a set of two connected cages. One cage was constantly set at 29 °C, while the other was adjusted daily at 26, 29, or 32 °C using water baths to control the temperatures. Every day, one piece of facial tissue paper (nesting material) and sawdust (bedding material) were replaced in both cages. After 2 weeks of adaptation to the experimental housing, the mice were gonadectomized (GDX) or sham operated (8 mice per group). One week later, the mice were investigated for 3 weeks. The location of each mouse was photographed by a webcam every 5 minutes, in both dark (active) and light (inactive) phases. In addition, food intake, fecal production, nest transfer and nest destruction were also recorded daily.

Results: All groups preferred a temperature higher than 26 °C ($p < 0.01$), especially in the inactive phase ($p < 0.001$). Moreover, daily food intake and nest destruction tended to decline when the environmental temperature increased. Female mice but not male mice preferred 32 °C rather than 29 °C. The time-weighted average preferred temperature was higher in female than male mice (31.16 ± 0.06 °C and 30.33 ± 0.12 °C respectively, $p < 0.001$). GDX did not affect the thermal preference in both sexes but it reduced other activities such as the transfer of nesting material from one cage to another.

Conclusions: Female mice prefer warmer environment than the males, but GDX does not affect this sex difference. The thermal sensing mechanism in the mice may be influenced by other sex-specific pathway or the set point has already been determined by sex hormones at the prepubertal stages.

7. Quantitative bone SPECT/CT clinically applied in patients with sternocostoclavicular hyperostosis

P.A. Valkema^{1,2}, M.H. van Heugten¹, M. Segbers², N.M. Appelman-Dijkstra¹, D. Vriens¹, P. Dibbets-Schneider¹, F.H.P. van Velden¹, P.C.W. Hogendoorn¹, N.A.T. Hamdy¹, R. Valkema²

1. Leiden University Medical Center, Leiden,
2. Erasmus MC University Hospital, Rotterdam.

Aim: There is a clinical need for an objective tool to assess disease activity and to monitor the efficacy of treatment in bone diseases. The aim of this retrospective study was to explore the performance of quantitative SPECT/CT (Q-SPECT/CT) measurements in the assessment of skeletal activity in a case series of patients with sternocostoclavicular hyperostosis (SCCH).

Methods: Two cameras (Symbia T series; Siemens Healthcare) were individually calibrated to convert measured cts/s/mL into kBq/mL using Jaszczak phantoms. Bone SPECT/CT studies and clinical data from 43 SCCH patients were collected retrospectively and reanalysed using Hermes SUV-SPECT software yielding Q-SPECT/CT images. Clinical measurements were expressed in terms of body weight corrected SUV_{max} , SUV_{peak} and lean body mass corrected SUL_{max} , SUL_{peak} . After exclusion of patients with incomplete CT data (n=16) or major extravasation (n=2), 25 patients had evaluable SPECT/CT studies (including 7 with baseline and follow-up scans 1.1 ± 0.3 years after bisphosphonate treatment). Skeletal activity was analysed using 3 cm spherical volumes of interest at clinically identified lesions and at a normal reference region (defined as average uptake in Th1-Th5 vertebral bodies without overt pathology).

Results: Vertebral reference activity was SUV_{max} 11.5 ± 2.4 , SUV_{peak} 9.3 ± 1.9 , SUL_{max} 7.3 ± 1.4 , SUL_{peak} 5.8 ± 1.1 (coefficients of variation: 0.19-0.21). Correction for lean body mass removed a small but significant correlation of SUV with body weight ($r^2 = 0.16$, $p = 0.05$ for SUV_{max}). Inter-observer variation (mean absolute difference; two independent observers) for reference SUL_{peak} was 0.17 (2.9%) for individual vertebrae and 0.12 (2.0%) on a per-patient basis (combined Th1-Th5 vertebrae). Vertebral reference SUL_{peak} was lower in patients previously treated with bisphosphonates (unpaired t-test, $\Delta = -1.1$, $p = 0.03$). However, it was not associated with eGFR (median 80 mL/min/1.73m², range 46-135). In clinical index lesions, SUL_{peak} was 16.2 ± 8.5 (coefficient of variation: 0.52). In 7 patients with follow-up scans, change in lesion SUL_{peak} over time was variable (median -3.8, range -9.6 to -0.03). Changes in vertebral reference SUL_{peak} were comparatively small (median -0.3, range -1.3 to -0.1).

Conclusions: Bone Q-SPECT/CT yields reproducible quantitative results in the clinical setting of SCCH. Reference vertebral SUL_{peak} lies within a narrow range across different patients, with the consistency of the reference parameter providing good contrast with active SCCH lesions. Our findings suggest that Q-SPECT/CT is a promising tool in the diagnosis and monitoring of disease activity in patients with SCCH and potentially other bone diseases. Prospective, confirmative studies are warranted to confirm these findings.

8. Various calibration procedures result in optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods

N.F. Dirks, H. Vesper, A.E. van Herwaarden, J.M. van den Ouweland, I.P. Kema, J.G. Krabbe and A.C. Heijboer

VU Medisch Centrum, Amsterdam

The variety of LC-MS/MS methods measuring total 25(OH)D used today is vast and despite efforts by various instances to standardize them, results also vary. Here we performed a comparison in samples of healthy donors between the currently routinely used 25(OH)D LC-MS/MS methods in the Netherlands and the NIST-Ghent University reference measurement procedures to address this issue (n = 40). Furthermore, an interlaboratory comparison in patient serum samples assessed agreement between the Dutch diagnostic methods (n = 37). The overall correlation of methods with the reference measurement procedures and with the mean of all diagnostic methods was excellent ($r > 0.993$ and $r > 0.987$, respectively). Three out of five methods aligned perfectly with both the reference values and the median of all methods. One method showed a positive bias (<10%), while another showed a negative bias (<10%) in both comparisons. These biases probably originated from differences in standardisation and may be obviated by reassessing calibration of stock standards or calibrator matrices. In conclusion, five diagnostic centers have performed a comparison with the 25(OH)D NIST-Ghent reference measurement procedures in healthy donor serum samples and a comparison among themselves in patient serum samples. Both analyses showed a high correlation and specificity of LC-MS/MS methods, yet did reveal some small standardization issues that could not be traced back to the technical details of the different methods. Hence, this study proves various calibration procedures can result in perfect alignment with the RMP.

9. Fluxomic characterization of metabolic reprogramming in activated brown adipose tissue

Ntsiki M. Held¹, Eline N. Kuipers², Michel van Weeghel¹, Patrick C.N. Rensen², Ronald J. Wanders¹, Arthur Verhoeven¹, Mariëtte R. Boon² and Riekelt H. Houtkooper¹

1. Laboratory Genetic Metabolic Diseases, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

2. Department of Medicine, Division of Endocrinology, and Einthoven Laboratory of Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

Brown adipose tissue (BAT) contributes to energy expenditure through energy dissipation as heat. Imaging techniques have showed high rates of glucose uptake in activated BAT, and BAT activation also leads to high fatty acid release from fat stores, suggesting an overall metabolic overload. Substrate overloading requires and induces metabolic reprogramming, but this effect in BAT has primarily been studied after prolonged cold exposure through gene expression arrays. These show increased expression of genes involved in glycolysis, β -oxidation, glycogen and fatty acid synthesis. However, how these distinct processes are regulated to occur simultaneously is poorly understood. Using fluxomics, we aim to estimate metabolic fluxes in the T37i brown adipocyte cell line during β 3-adrenergic activation. Using a set of specific substrate inhibitors and an extracellular flux analyzer, we found that the main substrates of BAT cells are fatty acids, which were equally oxidized during rest as well as during activation. Moreover, when BAT cells are activated they can increase the oxidation of other substrates, such as glutamine. Also, the rate of glycolysis increased by 60%, but interestingly most glucose is not converted to pyruvate to become fully oxidized. Therefore, in current studies we seek to further elucidate the metabolic pathways through which glucose is utilized. With [U-¹³C₆]-D-glucose labeling we perform stable isotope tracer-based metabolomics. This allows us to accurately calculate metabolic fluxes based on tracer incorporation in most central carbon metabolites. Gaining insight in BAT metabolism holds potential to accelerate the quest for pharmacological targets that collectively expand BAT volume as well as its function.

10. The effect of exenatide on brown adipose tissue and energy expenditure in healthy young men

Laura G.M. Janssen¹, Kimberly J. Nahon¹, Lenka M. Pereira Arias-Bouda², Lioe-Fee de Geus-Oei², Jimmy F.P. Berbée¹, Ingrid M. Jazet¹, Hildo J. Lamb³, Andrew G. Webb⁴, Jedrek Burakiewicz⁴, Hermien E. Kan⁴, Mariëtte R. Boon¹, Patrick C.N. Rensen¹

1. Department of Medicine, Division of Endocrinology, Leiden University Medical Center (LUMC), Leiden, the Netherlands
2. Department of Nuclear Medicine, LUMC, Leiden, The Netherlands
3. Department of Radiology, LUMC, Leiden, the Netherlands
4. Department of Radiology, C. J. Gorter Center for High-Field MRI, LUMC, Leiden, the Netherlands

Background & objectives: Brown adipose tissue (BAT) is an endocrine organ that combusts fatty acids into heat upon activation. This physiological function of BAT makes it a promising target to treat the consequences of a positive energy balance in humans, namely obesity and its related disorders such as type 2 diabetes mellitus (T2DM), dyslipidemia and cardiovascular disease. Especially the South Asian population is excessively prone to develop a disadvantageous metabolic phenotype, accompanied by these diseases.

We have recently shown that central agonism of the receptor for the incretin hormone glucagon-like peptide 1 (GLP-1) activates BAT in mice. In addition, the GLP-1 receptor agonist exenatide, which is currently used to treat T2DM, was shown to lower body weight and improve dyslipidemia in these patients. The underlying mechanisms of these beneficial effects of GLP-1 receptor agonism in humans have yet to be elucidated. Therefore, the aims of this study are to investigate the effect of exenatide on BAT activity and energy expenditure in healthy young men and look into possible differences herein between South Asian and white Caucasian ethnicities. Moreover, the golden standard method to measure BAT (¹⁸F-FDG PET-CT scan) will be compared to a novel technique (MRI scan).

Subjects and methods: We are currently performing an open-label single arm study in 24 adult male volunteers, aged 20-30 years, of whom 12 from South Asian and 12 from white Caucasian descent. These subjects will receive exenatide for 12 weeks via a weekly subcutaneous injection. BAT volume and activity via a cold-induced ¹⁸F-FDG PET-CT scan versus an MRI scan and resting energy expenditure via indirect calorimetry will be compared before and after treatment. Moreover, blood samples will be taken at these moments to investigate effects of exenatide on amongst others plasma lipid and glucose metabolism.

Conclusion: As this clinical trial is still ongoing, we are unable to show results just yet. During this meeting, we aim to discuss the study design with other researchers and to generate ideas on possible additional measurements and relevant research questions. This study will compare different methods to quantify BAT activity and will offer valuable unravelling insights in the effects of pharmacological activation of BAT by GLP-1 receptor agonism in healthy adult males from different ethnicities. This might result in a novel treatment strategy to combat obesity and its associated disorders.

11. Effect of Pre-analysis Conditions and Performance of Different Plasma n-oxPTH Assays

Stan R. Ursem, Marc G. Vervloet, Jacquélien Hillebrand, Renate T. de Jongh, Annemieke C. Heijboer

VU Medisch Centrum, Amsterdam

Introduction: PTH can be subject to oxidation at methionine residues 8 and 18. After this post-translational modification PTH loses its biological activity. Discriminating between biologically inactive, oxidized PTH (oxPTH) and biologically active, non-oxidized (n-ox)PTH may be of interest when assessing bone and mineral disorders. Recently, an affinity column was developed to separate the oxPTH from the n-oxPTH and to measure n-oxPTH subsequently with a routine PTH immunoassay. It is however debated whether oxidation of PTH mainly occurs *ex vivo*, which would limit its clinical significance. The aim of this study was to investigate influences of different pre-analytical conditions on n-oxPTH measurements and compare the appropriateness of several routine PTH assays. For this purpose, we determined PTH and n-oxPTH in patients at different levels of PTH and oxidative stress (hemodialysis (HD) and controls).

Methods: N-oxPTH was separated from its oxidized form using an affinity column (K1512SI, Immundiagnostik AG). The study included EDTA plasma samples of 17 HD patients undergoing hemodialysis (HD) and 32 controls. The effect of storage temperature, the effect of time until centrifugation and the effect of freeze-thaw cycles were determined. PTH and n-oxPTH was measured in each sample using four automated intact PTH (iPTH, 7-84) immunoassays (Architect; Centaur; Cobas; Immulite), one automated whole PTH (1-84) immunoassay (Liaison) and one manual iPTH immunoassay (Immundiagnostik).

Results: N-oxPTH concentrations were not affected up to 180 minutes until centrifugation or storage at -20°C or -80°C up to 79 days. N-oxPTH concentrations decreased after three freeze-thaw cycles (8% after 3 and 21% after 5 freeze-thaw cycles). Method comparison for both iPTH and n-oxPTH showed relatively good correlations (R 0.983 to 0.997 for iPTH and R 0.952 to 0.995 for n-oxPTH; yet for the manual assay R=0.935 and 0.576, respectively). Known standardisation differences between the different iPTH assays were confirmed (Architect and Centaur 50% higher; Immulite 60% higher and Liaison 45% lower compared to the Roche iPTH assay). Different standardisation differences between the measured n-oxPTH were shown (Architect 100% higher, Centaur 50% lower; Immulite 60% lower and Liaison 70% lower compared to the Roche assay). Plasma n-oxPTH concentrations in controls were frequently below the detection limit of the Liaison (whole PTH), Immulite (iPTH) and Centaur (iPTH).

Conclusion: Our study showed that n-oxPTH is rather stable in plasma samples. PTH levels are also known to be stable under these conditions. Hence, the results of this study indicate that further oxidation of PTH does not occur *ex vivo* under the circumstances of our study. In addition, we showed that routinely used iPTH immunoassays have a different sensitivity for n-oxPTH, showing the highest sensitivity in the Architect assay. Future studies will have to point out whether n-oxPTH measurements can contribute in assessing the rate of bone turn-over.

12. Thyroid signaling, insulin resistance and type 2 diabetes mellitus: a Mendelian randomization study

Maxime M Bos¹, Roelof AJ Smit^{1,2}, Stella Trompet^{1,2}, Diana van Heemst¹, Raymond Noordam¹

Department of Internal Medicine, section of Gerontology and Geriatrics¹, Cardiology², Leiden University Medical Center, Leiden, the Netherlands.

Background: Increasing observational evidence suggests an association between thyroid stimulating hormone (TSH), free thyroxine (fT4) and deiodinases with insulin resistance, but results are conflicting and causality has not yet been ascertained. In the present study, we applied Mendelian randomization (MR) to assess whether circulatory levels of the thyroid parameters TSH and fT4 and deiodinase activity causally associated with insulin resistance in euthyroid individuals.

Methods: Twenty genetic variants for TSH level and four for fT4 level (identified in a GWAS meta-analysis of European-ancestry cohorts) were used as instrumental variables in the MR analysis. Summary data from genome-wide association studies (GWAS) on type 2 diabetes mellitus (T2D) (DIAGRAM; 12,171 cases, 56,862 controls) and glycemic traits in nondiabetics (MAGIC; N=46,186 for fasting glucose and insulin and N=46,368 for HbA1c) were used as outcomes. The combined effect of the genetic instrumental variables on the outcomes was determined using inverse-variance weighted (IVW), and MR-Egger regression analyses. Furthermore, we examined the association between 16 variants in *DIO1*, *DIO2*, and *DIO3* with T2D and glycemic traits.

Results: We found no evidence of an association between the combined genetic instrumental variables for TSH and fT4 and the study outcomes using both IVW and MR-Egger regression analyses. For example, with IVW analyses, higher TSH was not associated with a higher risk of T2D (Odds ratio: 0.91 per standard deviation TSH increase; 95% confidence interval: 0.78; 1.07). No evidence for directional pleiotropy was found with MR-Egger regression analyses. On the other hand, selected genetic variants in *DIO1* (e.g., rs7527713) were associated with measures of insulin resistance.

Conclusion: We found no evidence of a causal association between circulatory levels of TSH and fT4 with insulin resistance, but found suggestive evidence that *DIO1* affects glucose metabolism, suggesting a role for thyroid metabolism in target tissues in glucose metabolism.

13. A novel L341V mutation of the thyroid hormone (TH) receptor β gene in a girl with resistance to TH

Karn Wejaphikul^{1,2}, Stefan Groeneweg¹, W. Edward Visser^{1,3}, V. Krishna Chatterjee³, Prapai Dejkharnon², Kevallee Unachak², Theo J. Visser¹, Marcel E. Meima¹, Robin P. Peeters¹

1. Department of Internal Medicine, Erasmus University Medical Center Rotterdam, the Netherlands
2. Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
3. Wellcome-MRC Institute of Metabolic Science, University of Cambridge, United Kingdom

Introduction: Mutations in the TH receptor β gene (*THRB*) cause resistance to TH β (RTH β). The clinical phenotypes include goiter, tachycardia, and elevated FT4 and FT3 with non-suppressed TSH. Here, we present the clinical phenotype and biochemical characteristics of a novel mutation, L341V-TR β 1, found in a 12-year-old Thai girl with RTH β .

Methods: Genomic DNA was sequenced for mutations in the exons of the *THRB* gene. To explore its pathogenic mechanism, the L341V mutation was modeled into a wild-type (WT) TR β 1 crystal structure (3GWS) using YASARA Structure and the L341V, L341A, L341I, L341F mutants were introduced in a FLAG-tagged TR β 1 construct. Binding affinity of *in vitro* translated L341V-TR β 1 was determined using competitive binding assays. Upon expression in JEG3 cells, transcriptional activity of WT and mutant TR β 1 were measured after 24 hours stimulation with 0-10,000 nM T3, using thyroid response element (TRE) luciferase reporter constructs (DR4, IRO and ER6) and pMAXGFP as control.

Results: Our patient presented with diffuse goiter, tachycardia, palpitations and high serum FT4 [5.37 ng/dL (0.7-2.1)] and FT3 [14.31 pg/mL (2.7-5.2)] with non-suppressed TSH [3.29 μ IU/mL (0.7-6.4)], indicative of RTH β . A heterozygous missense mutation in *THRB* (c.1021C>G; p.L341V) was found. Structure modeling of this mutation showed changes in side chain orientation and distance to the inner ring iodine of T3, suggesting interference with substrate binding. Indeed, the dissociation constant (Kd) for the L341V-TR β 1 mutant was 4-fold higher than for WT TR β 1 (7.0 vs. 1.6 nM, $p < 0.01$) and transcriptional activity was impaired, as indicated by higher EC50 than WT on all TREs tested (DR4: 19.8 vs. 0.27 nM, IRO: 35.1 vs. 1.04 nM, and ER6: 359.2 vs. 1.65 nM; $p < 0.001$). The EC50s for activation of the DR4-TRE by the mutants were: 0.2 nM for Leu (WT); 17.4 nM for Val (patient); 17.1 nM for Ala; 6.5 nM for Ile; 2.0 nM for Phe, $p < 0.001$). Thus, in particular, substitution of Leu341 by smaller amino acids result in impaired TR β 1 function.

Conclusions: We report a novel L341V-TR β 1 mutation as a cause of RTH β . Functional studies confirmed the impaired binding and transcriptional activity, and illustrate the importance of L341 side chain length and orientation for TR β 1 function.

14. Mutations in TBL1X are associated with central hypothyroidism

Charlotte Heinen¹, Monique Losekoot², Yu Sun², Peter Watson³, Louise Fairall³, Sjoerd Joustra², Nitash Zwaveling-Soonawala¹, Wilma Oostdijk², Erica van den Akker⁴, Mariëlle Alders¹, Gijs Santen², Rick van Rijn¹, Wouter Dreschler¹, Olga Surovtseva¹, Nienke Biermasz², Raoul Hennekam¹, Jan Maarten Wit², John Schwabe³, Anita Boelen¹, Paul van Trotsenburg¹, Eric Fliers¹

1. Academic Medical Centre, Amsterdam, Netherlands
2. Leiden University Medical Center, Leiden, Netherlands
3. Henry Wellcome Laboratories of Structural Biology, University of Leicester, Leicester, UK
4. Erasmus MC, Rotterdam, Netherlands

Background: Congenital central hypothyroidism (CeH) may occur isolated, or in combination with other pituitary hormone deficiencies. Although a third causative gene for CeH was recently reported (*IGSF1*), the aetiology of isolated CeH has remained unexplained in most cases.

Methods: We studied a family with three relatives with isolated CeH, in whom mutations in all known causative genes for CeH were excluded. Using X-exome sequencing in these three patients, we identified a missense mutation in the Transducin β -like protein 1, X-linked (*TBL1X*) gene. The *TBL1X* protein is part of the thyroid hormone receptor corepressor complex. Sanger sequencing of this gene in unrelated cases of unexplained isolated CeH revealed five additional missense mutations. We performed clinical and biochemical characterization of the probands and relatives with a mutation identified by family screening. We investigated the functional consequences of the mutations *in vitro*, and used qPCR and immunostaining to study *TBL1X* expression in post-mortem human hypothalamus and pituitary tissue.

Results: All probands (n=8, 6 males) had CeH with plasma free thyroxine (FT4) concentrations below the reference interval accompanied by thyrotropin concentrations within the reference interval. Family screening identified mutations in 9 females and 2 males, all with FT4 concentrations in the lower half of the reference interval. Eleven out of 15 evaluated individuals with a mutation had hearing loss. The *TBL1X* mutations were located in the highly conserved WD40-repeat domain of the protein and influenced its expression and thermal stability. *TBL1X* mRNA and protein were expressed in the human hypothalamus and pituitary.

Conclusion: Mutations in *TBL1X* are associated with a novel syndrome of familial isolated CeH and hearing loss, presumably resulting from impaired function of the nuclear NCoR/SMRT corepressor complex.

Funding: Wellcome Trust, Biological Sciences Research Council Project Grant; AMC Foundation Grant.

15. Incidence and prognostic value of serotonin-secretion in pancreatic neuroendocrine tumors

W.T Zandee, R.C.A. van Adrichem, K. Kamp, R.A. Feelders, W.W. de Herder

Erasmus MC

Introduction: Neuroendocrine tumors from the pancreas (PNETs) can secrete a wide variety of hormones. Serotonin-secretion is found in approximately 1% of PNETs, but different standards for diagnosis are being used. It causes the carcinoid syndrome and can be quantified measuring the urinary 24-hour 5-hydroxyindoleacetic acid excretion (u5-HIAA).

Aim: to determine the incidence of serotonin secretion (with or without the carcinoid syndrome) and its effect on overall survival in patients with a PNET

Methods: in our tertiary referral center u5-HIAA is routinely measured before treatment with Peptide Receptor Radionucleotide Therapy or when symptoms are compatible with the carcinoid syndrome. In all patients with a PNET and available u5-HIAA, incidence of carcinoid syndrome and incidence of serotonin-secretion (u5-HIAA >3x upper limit of normal of 50umol/24hour) were assessed. Overall survival was compared using a Kaplan-Meier with log rank test and hazard ratios were calculated using Cox-regression for univariate and multivariate analyses.

Results: 255 patients were included, mean age 56.3 years, 48.6% female, 85.5% had stage IV disease. Serotonin-secretion was diagnosed in 22 patients (8.6%), 2 patients (0.8%) were diagnosed with carcinoid syndrome. Serotonin-secretion was a negative predictor for survival (HR 2.2, 95% CI: 1.27-3.81). When correcting for age, sex, stage, chromogranin A and neuron-specific enolase, serotonin-secretion is no longer a prognostic factor (HR 1.61 95% CI: 0.87-3.00).

Conclusion: Occurrence of the carcinoid syndrome is rare, but serotonin-secretion is prevalent and a negative prognostic sign. However is it probably a epiphenomenon rather than a solitary prognostic factor for overall survival.

16. Toward the Understanding and Treatment of Pheochromocytoma

Marjolein C. ter Laak, Selma Waaijers, Henri J.L.M. Timmers, Peter M.T. Deen

UMC st. Radboud, Nijmegen

Introduction: Pheochromocytomas are rare tumours that originate from adrenal chromaffin cells. The strongest indicator of malignancy of these tumours is a mutation in subunit B of the succinate dehydrogenase protein complex (SDHB). Insight in mechanisms causing these pheochromocytoma is needed to develop therapeutics for this disease, but proper models are lacking. Therefore, we are working on the generation of cell models to study the underlying mechanisms of pheochromocytoma and test potential therapeutics.

Objective: The generation of cell models to study the underlying molecular mechanisms of pheochromocytoma development and test potential therapeutics.

Methods: For the development of such cell models, different approaches will be used:

1. Adrenal chromaffin murine cell line: tsAM5NE cells

By using the CRISPR/Cas9 technology, a bi-allelic SDHB mutation will be introduced in this cell line to create adrenal chromaffin SDHB knockout cells. Other pheochromocytoma-related mutations will be introduced for comparison.

2. Compound heterozygous SDHB-mutated patient fibroblasts

Both SDHB alleles are mutated in these fibroblasts (c.143A>T (p.Asp48Val) and c.308T>C (p.Met103Thr)). The suitability of these cells as an appropriate model will be investigated by comparison of these fibroblasts to control fibroblasts and, eventually, to SDHB-rescued fibroblasts. Different pheochromocytoma-related parameters will be measured and compared between cells with and without SDHB mutation including catecholamine production/release, succinate/fumarate ratio and expression of pheochromocytoma-related genes (HIF, proliferation markers, and protein succinylation). Based on these results, we will decide whether we will continue with further development and characterisation of these cell models. The most promising cell model(s) will be studied using metabolic flux studies after which a mice or zebrafish graft study will be performed. When successful, the cell model will be used for drug screening/testing purposes.

Results and conclusions: Culturing the tsAM5NE cells proves difficult, since they grow slow and require very specific culturing conditions. Catecholamine measurements in these cells and medium show the expected norepinephrine and dopamine production. For the introduction of an SDHB mutation in the genome CRISPR/Cas9 technology will be used. We designed and tested 5 different gRNAs targeting SDHB, since the efficiency of a gRNA is difficult to predict *in silico*. Simultaneously, gRNAs for other pheochromocytoma related genes (SDHA, VHL and NF1) were designed and tested (4 gRNAs/gene). These gRNAs will be used to create cell lines that will function as comparison to the SDHB mutant cells. All 17 gRNAs were tested and analysed for the occurrence of a mutation at the desired gene. Efficiencies ranged between 0 -83%. Currently, gRNAs that gave the highest efficiencies are used to introduce a mutation in the tsAM5NE cells.

To test the SDHB-mutated patient fibroblasts for SDHB-related marker proteins, we cultured the patient's fibroblasts and control fibroblasts under different culture conditions (normal, hypoxia, and starvation) and analyzed the cells for SDHB, HIF-1 α , HIF-2 α and Cyclin D (proliferation marker) levels. Whereas SDHB was absent in patients fibroblasts, no differences in expression of the marker genes were observed between SDHB mutant and control fibroblasts. Rescue to a control phenotype after reintroduction of SDHB will provide insight on whether this cell model (partially) expresses a mutant SDHB phenotype as in pheochromocytoma.

17. Genotype-dependent Brown Adipose Tissue Activation in Patients with Pheochromocytoma and Paranglioma

Troy Puar^{1,2}, Anouk van Berkel¹, Martin Gotthardt³, Bas Havekes⁴, Ad R.M.M. Hermus¹, Jacques W.M. Lenders^{5,6}, Wouter D. van Marken Lichtenbelt⁷, Ying Xu⁸, Boudewijn Brans⁹, Henri J.L.M. Timmers¹

1. Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Centre, The Netherlands
2. Department of Endocrinology, Changi General Hospital, Singapore
3. Department of Radiology and Nuclear Medicine, Radboud University Medical Centre, The Netherlands
4. Department of Internal Medicine, Division of Endocrinology, Maastricht University Medical Centre, Maastricht, Netherlands.;
5. Department of Internal Medicine, Division of Vascular Medicine, Radboud University Medical Centre, The Netherlands
6. Department of Medicine and Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Dresden, Germany
7. Department of Human Biology, NUTRIM school for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands
8. Centre for Quantitative Medicine, Duke-National University Singapore Graduate Medical School, Singapore
9. Department of Medical Imaging, Division of Nuclear Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands

Context: Patients with pheochromocytomas and paragangliomas (PGLs) may have brown adipose tissue (BAT) activation induced by catecholamine excess. 18F-fluorodeoxyglucose (18F-FDG) PET/CT can be used for the localization of both PGLs and BAT. It is unknown whether BAT is specifically affected by altered cellular energy metabolism in patients with *SDHx* and *VHL*-related PGLs.

Objective: To determine endocrine and paracrine effects of catecholamine excess on BAT activation in patients with PGLs as detected by 18F-FDG PET/CT, taking into account genetic variation.

Design: Patients with PGLs who were fully genetically characterized underwent pre-surgical 18F-FDG PET/CT imaging for tumor localization and to quantify BAT activation.

Setting: Single Dutch tertiary referral centre.

Patients and Intervention: 73 patients, age 52.4±15.4 yr, BMI 25.2±4.1 kg/m², mean±SD, were grouped into sporadic, cluster 1 (*SDHx*, *VHL*) and cluster 2 (*RET*, *NF1*, *MAX*) mutations.

Main outcome measures: 18F-FDG mean standard uptake values (SUV_{mean}) were assessed in predefined BAT locations, including perirenal fat.

Results: 21/73 (28.8%) patients exhibited BAT activation. BAT activation was absent in all six patients with non-secreting PGLs. No difference in 18F-FDG uptake by perirenal fat on the side of the pheochromocytoma and the contralateral side was observed (SUV_{mean} 0.80 vs. 0.78 respectively, *P*=0.42). The prevalence of BAT activation did not differ between sporadic (28.9%), cluster 1 (40.0%) and cluster 2 patients (15.4%), *P*=0.36.

Conclusion: Patients with PGLs exhibit a high prevalence of BAT activation on 18F-FDG PET/CT. This is likely due to systemic catecholamine excess. BAT activation is not associated with specific germline mutations.

18. Hypoxia-inducible factor 2a mutation-related paragangliomas classify as discrete pseudohypoxic sub-cluster

Stephanie M.J. Fliedner^{1, 2}, Uma Shankavaram³, Geena Marzouca², Abdel Elkahloun⁴, Ivana Jochmanova^{2, 5}, Roland Daerr^{2,13}, W. Marston Linehan⁶, Henri Timmers⁷, Arthur S. Tischler⁸, Konstantinos Papaspyrou⁹, Jürgen Brieger⁹, Ronald de Krijger^{10,11}, Jan Breza¹², Graeme Eisenhofer¹³, Zhengping Zhuang¹⁴, Hendrik Lehnert¹, Karel Pacak²

1. Department of Medicine, University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany.
2. Section of Medical Neuroendocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA.
3. Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.
4. Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.
5. 1st Department of Internal Medicine Medical Faculty of P. J. Šafárik University in Košice, Košice, Slovakia.
6. Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.
7. Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands.
8. Tufts Medical Center, Boston, MA, USA.
9. Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany.
10. Department of Pathology, Josephine Nefkens Institute, Erasmus MC-University Medical Center, Rotterdam, The Netherlands.
11. Department of Pathology, Reinier de Graaf Hospital, Delft, The Netherlands.
12. Department of Urology, Comenius University, Bratislava, Slovak Republic.
13. Institute of Clinical Chemistry & Laboratory Medicine and Department of Medicine III, University Hospital Carl Gustav Carus, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.
14. Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA.

Recently, activating mutations of the hypoxia-inducible factor 2 α gene (HIF2A/EPAS1) have been recognized to predispose to multiple paragangliomas (PGLs) and duodenal somatostatinomas associated with polycythemia, and ocular abnormalities.

Previously, mutations in the SDHA/B/C/D, SDHAF2, VHL, FH, PHD1, and PHD2 genes have been associated with HIF-activation and the development of pseudohypoxic (cluster-1) PGLs. These tumors overlap in terms of tumor location, syndromic presentation, and noradrenergic phenotype to a certain extent. However, they also differ especially by clinical outcome and by presence of other tumors or abnormalities. In the present study, we aimed to establish additional molecular differences between HIF2A and non-HIF2A pseudohypoxic PGLs.

RNA expression patterns of HIF2A PGLs (n=6) from 2 patients were compared to normal adrenal medullas (n=8) and other hereditary pseudohypoxic PGLs (VHL: n=13, SDHB: n=15, and SDHD: n=14). Unsupervised hierarchical clustering showed that HIF2A PGLs made up a separate cluster from other pseudohypoxic PGLs. Significance analysis of microarray yielded 875 differentially expressed genes between HIF2A and other pseudohypoxic PGLs after normalization to adrenal medulla (false discovery rate 0.01). Prediction analysis of microarray allowed correct classification of all HIF2A samples based on as little as 3 genes (TRHDE, LRRC63, IGSF10; error rate: 0.02). Genes with the highest expression difference between normal medulla and HIF2A PGLs were selected for confirmatory qRT-PCR.

In conclusion, HIF2A-PGLs show a characteristic expression signature that separates them from non-HIF2A pseudohypoxic PGLs. Unexpectedly, the most significantly differentially expressed genes have not been previously described as HIF target genes.

19. McTOM: Metformin and Core body temperature in lean and obese males

L. Bahler¹, M.W. Chan¹, J. Booi², J.B. Hoekstra¹, F. Holleman¹, H.J. Verberne²

1. Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands
2. Nuclear Medicine, Academic Medical Center, Amsterdam, The Netherlands

Background: Physiological uptake of ¹⁸F-FDG in the colon is a frequent finding on ¹⁸F-FDG PET-CTs. The use of metformin is retrospectively associated with an increased ¹⁸F-FDG uptake in the colon. Interestingly, metformin is a glucose lowering drug associated with moderate weight loss. Increased colonic glucose disposal could partly explain the weight losing effect of metformin.

Therefore, we aimed to determine whether metformin modifies the metabolic activity of the colon by increasing glucose uptake.

Methods: We included 8 lean (22.1 [21.4-22.6] kg/m², age 60 [54-66] years) and 8 obese males (BMI 31.3 [28.9-33.4] kg/m², age 63 [53-68] years). We measured ¹⁸F-FDG uptake in the colon on PET-CT, energy expenditure and core body temperature before and after using metformin. The maximal colonic ¹⁸F-FDG uptake was measured in 5 separate segments (caecum, colon ascendens, - transversum, - descendens and sigmoid).

Results: The maximal ¹⁸F-FDG uptake in the colon increased significantly in all separate segments after the use of metformin. There was no difference in energy expenditure or core body temperature after the use of metformin. There was no correlation between maximal ¹⁸F-FDG uptake in the colon and energy expenditure or core body temperature.

Conclusion: Metformin significantly increases the ¹⁸F-FDG uptake in the colon, but this is not associated with an increase in energy expenditure or core body temperature.

20. G protein-coupled receptor 120 signaling activates brown adipocytes

Maaïke Schilperoort^{1,2}, Andrea van Dam¹, Geerte Hoeke¹, Sander Kooijman¹, Mark Christian², Patrick C.N. Rensen¹.

1. Dept. Medicine, Div. Endocrinology, Leiden University Medical Center, Leiden, the Netherlands.
2. Dept. Metabolic and Vascular Health, the University of Warwick, Coventry, the United Kingdom.

Aim: Brown adipose tissue (BAT) has recently been shown to contribute to total energy expenditure in human adults, which makes it an attractive target to combat obesity and related disorders. Recent studies have shown that BAT activation by cold exposure strongly increases expression of the G protein-coupled receptor 120 (GPR120). Interestingly, both GPR120-deficient mice and humans carrying a mutation associated with decreased GPR120 signaling are predisposed to obesity. Collectively, these data suggest a role of GPR120 in BAT activation. Therefore, the aim of this study was to investigate whether GPR120 signaling could have beneficial metabolic effects by increasing the activity of brown adipocytes.

Methods & Results: Classical activation of murine brown adipocytes by a β 3-adrenergic receptor agonist increased *Gpr120* gene expression. Also, induction of browning of subcutaneous and mesenteric white adipocytes by rosiglitazone induced *Gpr120* expression, underlining the importance of GPR120 for brown adipocyte function. In addition, the GPR120 agonist TUG-891 strongly increased the oxygen consumption rate of brown adipocytes, as demonstrated by using the Seahorse XF24 Analyzer. To elucidate the pathway by which GPR120 agonism activates brown adipocytes, we assessed the effects of TUG-891 on downstream targets of $G\alpha_q$ signaling. Indeed, TUG-891 stimulated intracellular calcium release as well as phosphorylation of both ERK and AKT. Pre-treatment with the calcium chelator BAPTA-AM completely abrogated the GPR120-dependent increase in oxygen consumption, while inhibition of the ERK or AKT pathways had no effect. A pilot study in which C57Bl/6J mice were injected intraperitoneally with TUG-891 demonstrated that TUG-891 increased fat oxidation *in vivo*, which is fully consistent with BAT activation.

Conclusion: GPR120 signaling stimulates the metabolic activity of brown adipocytes. Mechanistically, this effect is dependent on an increase in intracellular calcium levels as a downstream target of $G\alpha_q$ signaling. These data suggest that activation of GPR120 on brown adipocytes is a promising novel therapeutic strategy to increase energy expenditure and combat the obesity epidemic.

21. Insulin resistance in overweight and obese adolescents is associated with gender, puberty stage, BMI z-score and fat mass - a PREVIEW study

E. Dorenbos^{1,2}, M. Drummen², J.M. Rijks^{1,2}, J.A. Martínez³, S. Navas-Carretero³, G. Stratton⁴, A. Raben⁵, M. Fogelholm⁵, T. Adam², M.S. Westerterp-Plantenga², A.C.E. Vreugdenhil^{1,2}

1. Centre for Overweight Adolescent and Children's Healthcare (COACH), department of Paediatrics, Maastricht University Medical Centre, Maastricht, the Netherlands
2. Department of Human Biology, NUTRIM School of Translational Research in Metabolism, Maastricht University, the Netherlands
3. Department of Nutrition, Food Science, Physiology and Toxicology, Universidad de Navarra, Pamplona, Spain
4. Research Centre in Applied Sports, Technology, Exercise and Medicine (A-STEM), Swansea University, Swansea, United Kingdom
5. Department of Nutrition, Exercise and Sports, SCIENCE, University of Copenhagen, Copenhagen, Denmark

Background: During puberty a physiological transient insulin resistance occurs. Adolescents with overweight and obesity are at risk for higher levels of insulin resistance, and therefore increased risk of developing diabetes earlier in life. In the light of the current childhood obesity epidemic it is essential to determine which factors contribute to insulin resistance of puberty, so that effective treatment strategies for diabetes prevention can be developed.

Objectives: The PREvention of diabetes through lifestyle Intervention in Europe and around the World study (PREVIEW, FP7 grant no. 312057) and the Centre for Overweight Adolescent and Children's Healthcare (COACH) collaborate to identify effective lifestyle components in overweight and obese adolescents to prevent type-2 diabetes later in life. The aim of this study was to evaluate associations between anthropometric characteristics, body composition, puberty stages, physical activity and food intake on insulin resistance in overweight and obese adolescents.

Methods: Anthropometric characteristics, body composition, Tanner stages, physical activity (Baecke questionnaire), and food intake behaviour (Three Factor Eating Questionnaire) were determined, and tested for associations with homeostatic model assessment of insulin resistance (HOMA-IR).

Results: 134 adolescents (54 ♂; 80 ♀, age 13.6±2.3y, BMI z-score 3.0±0.7, HOMA-IR 3.5±1.7) were eligible for this study. HOMA-IR was not significantly different between boys and girls (♂ 3.1±1.5, ♀ 3.7±1.8, p=0.099). HOMA-IR was positively associated with BMI z-score (♂ r=0.29, p<0.001; ♀ r=0.39, p=0.001), puberty (♂ r=0.56, p=0.001; ♀ r=0.29, p=0.047), waist circumference (♂ r=0.34; p=0.016; ♀ r=0.35, p=0.003), and fat mass (♂ r=0.24; p=0.135; ♀ r=0.41, p=0.001). After correction for age, gender, puberty stage, and BMI z-score, fat mass was an independent contributor to HOMA-IR in girls (β=0.34, p=0.005) but not in boys (β=0.28, p=0.135). No associations with lifestyle factors were observed.

Conclusion: Gender, puberty stage, BMI z-score and fat mass are independent contributors to pubertal insulin resistance in overweight and obese adolescents.

22. Evaluating the role of the E3-ubiquitin ligase IDOL in diet-induced diabetes

Nienke M. van Loon & Noam Zelcer

Department of Biochemistry, AMC, Amsterdam

The prevalence of obesity is increasing worldwide paired with a global rise in associated cardiovascular disease and diabetes. Disturbed cholesterol metabolism is causally implicated in both pathologies. Herein, the low density lipoprotein receptor (LDLR) plays an important role owing to its ability to promote cellular uptake of LDL.

A recent report described that mice lacking the secreted protein Pcsk9, a post-transcriptional regulator of LDLR abundance, develop hyperglycemia, hypo-insulinemia and alterations in pancreatic islet morphology. Similar to PCSK9, the Inducible Degradator of the LDLR (IDOL), acting as an E3 ubiquitin ligase, promotes degradation of the LDLR. We therefore hypothesise that regulation of LDLR by IDOL in pancreatic β -cells will impact systemic glucose metabolism. To test this hypothesis we will challenge IDOL^(-/-) and WT mice with a high fat diet and evaluate their systemic glucose metabolism. Our results will contribute to unravelling the interplay between cellular cholesterol and systemic glucose metabolism.

23. How Obesity May Predispose for Anxiety: Review of the Literature and Presence of Metabolic Receptors in the Fear Circuitry

Lisa L. Koorneef^{1,2}, Ahmed Mahfouz^{3,4}, Marcel J.T. Reinders³, Boudewijn P.F. Lelieveldt⁴, Onno C. Meijer^{1,2,5}

1. Division of Endocrinology, Dept Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands
2. Eindhoven Laboratory of Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands
3. Delft Bioinformatics Laboratory, Delft University of Technology, Delft, The Netherlands
4. Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
5. Leiden Institute for Brain and Cognition, Leiden University Medical Center, Leiden, The Netherlands

It is well established that hunger reduces anxiety levels to promote risk-taking behaviour for the acquisition of food. Conversely, obesity may predispose to anxiety, as part of a mechanism that prevents unnecessary risk taking. We reviewed the literature on neurobiological processes that could underlie anxiety that is related to obesity and – as part of the metabolic syndrome spectrum – type 2 diabetes. The majority of findings pointed to disturbances in serotonergic and dopaminergic neurotransmission. To gain insight in these and less explored mechanisms, we performed a de novo genome-wide expression analysis to identify brain regions that are sensitive to obesity-associated factors. We selected metabolism-related hypothalamic peptides and peripheral factors that 1) can cross the blood-brain-barrier and 2) have a cognate receptor that is expressed in anxiety-related brain regions. Brain transcriptome analysis showed that many of these ‘metabolic receptor’ genes were significantly co-expressed with the serotonergic marker *Slc6a3*. In contrast, expression of the noradrenergic marker *Dbh* showed strong correlation with the hypocretin receptor only. We next hierarchically clustered expression of metabolic receptors in the amygdala and two major afferent areas (hippocampus and prefrontal cortex). We also evaluated coexpression of metabolic receptors with important mediators of anxiety responses, *Crh*, *Crhr1*, *Crhr2*. We observed overlapping but region-specific abundance of metabolic receptors, with a strikingly strong correlation with midbrain *Crh* expression. Taken together, we show a distributed neurobiological basis for an effect of obesity on anxiety, with a clear link to the serotonin and dopamine systems that emerged from the literature. Candidate obesity factors are brought forward which could be used as starting point for future obesity-related anxiety research.

24. Markers for inflammation and endothelial dysfunction are associated with deteriorated augmentation indices in overweight, obese and morbidly obese boys

K. Karnebeek^{1,2}, J. Plat², J.M. Rijks^{1,2}, R.P. Mensink², A.C.E. Vreugdenhil^{1,2}

1. Centre for Overweight Adolescent and Children's Healthcare (COACH), Department of Paediatrics, Maastricht University Medical Centre, Maastricht, The Netherlands
2. Department of Human Biology, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands

Background: The development of (cardiovascular) comorbidities in overweight and obese individuals is associated with low-grade inflammation. It is yet unknown to what extent an inflammatory profile is associated with vascular function in overweight and obese children. Here, we evaluated the association between arterial stiffness and classical cardiovascular risk parameters, enriched with a wide panel of markers for inflammation and endothelial dysfunction, in overweight and (morbidly) obese children

Methods: 196 overweight (18%), obese (47%) and morbidly obese (35%) children (45% boys) were included in this study. Augmentation index (Alx) was evaluated as a measure for arterial stiffness. Additionally, blood pressure, lipids and lipoproteins, monocyte chemoattractant protein 1 (MCP-1), E-selectin, high sensitive C-reactive protein (hs-CRP), serum amyloid A (SAA), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor α (TNF- α) concentrations were evaluated in these children. The association of these parameters with Alx was analyzed in all children, and separately for boys and girls.

Results: The Alx was significantly higher in girls (23.0% (interquartile range (IQR) 15.0;29.8)) than in boys (13.5% (IQR 5.0;22.0))(p<0,001). Also, hs-CRP and IL-6 concentrations were higher in girls, despite a lower BMI z-score. Interestingly, the Alx was comparable between overweight, obese and morbidly obese children. Forward linear regression analysis showed that height (cm; β =-0.313; p<0.001) and diastolic blood pressure z-score (β =1.795; p=0.034) were significant predictors for Alx in girls. In boys, height (cm; β =-0.336; p<0.001), sICAM-1 (ng/mL; β =0.039; p=0.001) and TNF- α (pg/mL; β =-3.979; p=0.049) were significant predictors for Alx.

Conclusion: No association was found between BMI z-score and Alx in overweight and (morbidly) obese children. Interestingly, Alx was higher in girls compared with boys, despite having a lower BMI z-score. In boys rather than girls, markers for inflammation and endothelial dysfunction contributed significantly to the augmentation index.

25. A-Kinase Anchoring Proteins in the Protection of Pancreatic Beta-cells from Cytokines

Johanne H. Ellenbroek*^{1,2}, Lorna M. Dickson², Rob C. Hoeben¹, Eelco J.P. de Koning^{1,3}, Chris J. Rhodes², Barton Wicksteed^{2,4}

1. Leiden University Medical Center, Leiden, The Netherlands.
2. University of Chicago, Chicago, IL, USA.
3. Hubrecht Institute, Utrecht, The Netherlands.
4. Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Diabetes mellitus results from loss of functional insulin-producing beta-cells in the pancreas. Therefore, therapies are needed that improve beta-cell survival. cAMP-protein kinase A (PKA) signaling improves beta-cell survival and function. Signaling downstream of PKA is coordinated by A-kinase anchoring proteins (AKAPs) that determine where and when PKA meets downstream effectors. For example, in cardiomyocytes, AKAPs are located at specific cellular sites to coordinate critical functions of the heart, including excitation-contraction coupling, oxygen homeostasis and transcriptional control. In beta-cells, the full complement of AKAPs is unknown. Our aim is to elucidate the role of PKA-AKAP signaling in beta-cell survival.

MIN6 cells were infected with adenoviruses expressing a dominant negative PKA or an AKAP inhibitory sequence to reduce PKA activity or PKA signaling through AKAPs, respectively. Cells were exposed to cytokines for 4 hours followed by detection of caspase 3 activity. Loss of PKA signaling through expression of PKA-DN or AKAP-IS aggravated cytokine-induced apoptosis of MIN6 cells.

To identify AKAPs, MIN6 cells were transfected with a FLAG-tagged PKA regulatory subunit (PKA-R11a-FLAG) to allow pull-down of associated proteins that were analyzed by mass spectrometry. Analysis of associated proteins led to the identification of 3 AKAPs (AKAP1, AKAP10 and AKAP13) and an endogenous AKAP disruptor, that have not been associated with beta-cells before.

The role of the identified AKAPs in beta-cell survival was assessed by lentivirus-mediated shRNA knockdown in MIN6 followed by exposure to cytokines. Loss of AKAP1 and AKAP13 aggravated cytokine-induced apoptosis of MIN6 cells. In contrast, loss of the AKAP disruptor led to an improvement of MIN6 cell survival after cytokine exposure.

We show that PKA-AKAP signaling in MIN6 cells is involved in cell survival. AKAP1, AKAP10, AKAP13 and an endogenous AKAP disruptor were associated with beta-cells for the first time. Elucidating the role of PKA-AKAP signaling in beta-cell biology is central to understand the mechanisms by which cAMP-PKA signaling improves beta-cell survival and function.

26. Fasting relative proinsulin secretion as a marker for β -cell secretory strain and β -cell function in patients with type 1 diabetes

Bastiaan S. Uitbeijerse¹, Michiel F. Nijhoff¹, Petra Sonneveld², Tanja Nikolic², Henk-Jan Aanstoot³, Bart O. Roep², Jaap J. Zwaginga², Eelco J. P. de Koning¹

1. Department of Internal Medicine, LUMC

2. Department of Immunohematology and Blood Transfusion, LUMC 3Diabeter, Rotterdam

Background: Sensitive assays indicate that in the majority of patients with type 1 diabetes circulating C-peptide is present but there is lack of insight in the functional aspects of these remaining β -cells. Stimulation tests can be used to assess the responsiveness of these β -cells but these tests are cumbersome. Proinsulin is the uncleaved propeptide of insulin and has a higher concentration in immature granules. Here we investigated fasting proinsulin as an indicator of residual insulin secretory capacity in type 1 diabetes.

Methods: Sixteen patients with type 1 diabetes (7M/9F, age 21.8 ± 3.4 years, BMI 24.9 ± 3.1 kg/m², diabetes duration 9.4 ± 5.5 years, autoantibody-positive) underwent a mixed meal tolerance. Glucose, C-peptide and proinsulin were measured before and after the mixed meal stimulus. A linear regression model was used with the stimulated C-peptide as independent variable and fasting C-peptide and proinsulin as dependant variable.

Results: In 6 out of 16 patients both proinsulin and C-peptide were detected during the test (1M/5F male, age 20.6 ± 1.3 years, BMI 25.6 ± 4.1 kg/m² diabetes duration: 4.5 ± 2.8 years). At baseline the glucose was 9.8 ± 2.3 mmol/l, C-peptide 0.27 ± 0.16 nmol/L and proinsulin 13.4 ± 9.3 pmol/L. After stimulation, glucose increased to 18.9 ± 3.3 mmol/L, C-peptide to 0.74 ± 0.87 nmol/L and proinsulin to 36.1 ± 20.8 pmol/L. In the multivariate model, there was a significant association between fasting C-peptide and stimulated C-peptide (Beta=5.68 p=0.021) and statistical insignificant association between fasting proinsulin and stimulated C-peptide (Beta -0.037 p=0.201).

Conclusion: Proinsulin can be detected in the circulation of patients with type 1 diabetes and measurable circulating C-peptide. As increased fasting proinsulin is a marker of beta cell dysfunction, proinsulin together with fasting C-peptide may be an indicator of secretory capacity in type 1 diabetes but larger groups of patients are needed to assess its usefulness.

27. High-intensity interval exercise reduces awareness of hypoglycemia in patients with type 1 diabetes

Hanne M.M. Rooijackers¹, Evita C. Wiegers², Marinette van der Graaf^{2,3}, Cees J. Tack¹ and Bastiaan E. de Galan¹

1. Department of Internal Medicine, Radboud university medical center, Nijmegen, the Netherlands
2. Department of Radiology and Nuclear Medicine, Radboud university medical center, Nijmegen, the Netherlands
3. Department of Pediatrics, Radboud university medical center, Nijmegen, the Netherlands

Background: Regular exercise is recommended for patients with type 1 diabetes (T1DM). High-intensity interval exercise is a relatively new training modality with increasing popularity, which markedly increases plasma lactate levels. Interestingly, intravenous administration of lactate is known to suppress symptomatic perception of and counterregulatory hormone responses to hypoglycemia, while preserving cognitive function, possibly because lactate provides the brain with an alternative fuel. We therefore hypothesized that a high-intensity interval training (HIIT) could mediate a suppressive effect on awareness of subsequent hypoglycemia.

Methods: In a randomized cross-over trial, healthy controls, T1DM patients with normal awareness of hypoglycemia (NAH) and T1DM patients with impaired awareness of hypoglycemia (IAH) (n=10 per group) underwent a hyperinsulinemic hypoglycemic (nadir, 2.6mmol/L) clamp, either after HIIT or after an equivalent rest period. HIIT consisted of three 30-second all-out sprints interspersed with 4 min active recovery. Counterregulatory hormones, hypoglycemic symptoms and cognitive function were measured at regular intervals.

Results: HIIT markedly increased plasma lactate levels (from 1.2 ± 0.1 to 13.1 ± 0.5 mmol/L), which remained elevated during subsequent hypoglycemia. Compared to rest, HIIT suppressed hypoglycemic symptoms in patients with NAH (25.3 ± 4.0 vs. 17.5 ± 3.3 , $p=0.01$) and numerically, but not significantly in healthy controls (17.1 ± 3.5 vs. 14.1 ± 2.2 , $p=0.19$). HIIT did not affect symptom scores in patients with IAH (7.2 ± 0.9 vs. 8.0 ± 1.5 , $p=0.80$). HIIT also suppressed cortisol and growth hormone responses to hypoglycemia, but had no effect on catecholamine responses. The decline in cognitive function during hypoglycemia was less after HIIT than rest in patients with NAH (Z-scores: -1.9 ± 0.5 vs. -3.2 ± 0.6 , $p=0.04$), but did not change significantly in healthy controls or patients with IAH.

Discussion/Conclusion: A short bout of high-intensity interval exercise is able to rapidly blunt defenses against hypoglycemia in type 1 diabetes patients with normal awareness of hypoglycemia, which may increase the risk of post-exercise hypoglycemia. Effects may be mediated by exercise-induced release of lactate, which can provide an alternative fuel for the brain when glucose supply is low.

28. The diurnal rhythm of adipose tissue gene expression is reduced in patients with type 2 diabetes

Dirk J. Stenvers^{1*}, Aldo Jongejan¹, Sadaf Atiqi¹, Jeroen P. Vreijling¹, Eelkje J. Limonard¹, Erik Endert¹, Frank Baas¹, Perry D. Moerland¹, Eric Fliers¹, Andries Kalsbeek^{1,2}, Peter H. Bisschop¹

1. Academic Medical Center (AMC), University of Amsterdam

2. Netherlands Institute for Neuroscience (NIN)

Animal studies have shown that genetic modification of the molecular clock can induce metabolic syndrome, and that increasing clock rhythm amplitude with the small molecule nobiletin is an effective treatment for obesity and type 2 diabetes. There is no evidence so far of altered molecular clock rhythms in humans with the metabolic syndrome.

Therefore we compared diurnal mRNA expression profiles in subcutaneous adipose tissue, between obese patients with type 2 diabetes and age-matched healthy lean control subjects, using RNA sequencing. We also assessed the diurnal rhythm in postprandial glucose tolerance. All subjects received three identical mixed meals per day at evenly spaced time points.

In patients, 1.8% (303 transcripts) of expressed transcripts showed time dependent oscillations, compared to 8.4% (1421 transcripts) in healthy subjects. The core clock genes showed reduced amplitude oscillations in patients compared to healthy subjects. 184 genes showed a rhythm in both groups, and enriched canonical pathways for these common rhythmic genes included *circadian rhythm signaling* and *adipogenesis*. Enriched rhythmic pathways in controls only (i.e. with a loss of rhythmic enrichment in patients) included *AMPK signaling*. Furthermore, patients with type 2 diabetes showed a reduced diurnal rhythm in postprandial glucose excursions.

In conclusion, we provide the first evidence of decreased diurnal gene expression rhythms in subcutaneous adipose tissue of obese patients with type 2 diabetes.

Last name	Surname	e-mail
Akintola	Abimbola	a.a.akintola@lumc.nl
Akker van den	Erica	e.l.t.vandenakker@erasmusmc.nl
Bahler	Lonneke	l.bahler@amc.nl
Berkel van	Anouk	Anouk.vanBerkel@radboudumc.nl
Bilsen	Manu	m.p.bilsen@lumc.nl
Boertien	Tessel	t.m.boertien@amc.nl
Boon	Mariette	m.r.boon@lumc.nl
Bos	Maxime	m.m.Bos@lumc.nl
Dirks	Niek	n.dirks@vumc.nl
Dorenbos	Elke	elke.dorenbos@mumc.nl
Ellenbroek	Ellen	jhellenbroek@gmail.com
Fliedner	Stephanie	stephanie.fliedner@uksh.de
Heinen	Charlotte	c.a.heinen@amc.uva.nl
Held	Ntsiki	n.m.held@amc.uva.nl
Janssen	Laura	L.G.M.Janssen@lumc.nl
Kaikaew	Kasiphak	k.kaikaew@erasmusmc.nl
Karnebeek	Kylie	kylie.karnebeek@mumc.nl
Koorneef	Lisa	L.L.Koorneef@lumc.nl
Links	Thera	t.p.links@umcg.nl
Loon van	Nienke	n.m.vanloon@amc.uva.nl
Muhammed	Ammar	a.muhammad.1@erasmusmc.nl
Nauta	Lucres	l.nauta@debascule.com
Nijhoff	Michiel	m.f.nijhoff@lumc.nl
Osinga	Thamara	t.osinga@umcg.nl
Peters	Toine	t.pieters@uu.nl
Rao	Dipti	diptirao@live.nl
Rooijackers	Hanne	Hanne.Rooijackers@radboudumc.nl
Rotman	Marlous	m.rotman@lumc.nl
Schilderpoort	Maaïke	m.schilperoort@lumc.nl
Snaterse	Gido	g.snaterse@erasmusmc.nl
Spoel van der	Evie	E.van_der_Spoel@lumc.nl
Ter Laak	Marjolein	marjolein.terLaak@radboudumc.nl
Thaiss	Christoph	christoph.thaiss@weizmann.ac.il
Toorop	Alyssa	alyssatorop@hotmail.com
Uijtbeijerse	Bas	B.S.Uitbeijerse@lumc.nl
Ursem	Stan	s.ursem@vumc.nl
Valkema	Pieter	pavalkema@gmail.com
Vervloet	Marc G	m.vervloet@vumc.nl
Vlot	Mariska	m.vlot@vumc.nl
Vonk-Noordegraaf	Magreet	m.vonknoordegraaf@erasmusmc.nl
Weert van	Lisa	L.T.C.M.van_Weert@lumc.nl
Wolters	Thalijn	thalijn.wolters@radboudumc.nl
Zandee	Wouter	w.zandee@erasmusmc.nl

