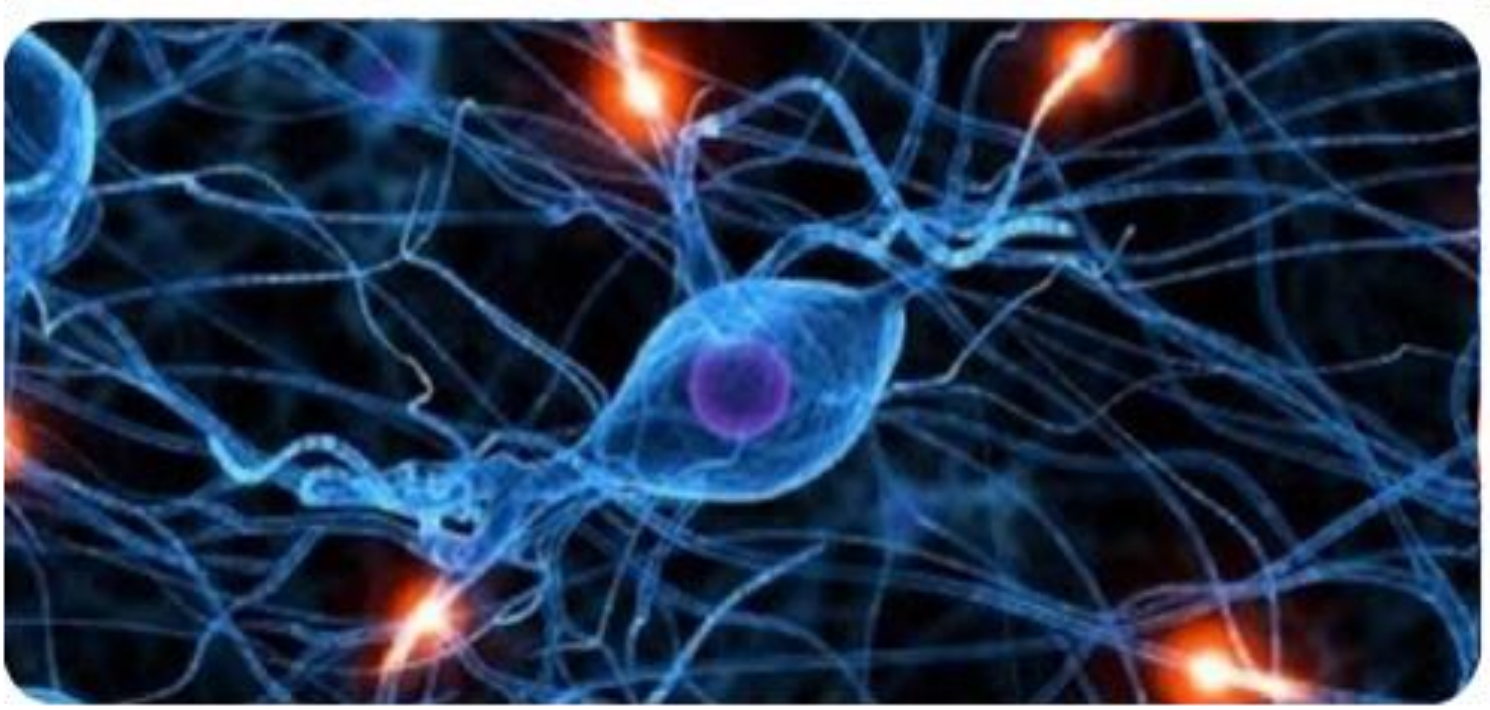
2nd JNVE conference

October 29 and 30, 2015

Leiden



JNVE
JONGE NEDERLANDSE VERENIGING
VOOR ENDOCRINOLOGIE

This meeting is kindly sponsored by the Dutch Endocrine Society (NVE) and the European Society of Endocrinology who has provided financial support through an ESE Small Meeting Grant.

JNVE meeting 2015

Table of contents

Introduction

Dear colleagues,

Welcome at the second 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 and presentations of 34 young endocrine researchers and clinicians from all over the Netherlands. We also welcome two colleagues from Poland who have come to share some of their 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 and specifically this 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: ENGIOl 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 two members of our polish counterpart Klub 30 will present their 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 2015 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 JVNE torch.

The JNVE-board

Mariëtte Boon (MUMC, Maastricht and LUMC, Leiden), *main organizer JNVE 2015 meeting*
Rahel Büttler (VUMC, Amsterdam), *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)

Program JNVE meeting 2015

Day 1: Thursday 29th October 2015

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 Hotel Leiden

13.00 **Opening and Introduction**
Rahel Büttler (Chair JNVE)
Mariëtte Boon (main organizer JNVE 2015 meeting)
Anneke van den Beukel (Board member European Young Endocrine Scientists (EYES))

13.30 **Invited lecture 1: Circadian Control of hormone rhythms**
Prof. Dr. Andries Kalsbeek *(AMC, Amsterdam)*
Chair: Angela Sarabdjitsingh *(UMC Utrecht, Utrecht)*

14.15 **Delegate session 1: Biological clock and HPA axis hormones**
Chairs: Angela Sarabdjitsingh *(UMC Utrecht, Utrecht)* and Mariëtte Boon *(LUMC, Leiden)*

15.30 **Tea and coffee**

16.00 **Interactive seminar: The value of the P-value**
Dr. Olaf Dekkers *(LUMC, Leiden)*
Chair: Michiel Nijhoff *(LUMC, Leiden)*

16.45 **Delegate Session 2**: **Growth, bone and sex hormones**
Chairs: Rahel Büttler *(VUMC, Amsterdam)* and Anneke van den Beukel *(Erasmus MC, Rotterdam)*

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

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

21.00 **Social evening program and party at 'Haarlem' venue**

Delegate Session 1:

Biological clock and HPA axis hormones

Chair: Angela Sarabdjitsingh *(UMC Utrecht, Utrecht)* and Mariëtte Boon *(LUMC, Leiden)*

1. **Diurnal rhythmicity of cortisol and cortisone in breast milk.**
   Bibian Metselaar- Van der Voorn et al. *(VUMC, Amsterdam)*

2. **A diurnal rhythm of cardiac troponin: consequences for the diagnosis of acute myocardial infarction**
   Lieke Klinkenberg et al. *(MUMC, Maastricht)*

3. **Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity**
   Dirk van Moorsel et al. *(MUMC, Maastricht*)

4. **Biological clock strongly regulates brown adipose tissue activity: implications for postprandial triglyceride metabolism**
   Rosa van den Berg et al. *(LUMC, Leiden)*

5. **Hitting the brake and the gas at the same time; measuring endogenous cortisol production with LCMS during glucocorticoid therapy**
   Jeroen van den Wijngaard et al. *(LUMC, Leiden)*

6. **A cis-element that is associated with selectivity for mineralocorticoid over glucocorticoid receptor binding in the brain**
   Lisa van Weert et al. *(LUMC, Leiden)*

7. **Reducing unnecessary low dose ACTH stimulation testing in an outpatient setting; an assessment of basal cortisol cut-off values and other predictive variables**
   Frank Perton et al. *(Isala, Zwolle)*

Delegate Session 2:

Growth, bone and sex hormones

Chair: Rahel Büttler *(VUMC, Amsterdam)* and Anneke van den Beukel *(Erasmus MC, Rotterdam)*

8. **How does living in plastic world influence women's hormonal profile?**
   Aleksandra Szybiak et al. *(Medical University of Gdansk, Poland)*

9. **Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers and BMAD in transgender adolescents.**
   Mariska Vlot et al. *(VUMC, Amsterdam)*

10. **Associations of vitamin D status and vitamin D-related polymorphisms with sex hormones in older men**
    Rachida Rafiq et al. *(VUMC, Amsterdam*)

11. **Growth patterns in very preterm and/or very low birth weight infants: an analysis from birth to adolescence.**
    Jonneke Hollanders et al. *(VUMC, Amsterdam)*

12. **Growth hormone in human familial longevity**
    Evie van der Spoel et al. *(LUMC, Leiden)*

13. **Genetic analysis of IRF6, a gene involved in craniofacial midline formation, in relation to pituitary and facial morphology of patients with idiopathic growth hormone deficiency**
    Laura de Graaff-Herder et al. *(Erasmus MC, Rotterdam)*

14. **Association between bone marrow adiposity and bone turnover before and after raloxifene.**
    Kerensa Beekman et al. *(VUMC, Amsterdam)*

Program JNVE meeting 2015

Day 2: Friday 30th October 2015

Lectures at 'Amsterdam' venue at Holiday Inn Leiden

8.30 **Invited lecture 2: Translational research in thyroid hormone regulation**
Dr. Edward Visser *(Erasmus MC, Rotterdam)*
Chairs: Michiel Nijhoff *(LUMC, Leiden)* and Angela Sarabdjitsingh *(UMC Utrecht, Utrecht)*

9.15 **Delegate Session 3: Diabetes**
Chairs: Mariëtte Boon *(LUMC, Leiden)* and Thamara Osinga *(UMCG, Groningen)*

10.30 **Coffee and tea**

10.45 **Invited lecture 3: Brown adipose tissue and metabolism**
Prof. dr. Wouter van Marken Lichtenbelt *(Maastricht University*)
Chair: Mariëtte Boon *(LUMC, Leiden)*

11.30 **Delegate Session 4: Metabolism and brown fat**
Chairs: Anneke van den Beukel *(Erasmus MC, Rotterdam)* and Lonneke Bähler *(AMC, Amsterdam)*

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

13.30 **Invited lecture 4: Androgens and estrogens: how a very similar structure makes a huge difference. Lessons from the trangener clinic**
Prof. dr. Martin den Heijer (*VUMC, Amsterdam*)
Chair: Rahel Büttler *(VUMC, Amsterdam)*

14.15 **Delegate Session 5: (Neuro)endocrine tumors**
Chairs: Michiel Nijhoff *(LUMC, Leiden)* and Thamara Osinga *(UMCG, Groningen)*

15.30 **Coffee and tea**

15.50 **Introducing BijnierNet: Patient-Provider network in The Netherlands**
Dr. Lisanne Smans *(UMCU, Utrecht)*
Chair: Thamara Osinga *(UMCG, Groningen)*

16.20 **Evaluation and farewell**

Delegate Session 3:

Diabetes

Chairs: Mariëtte Boon *(LUMC, Leiden)* and Thamara Osinga *(UMCG, Groningen)*

15. Predictors of need for insulin therapy in gestational diabetes mellitus
Sarah Koning et al. *(UMCG, Groningen)*

16. Clinical and lifestyle factors associated with skin autofluorescence in a population-based cohort study
Robert van Waateringe et al. *(UMCG, Groningen)*

17. Accuracy of continuous glucose monitoring measurements in normo-glycemic individuals
Abimbola Akintola et al. *(LUMC, Leiden)*

18. Sleep efficiency as a determinant of insulin sensitivity in overweight and obese adolescents
Elke Dorenbos et al. *(UMCG, Groningen)*

19. Hypoglycemia unawareness in an otherwise healthy 71 year old men.
Hester van der Valk et al. *(Isala, Zwolle)*

Delegate Session 4:

Metabolism and brown fat

Chairs: Anneke van den Beukel *(Erasmus MC, Rotterdam)* and Lonneke Bähler *(AMC, Amsterdam)*

20. **The effect of sitagliptin on brown adipose tissue and whole-body metabolism in overweight pre-diabetic men**
    Kimberly Nahon et al. *(LUMC, Leiden)*

21. **Fecal microbiota transplantation used to improve postprandial bacterial translocation; the RALSTONIA study**
    Guido Bakker et al. *(AMC, Amsterdam)*

22. **Children with morbid obesity benefit equally as children with overweight and obesity from an ongoing care program**
    Jesse Rijks et al. *(MUMC, Maastricht)*

23. **Brown fat activation enhances the lipid-lowering effect of statin treatment in APOE*3-Leiden.CETP mice**
    Geerte Hoeke et al. *(LUMC, Leiden)*

24. **The role of mitochondrial quality control in brown adipose tissue metabolism**
    Ntsiki Held et al. *(AMC, Amsterdam)*

25. **BCG vaccine lowers plasma cholesterol levels and atherosclerosis development in mice**
    Andrea van Dam et al. *(LUMC, Leiden)*

26. **The effect of short term high fat diet on mitochondrial function in brown adipose tissue**
    Eline Kuipers et al. *(LUMC, Leiden)*

27. **Leptin resistance in high-fat high-sucrose diet induced obesity: is there a role for hypothalamic inflammation?**
    Kathy de Git et al. *(UMCU, Utrecht)*

Delegate Session 5:

(Neuro)endocrine tumors

Chairs: Michiel Nijhoff *(LUMC, Leiden)* and Thamara Osinga *(UMCG, Groningen)*

28. Silencing of Tumor Suppressor Genes by gene promoter methylation – search for biomarkers differentiating follicular-cell derived thyroid tumors
Karolina Czarnecka et al. *(Medical University of Lodz, Poland)*

28. Thyroid carcinoma in families with DICER1 germline mutations
Karin van der Tuin et al. *(LUMC, Leiden)*

29. Characterization of the hyperparathyroid-jaw tumour (HPT-JT) syndrome in eight large Dutch kindreds
Karin van der Tuin et al. *(LUMC, Leiden)*

30. New CgA assay of Thermo Fischer in the monitoring of NET patient
Roseri Roelofsen- de Beer et al. *(Erasmus MC, Rotterdam)*

31. Multiple Endocrine Neoplasia type 1 redefined: a clinical comparison of mutation positive and mutation negative patients
Marieke de Laat et al. *(UMCU, Utrecht)*

32. No Association of Blood Type O with Neuroendocrine Tumors in Multiple Endocrine Neoplasia Type 1.
Rachel van Leeuwaarde et al. *(UMCU, Utrecht)*

Contact information:

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

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Organizing Committee

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Thamara Osinga	UMCG	t.osinga@umcg.nl

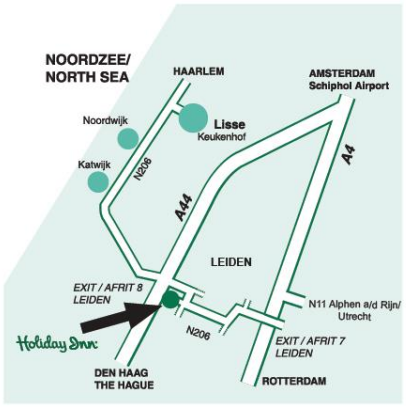
If there are any questions during the conference please contact:

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 (afslag 8). The hotel has its own parking and this can be freely used for guests. As a hotel guest, please temporarily park your car before the hotel when you arrive; you will receive a pass to open the gate during check-in. When you are not a hotel guest, you have to draw a ticket at the gate which you can freely exchange at the end of the day at the reception.

Public transport
From Leiden Central Station, you can take bus 43 in the direction of The Hague and take the stop ‘Holiday Inn’ (about 10 min drive).

Exchange in Endocrinology Expertise

Novo Nordisk, Novartis & the Section/Board of Endocrinology of the UEMS

In order to facilitate within Europe the exchange of trainees specialising in adult Endocrinology and to harmonise and improve the training of endocrinologists, the Section/Board of Endocrinology of the UEMS and Novo Nordisk A/S and Novartis have set up a fellowship program called '3E', the Exchange in Endocrinology Expertise.

The Exchange in Endocrinology Expertise program will support candidates for an assignment in a leading centre in Endocrinology, Diabetes and Metabolism in Europe. The task will include both clinical practice and research.

Depending on the requirement of each individual project the duration of assignment may last up to six months. Whenever possible, the assignment should include both clinical practice and research. Publication should be seeked, and if not relevant a report has to be submitted at the end.

The maximum support is EUR 18.000 for six months or the respective aliquot for shorter exchange periods.

The selection of the candidates will be done by a Committee from the Section/Board of Endocrinology.

The criteria for a candidate to be selected are the following:

- Education: minimum of 1 year in the specialty of endocrinology
- At least 1 publication in a peer reviewed journal
- Recommendation from the head of the department
- Language: incompatibility of language should be avoided
    - The UEMS Committee will evaluate case by case if knowledge of the receiving centre language is necessary
- Motivation proven with a **specific project proposal** (4-5 pages) including a proposal of centre and feasibility letter from the receiving centre

The country of origin as well as the receiving centre might be any of the countries represented in the UEMS Section/Board of Endocrinology.

The budget for the fellowship is limited to 18.000 EUR and can be used to cover salary, travel expenses (one return trip) and potential research expenses like bench fees.

The fellowship applications should be sent to the following address before November 27th, 2015:

Prof. Anton Luger, MD
President, UEMS Section/Board of Endocrinology
Division of Endocrinology and Metabolism
Department of Medicine III
Medical University and General Hospital of Vienna
Waehringer Guertel 18-20
1090 Vienna
Austria
Email: anton.luger@meduniwien.ac.at

Vienna, September 2015

Prof. Anton Luger, MD
President Section/Board of Endocrinology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1. Diurnal rhythmicity of cortisol and cortisone in breast milk

Bibian Metselaar - van der Voorn, Annemieke C. Heijboer , Marita A. de Waard, Hans B. van Goudoever, Joost Rotteveel and Martijn J.J. Finken

Background: Serum cortisol is higher in breast-fed than in formula-fed infants. This might be attributed to cortisol in breast milk. Milk glucocorticoids are suggested to influence the infant. Diurnal rhythmicity in breast milk glucocorticoids has, however, never been tested.
Objective and hypotheses: Our aim was to assess diurnal variation in cortisol and cortisone levels in breast milk and to compare this with maternal salivary levels. We expected that milk glucocorticoid levels follow a diurnal rhythm, reflecting maternal hormonal production.
Method: Ten paired breast milk and saliva samples from ten healthy mothers were obtained over a 24-hr period in the fourth week postpartum. After hexane extraction, cortisol and cortisone concentrations were measured by our recently developed LC-MS/MS method. Correlations between milk and saliva cortisol and cortisone concentrations, as well as the influence of time of collection, were analyzed by generalized estimating equations and presented as beta (95% CI).
Results: Milk and salivary glucocorticoid concentrations were correlated ($p \leq 0.001$): 0.86(0.79–0.95) for cortisol and 0.74(0.71–0.78) for cortisone. Milk cortisol and cortisone concentrations were dependent on the time of collection ($p \leq 0.001$): 0.92(0.88–0.96) and 0.96(0.93 – 0.98), respectively, with a peak around 7am. Peak cortisol and cortisone levels in breast milk were 10.3 (interquartile range:6.7–17.5) nmol/L and 38.6(33.1–41.5) nmol/L, respectively, between 6am–8am.
Conclusion: Breast milk cortisol and cortisone concentrations follow a diurnal rhythm, with a peak around 7am. Concentrations of cortisol and cortisone in breast milk and saliva were strongly correlated, with cortisone being higher than cortisol in both fluids. The results of this study have implications for the timing of collection of breast milk in future studies and for the interpretation of reported concentrations by previous studies. We are now exploring whether circadian variation in breast milk glucocorticoids have the potential to exert biologically relevant effects on the developing newborn.

2. A diurnal rhythm of cardiac troponin: consequences for the diagnosis of acute myocardial infarction

Lieke J.J. Klinkenberg#, MSc; Karin Wildi#, MD; Noreen van der Linden, MD; Imre W. K. Kouw, MSc; Marijke Niens, PhD; Raphael Twerenbold, MD; Maria Rubini Gimenez, MD; Christian Puelacher, MD; Jean Daniel Neuhaus, MD; Petra Hillinger, MD; Thomas Nestelberger, MD; Jasper Boeddinghaus, MD; Karin Grimm, MD; Jeroen D.E. van Suijlen, PhD; Frans E.S. Tan, PhD;
Joop ten Kate, PhD; Otto Bekers, PhD; Luc J.C. van Loon, PhD; Marja P. van Dieijen-Visser, PhD; Christian Mueller*, MD PhD; Steven J.R. Meex*, PhD

#Both authors contributed equally
*Both research groups contributed equally

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Department of Human Movement Sciences, School for Nutrition, Toxicology and Metabolism (NUTRIM), Maastricht University Medical Center (MUMC), Maastricht, the Netherlands (I.K., L.v.L.)
Department of Clinical Chemistry and Laboratory Hematology, Gelre ziekenhuizen, Apeldoorn/Zutphen, the Netherlands (M.N, J.v.S.).
Department of Methodology and Statistics, CAPHRI School for Public Health and Primary Care. Maastricht University, Maastricht, the Netherlands (F.T.).
Department of Clinical Chemistry and Hematology, Orbis Medical Center, Sittard-Geleen, the Netherlands (J.t.K.).

Background: Interpretation of serial high-sensitivity troponin (hs-cTn) measurements for the diagnosis of acute myocardial infarction (AMI) assumes random fluctuation of hs-cTn around an individual's homeostatic set-point.
Methods and Results: Two studies were conducted to challenge this diagnostic concept: Study-1 examined the presence of a diurnal hs-cTn rhythm by hourly blood sampling, day and night, in 24 individuals without a recent history of AMI. Study-2 assessed morning versus evening diagnostic accuracy of hs-cTnT and hs-cTnI in a prospective multicenter diagnostic study of 2782 unselected patients, presenting to the emergency department with acute chest pain. The final diagnosis was adjudicated by two independent cardiologists. In study-1, hs-cTnT, but not hs-cTnI, exhibited a diurnal rhythm, characterized by gradually decreasing concentrations throughout daytime, rising concentrations during nighttime, to peak concentrations in the morning (mean 16.2ng/L at 8:30A.M and 12.1ng/L at 7:30P.M.). In study-2, the hs-cTnT rhythm was confirmed by significantly higher hs-cTnT levels in early-morning presenters compared to evening presenters with an adjudicated diagnosis of non-cardiac disease. The diagnostic accuracy (area under the receiver-operator characteristic curve (AUC)) of hs-cTnT at presentation, 1hour, and for the combination of absolute changes with presenting concentration, were very high and comparable among patients presenting early-morning as compared to evening (all AUC>0.93). Hs-cTnI exhibited no diurnal rhythm with no differences in AUC among early-morning and evening presenters.
Conclusion: Rhythmic diurnal variation of hs-cTnT is a general phenomenon that is not seen with hs-cTnI. The diurnal hs-cTnT rhythm does not seem to affect the diagnostic accuracy of hs-cTnT for AMI.

3. Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity

Dirk van Moorsel[1,4], Jan Hansen[1], Bas Havekes[1,4], Frank Scheer[2], Bart Staels[3], Helene Duez[3], Matthijs Hesselink[1], Nicolaas Schaper[4], Patrick Schrauwen[1]

[1] Department of Human Biology and Movement Sciences, Maastricht University Medical Center, Maastricht, The Netherlands. [2] Division of Sleep Medicine, Harvard Medical School, Boston, MA, USA. [3] Institut Pasteur de Lille, Lille, France. [4] Department of Internal Medicine, Division of Endocrinology, Maastricht University Medical Center, Maastricht, The Netherlands.

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Background: Many processes in our body are governed by an endogenous circadian rhythm. However, in our modern "24/7" society, many individuals do not adhere to the day-night lifestyle imposed by nature. This circadian disruption is associated with many unfavorable effects such as obesity and type 2 diabetes mellitus. Since skeletal muscle mitochondrial function is a major determinant of insulin sensitivity, a disturbance in the day-night rhythm of skeletal muscle mitochondrial function could explain several the adverse metabolic consequences of circadian disruption. However, so far it is unknown if this mitochondrial function exhibits a day-night rhythm in humans.
Methods: Twelve healthy young male individuals (22.2±2.3 years) with a normal day-night rhythm were subjected to standardized meals (based on energy requirements) and physical activity. Five skeletal muscle biopsies were taken from the quadriceps muscle, each five hours apart, for measurement of ex vivo oxidative capacity by respirometry. Additionally, we measured energy expenditure and core body temperature.
Results: Core body temperature was lower during the early night, demonstrating a normal day-night rhythm. Skeletal muscle oxidative capacity exhibited profound differences over a 24h period ($p<0.05$, ANOVA). Mean ADP-driven coupled respiration was lowest at 1PM and highest at 23PM (80.6±4.0 vs. 95.8±4.7 pmol/mg/s). Resting energy expenditure was highest at 23PM and lowest at 4AM ($p<0.001$).
Discussion: Skeletal muscle oxidative capacity demonstrates a day-night rhythm in humans. Whether this rhythm is mainly caused by behavioral or circadian influences, or why the oxidative capacity is highest around midnight remain important questions to be addressed.

4. Biological clock strongly regulates brown adipose tissue activity: implications for postprandial triglyceride metabolism

Rosa van den Berg[1,2], Sander Kooijman[1,2], Ashna Ramkisoensing[3], Claudia P. Coomans[3], Johanna H. Meijer[3], Nienke R. Biermasz[1,2] and Patrick C.N. Rensen[1,2]

[1]Dept. Medicine, Div. Endocrinology, [2]Einthoven Laboratory for Experimental Vascular Medicine, and [3]Dept. Molecular Cell Biology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

Background: Brown adipose tissue is an attractive anti-dyslipidemia target, due to its capacity to burn high amounts of triglycerides (TG) into heat. In humans, plasma TG levels display 24 h variation independent of food intake, indicating a strong regulation by the biological clock. The main input for the biological clock is day light. Interestingly, we recently showed that prolonged day length decreases the ability of BAT to take up TG (Kooijman & Van den Berg, PNAS USA 2015). The present study aimed to investigate the 24 h rhythm of BAT activity, the effect of day length thereon, and the consequences for postprandial TG metabolism.
Methods and Results: We first determined the 24 h rhythm of BAT activity, and the effect of day length thereon. Male C57Bl/6J mice were exposed to a day length of 8 h (short), 12 h (normal), or 16 h (long). After 5 weeks, 24 h rhythm of BAT activity was determined by assessing its capacity to take up TG-derived fatty acids (FA) from plasma at Zeitgeber time points (ZT; h after lights on) 0, 4, 6, 8, 12 and 18. Hereto, fasted mice were injected with glycerol tri[3H]oleate-labeled VLDL-like particles and after 15 minutes various organs were taken out to determine uptake of [3H]oleate (FA). Mice exposed to normal day length displayed a remarkable diurnal pattern of [3H]FA uptake by BAT, but not by other metabolically active tissues. The peak [3H]FA uptake by BAT was reached at the onset of the dark (ZT12). Strikingly, short day length increased the amplitude of [3H]FA uptake by BAT by ~3-fold. Moreover, short day length advanced the peak of [3H]FA uptake, again to the onset of dark (ZT8). Conversely, long day length delayed the peak to onset of dark. Since BAT has a high capacity to take up TG-derived FA from plasma, we hypothesized that BAT rhythmicity would affect postprandial TG clearance. Therefore, we investigated the effect of short and long day length on postprandial TG clearance in dyslipidemic mice at different time points. To this end, APOE*3-Leiden.CETP female mice fed a western-type diet were exposed to either 8 h (short) or 16 h (long) day length. After 4 weeks, fasted mice were gavaged with olive oil at time points ZT0, ZT8 or ZT16 and plasma TG levels were measured after 0, 1, 2, 4, and 8 h. The postprandial TG excursion was quantified by calculating the area under the curve. Postprandial TG excursion displayed a ~3-fold difference between ZT0, ZT8 and ZT16. Strikingly, postprandial TG excursion was lowest before the onset of dark and highest before onset of light in both short and long day length. We next investigated the TG-derived [3H]FA uptake by BAT at ZT0, ZT8 and ZT16. Remarkably, [3H]FA uptake by BAT showed a strong negative correlation to the postprandial TG plasma levels ($p<0.005$).
Conclusion: This study is the first to show that day length dictates a diurnal rhythm in TG-derived FA uptake capacity of BAT, thereby determining postprandial TG metabolism. We propose that the diurnal variations in TG levels observed in humans may be explained by a diurnal rhythm in BAT activity.

5. Hitting the brake and the gas at the same time; measuring endogenous cortisol production with LCMS during glucocorticoid therapy

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Introduction: We present a case of rapid developing Cushing's syndrome as a consequence of adrenocortical carcinoma complicated by opportunistic pulmonary infection. A 35 year old female patient with Cushingoid appearance presented with dyspnea, pleural effusion and complaints of severe muscle weakness. Patient history further revealed hypertension, palpitations and weight loss. Laboratory analysis showed high serum cortisol (1,575 µmol/L, immunoassay on Roche Modular E170). CT-thorax revealed ground glass lesions in the lungs and a large left adrenal mass with pulmonary, hepatogenous, osseous and lymphoid metastases. Pneumocystis Jirovecii Pneumonia (PJP) was suspected. She was admitted to the ICU because of respiratory failure.
Methods: Immediate treatment was started, including metyrapon and etomidate, to block endogenous cortisol synthesis and cotrimoxazol for PJP. Following PJP confirmation, prednisone therapy commenced. To adequately monitor endogenous cortisol suppression while simultaneously supplementing prednisone, 24h urine collections were gathered and analyzed for free cortisol with selective Liquid Chromatography Mass Spectrometry (LCMS).
Results: At hospitalization, serum cortisol and urine free cortisol with LCMS, were strongly elevated at 1575 µmol/L (0,1–0,6 µmol/L) and 790 nmol/24h (ref 0-150 nmol/24h) respectively. During metyrapon/etomidate and prednisone therapy, urine cortisol measured with LCMS was in the normal range, whereas immunoassay-based serum cortisol remained elevated due to prednisol cross-reactivity. Notwithstanding adequate cortisol suppression, the patient's condition deteriorated further and the patient died.
Conclusion: We present a case with simultaneous treatment of the cortisol excess during supplementation of glucocorticoids. Whereas immunoassays exhibit interference from glucocorticoid therapy, LCMS does not and indicates adequate suppression of endogenous cortisol production.

6. A cis-element that is associated with selectivity for mineralocorticoid over glucocorticoid receptor binding in the brain

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Introduction: In the limbic brain, mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) both function as receptors for glucocorticoids, cortisol and corticosterone. MR and GR mediate distinct effects on cellular physiology via transcriptional mechanisms, but how target gene specificity comes about has remained enigmatic. We have identified DNA binding sites of MR and GR in the rat hippocampus, using chromatin immunoprecipitation followed by sequencing (ChIP-Seq) in order to identify the extent of MR/GR binding selectivity and underlying mechanisms.
Methods: To study MR and GR binding to the genome, adrenalectomized rats were injected IP with corticosterone (3 mg/kg) and sacrificed 60 min later. Hippocampi were freshly isolated, formaldehyde-fixated, chromatin was sheared and ChIP was performed with MR/GR antibodies. Samples were sequenced single end 35bp on an Illumina Genome Analyzer and reads were mapped on the reference genome (rn4) by Burrows-Wheeler Aligner. Peaks were identified with MACS. Overlap of binding sites was calculated with HOMER. De novo motif analysis was performed using MEME.
Results: In the ChIP-Seq dataset 1465 MR and 2460 GR binding sites were found in total, of which respectively 606 and 1450 sites were non-overlapping between MR and GR. De novo motif analysis of binding sites that were selective for either MR or GR resulted in a similar 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 or MR-GR overlapping sites. This motif also matched a known transcription factor consensus sequence and we identified a brain-specific family member showing hippocampal expression.
Conclusion: An additional motif near the GRE seems to drive specificity for MR binding at a fraction of the identified binding sites. This motif was not found around MR-GR overlapping target sites, pointing to GR exclusion rather than preferential MR recruitment. The data suggest that interactions between GR and the transcription factor(s) that bind to the identified motif result in MR selective signaling in the limbic brain..

7. Reducing unnecessary low dose ACTH stimulation testing in an outpatient setting; an assessment of basal cortisol cut-off values and other predictive variables.

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Objective: to find new guidelines for diagnosing adrenal insufficiency in order to reduce the number of low dose short Synacthen tests (LD-SST's) performed in an outpatient setting, firstly by determining optimal cut-off values for screening basal cortisol tests and secondly by analysing several variables for their role in predicting the outcome of the LD-SST.
Materials and methods: Optimal basal cortisol cut-off values were determined by ROC analysis of 145 outpatient LD-SST's. Subsequently the predictive value of several variables were analysed using univariable and multivariable logistic regression analysis. In a second study the predictive variables were analysed in a larger group consisting 329 outpatient LD-SST's, in which also tests without a known basal cortisol value were included.
Results: A basal cortisol lower cut-off value of 145 nmol/L (specificity 89.9%, positive predictive value 90.0%) and an upper cut-off value of 375 nmol/L (sensitivity 100%, negative predictive value 100%) were found. Chronic fatigue symptoms and symptoms of hypotension/ orthostasis as an indication for performing the test predicted a normal outcome of the LD-SST. The use of glucocorticosteroïds as a dichotomous variable predicted an abnormal outcome of the test. Oral, topical, nasal and inhaled glucocorticosteroïds are each significant predictors when analysed specifically for predicting the diagnosis central adrenal insufficiency.
Conclusions: By using basal cortisol cut-off values of 145 nmol/L and 375 nmol/L instead of the conventional cut-off values of 100 nmol/L and 500 nmol/L, the number of performed outpatient LD-SST's can be reduced by 12% maintaining high sensitivity and specificity. An abnormal LD-SST outcome is less likely when the main indications for performing the test are chronic fatigue symptoms or symptoms of hypotension/orthostasis. An abnormal outcome of the test can be predicted by the use of glucocorticosteroïds. Moreover, different forms of administration of glucocorticosteroïds such as inhalation or topical use should be taken into account when central insufficiency is suspected.

8. How does living in plastic world influence women's hormonal profile?

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In everyday life we are exposed to plastics containing many chemicals, among which are bisphenol A (BPA) and the esters of the phthalic acid referred to as phthalates (PAE). These plasticizers are commonly used in the production of plastic packages, including the food ones, bottles, canned in which food is stored, enteric coatings of pharmaceutical pills, toys, electronic equipment, cosmetics, personal-care products, and various medical devices. Therefore humans are exposed to them daily, not only through dermal route but also an inhalation of the home dust and consumption of tainted food. PAE and BPA due to their phenolic structures belong to endocrine disrupting chemicals (EDCs) and may be involved in the pathogenesis of hormonal disturbances including the polycystic ovary syndrome (PCOS). PCOS is the most common endocrinopathy in women of reproductive age, inevitably leading to fertility problems, type 2 diabetes mellitus and coronary heart disease.
The aim of the study was to evaluate serum concentrations of selected plasticizers that are supposed to be EDCs in healthy women and women with PCOS and to estimate how increased exposure to plastic packaged and canned food influence on serum BPA concentrations.
Materials and methods: A total of 133 women of reproductive age with (n=56) and without (n=77) PCOS were included to studies. PCOS was diagnosed according to the ESHRE/ASRM criteria, healthy controls didn't present any endocrinopathies and additionally did not take any oral contraceptives. Fasting serum levels of prolactin, luteinizing hormone, follicle-stimulating hormone, 17OH-progesteron, total testosterone, dehydroepiandrosterone sulfate, insulin and sex hormone binding globulin were measured between 6th and 10th day of menstrual cycle. To estimate the effects of higher exposure to plasticizers on changes of BPA serum concentrations 20 women were selected from healthy controls (22-25 years old) and divided into two equal groups (study and control). As dietary sources are the main route of exposure to BPA, both groups applied special menu for one week, which increased or limited the exposure to BPA from canned foods and plastic packages, respectively. BPA was analysed in serum collected at baseline and after seven days of individual dietary intervention. All analyses of serum BPA and bisphenol S (BPS) concentrations were conducted using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Serum concentrations of PAE were analysed in biological samples using gas chromatography (GC) combined with mass spectrometry.
Results: BPS was detected in 35% of serum samples and BPA and PAE in 90% of samples, respectively. Serum BPA concentrations in women with PCOS were higher in comparison with healthy controls. The study has shown a strong positive correlation between serum concentrations of BPA and BPS with free androgen index and negative correlation with serum oestradiol level in PCOS women. The results of 7-day exposure of healthy women to food containers with BPA pointed the significantly twice fold increase in BPA levels in serum (57±57 ng/ml at baseline to 121±119 ng/ml, p=0.007). There were no differences in BPA serum concentrations in the group with limited BPA exposure from food (55±63 ng/ml vs. 48±48 ng/ml, p=0.74).
Conclusion: Plasticizers are detected in great majority of analysed samples what suggests widespread exposure to these EDCs among women. What is more, the higher exposure to plastic packaged and canned food, the higher concentrations of the plasticizers in serum. Their significant influence on the hormonal profile in women with PCOS confirms also their endocrine disrupting potential. Thus, further studies and increasing the consumers' awareness are necessary.

9. Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers and BMAD in transgender adolescents.

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Background: Puberty is important for the accumulation of bone mass. Postponing puberty by gonadotrophin-releasing hormone analogues (GnRHa), followed by treatment with cross sex hormone therapy (CSHT) in transgender adolescents can therefore affect bone turnover markers (BTMs) and bone mineral apparent density (BMAD) during treatment.
Objectives: To investigate the effect of GnRHa and CSHT on BTMs and BMAD during puberty in transgender adolescents.
Methods: 34 Female-to-males (FtMs) and 22 male-to-females (MtFs) were divided into a young and old pubertal group, based on the bone age of 14 years in FtMs and 15 years in MtFs respectively. All patients received the GnRHa triptorelin 3.75 mg every 4 weeks (D0). CSHT was prescribed in incremental doses from the age of 16 years (C0). FtMs received testosterone ester mixture 25 mg/m2 BSA intramuscularly every 2 weeks. MtFs were treated with 5 micrograms/kg 17-β estradiol orally every day. Bone formation markers P1NP and osteocalcin and bone resorption marker ICTP were measured at D0, C0 and C24 (24 months after C0). Also, BMD of lumbar spine and hip were measured by DEXA and BMAD and Z-scores were calculated according to natal sex.
Results: P1NP and 1CTP decreased during GnRHa treatment (D0-C0), indicating decreased bone turnover and remained low during CSHT (C0-C24). The highest levels of BTMs were observed in all the young pubertal groups and especially in the MtFs.
During GnRHa treatment (D0-C0) BMAD Z-scores of spine and hip either did not change or decreased. During CSHT (C0-C24) BMAD Z-scores increased, especially in the spine region. A low BMAD Z-score was more often observed in the MtFs.
Conclusion: Changes in BMAD Z-scores as a result of treatment with GnRHa and CSHT are not reflected by BTMs. Thus, repeated DEXA scans remain important for the follow-up of transgender adolescents.

10. Associations of vitamin D status and vitamin D-related polymorphisms with sex hormones in older men

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Objective: Evidence regarding relationships of serum 25-hydroxyvitamin D (25(OH)D) with sex hormones and gonadotropin concentrations remains inconsistent. Polymorphisms in vitamin D-related genes may underly these relationships. Our aim was to examine the relationship of vitamin D status and polymorphisms in vitamin D-related genes with sex hormone and gonadotropin levels.
Design and measurements: We analysed data from the Longitudinal Aging Study Amsterdam, an ongoing population-based cohort study of older Dutch individuals (65-89 years). We included data of men with measurements of serum 25-hydroxyvitamin D (25(OH)D) (n=643) and determination of vitamin D-related gene polymorphisms (n=465). 25(OH)D concentrations were classified into four categories: <25, 25-50, 50-75 and >75 nmol/L. Outcome measures were total testosterone, calculated bioavailable and free fraction testosterone, SHBG, estradiol, LH and FSH concentrations. Hypogonadism was defined as a total testosterone level <8.0 nmol/L.
Results: Serum 25(OH)D was positively associated with total and bioavailable testosterone levels. After adjustments for confounders, men with serum 25(OH)D less than 25 (n=56), 25-50 (n=199) and 50-75 nmol/L (n=240) had lower total testosterone levels compared to men with serum 25(OH)D higher than 75 nmol/L (n=148) (Bèta(95% confidence interval): -2.1(-3.7 to -0.4 nmol/L), -0.8(-1.9 to 0.4 nmol/L) and -1.4 (-2.4 to -0.3 nmol/L), respectively). For bioavailable testosterone the association was significant only for men with serum 25(OH)D less than 25 nmol/L (-0.8 (-1.4 to -0.1 nmol/L)) compared to men with serum 25(OH)D >75 nmol/L. Serum 25(OH)D was not related to SHBG, estradiol or gonadotropin levels. Hypogonadism was not associated with lower serum 25(OH)D. No significant differences were found in hormone levels between the different genotypes of the vitamin D-related gene polymorphisms. Also, the polymorphisms did not modify the relationships of serum 25(OH)D with sex hormones or gonadotropins.
Conclusion: Vitamin D status is positively associated with testosterone levels. No association was found between vitamin D-related gene polymorphisms and hormone levels.

11. Growth patterns in very preterm and/or very low birth weight infants: an analysis from birth to adolescence

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Introduction – At birth, preterm infants can be classified as very preterm (VP; i.e., <32 weeks) and/or very low birth weight (VLBW; i.e., <1,500 grams). Postnatal growth restriction followed by late catch-up growth is often observed in these infants. It is not known whether the growth patterns between birth and young adulthood of infants classified as VP and/or VLBW at birth are different.
Methods – This was a prospective study among subjects born VP and/or VLBW from the Project On Preterm and Small-for-gestational-age infants (POPS) cohort that comprised of 1,338 subjects. For our study, we used the data of all the infants who survived the initial hospital stay (n=998). Enrolled subjects were divided into three groups: 1) VP and VLBW (n=495), 2) VP but not VLBW (n=207) and 3) VLBW but not VP (n=296) infants. Weight, length/height and head circumference (HC) were measured at birth, at 3, 6, 12 and 24 months corrected age, and at 5 and 19 years. All parameters were expressed as standard deviations (SD). Growth from birth to 19 years was compared between the three groups using a generalized estimating equation (GEE).
Results – At birth, weight was -1.4±0.1, 0.1±0.1 and -4.6±0.1 SD, length was -1.2±0.2, 0.3±0.3 and -4.2±0.2 SD, and HC was -0.7±0.1, 0.2±0.1 and -2.3±0.2 SD in the VP/VLBW, VP and VLBW group respectively. Between birth and age 19, weight changed +0.7SD (95% confidence interval (CI): 0.6 to 0.9, P<0.001), -0.3SD (95% CI: -0.5 to -0.1, P=0.12) and +3.6SD (95% CI: 3.4 to 3.8, P<0.001), while length/height changed +0.4SD (95% CI: 0.2 to 0.7, P<0.001), -0.6SD (95% CI: -0.9 to -0.3, P<0.001) and +2.9SD (95% CI: 2.7 to 3.2, P<0.001) in the VP/VLBW, VP and VLBW group respectively. Between birth and age 5, HC changed +0.5SD (95% CI: 0.4 to 0.7, P<0.001), +0.1SD (95% CI: -0.1 to 0.3, P=0.300) and +1.7SD (95% CI: 1.5 to 1.8, P<0.001) in the VP/VLBW, VP and VLBW group respectively. Catch-up growth was most observed in the VLBW group, especially between birth and 3 months. At age 19, weight and height were still significantly different between the groups, while at age 5 HC was also significantly different.
Discussion – Infants born VP, with a VLBW or with both characteristics have significantly different growth patterns, reflecting the importance of the prenatal situation as well as an unequivocal classification system at birth for the description of growth until young adulthood in preterm born subjects. The terms VP and VLBW cannot be used interchangeably.

12. Growth hormone in human familial longevity

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Background: Numerous studies have shown that the insulin/insulin-like growth factor (IGF-1) pathway is associated with longevity in various model organisms. Reduced growth hormone (GH) secretion has been associated with extended lifespan in mouse models. Ames dwarf mice, which are deficient in GH, thyroid stimulating hormone (TSH) and prolactin (PRL), live ~50% longer than wild-type mice. To study healthy longevity in humans, the Leiden Longevity Study (LLS) included offspring of long-lived families and partners thereof, serving as a control group. Offspring have the propensity to reach old age in good health, are metabolic healthier and have a lower mortality compared to their partners. Thereby, it was found that the total secretion of TSH was higher in the offspring compared to their partners. The involvement of GH in human familial longevity has not yet been investigated.
Research aim: The aim of this study is to investigate 24 hour secretion parameters of GH in offspring of long-lived families compared to partners.
Methods: In this cross-sectional study, we included 19 middle-aged offspring from long-lived siblings from the LLS together with 18 partners thereof. Blood was sampled every 10 minutes over 24 hours and GH was measured. IGF-1 and IGFBP3 were measured every 4 hours. The regularity of the 24-hour GH concentration profile was assessed by Jack Approximate Entropy (Jack ApEn). GH secretion parameters were assessed using deconvolution analysis.
Results: Mean (95% confidence interval (CI)) Jack ApEn was significantly lower in the offspring compared to the partners (0.45 (0.39 - 0.53) vs 0.66 (0.56 - 0.77), p=0.001). Furthermore, mean (95% CI) total GH secretion was lower (p=0.04) in offspring (171.7 mU/L (127.7 – 215.8)) compared to partners (238.1 mU/L (192.7 – 283.5)). Mean (95% CI) basal GH secretion was also lower (p=0.03) in offspring (14.5 mU/L (9.8 - 21.5)) compared to partners (26.9 mU/L (17.9 - 40.4)). The number of pulses was equal between the groups.
Discussion: We found that human familial longevity is associated with enhanced regularity of the 24-hour GH concentration profile and reduced GH secretion. Thus, our data suggest that GH signalling is a conserved mechanism implicated in mammalian longevity.

13. Genetic analysis of IRF6, a gene involved in craniofacial midline formation, in relation to pituitary and facial morphology of patients with idiopathic growth hormone deficiency

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Introduction Growth hormone is secreted by the pituitary gland, which forms part of the craniofacial midline. Defects in the craniofacial midline are associated with pituitary hormone deficiencies. Since IRF6 is a transcription factor involved in the development of the craniofacial midline, we hypothesized that variants in IRF6 could explain part of the cases with idiopathic growth hormone deficiency and might be related with pituitary and facial morphology.
Materials and methods We sequenced all coding exons and exon-intron boundaries in 81 patients with isolated growth hormone deficiency. We performed a Multiplex Ligation-dependent Probe Amplification in order to exclude copy number variations in IRF6. We analyzed facial measurements taken from standardized frontal and lateral digital pictures of 48 patients.
Results We found two new heterozygous missense variants in IRF6 (Arg233Cys and Pro456Ser) and 11 known polymorphisms. Arg233Cys was reported as extremely rare in exome databases (1 allele out of 120.852 alleles sequenced), strictly conserved among species and was predicted deleterious by all variant predictor programs. Pro456Ser was also extremely rare, but not strictly conserved among species and predicted to be benign. MLPA did not reveal any exon deletions or duplications in any of the patients. SNP rs2235371, reported to have a protective effect on orofacial cleft formation, was present in 5 patients, who showed a slightly less severe phenotype.
Conclusion This is the first report of IRF6 analysis in a IGHD cohort. Apart from one new variant which, based on in silico analysis, could be of functional relevance, we did not find any mutations. Therefore we conclude that IRF6 defects are rare in IGHD patients and further research should focus on new candidate genes.

14. Association between bone marrow adiposity and bone turnover before and after raloxifene.

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Background Bone marrow fat is inversely related to bone mass in patients with osteoporosis, but the association between bone marrow fat and bone turnover is not known. 17beta-estradiol is known to decrease bone marrow fat fraction in postmenopausal women, but the effect of selective estrogen receptor modulators like raloxifene on bone marrow fat remains unknown.
Objective To determine the association between marrow adipose tissue and bone turnover. In addition we determined the effect of raloxifene on bone marrow fat content.
Methods 26 paired iliac crest biopsies from postmenopausal osteoporotic women, all enrolled in the MORE-trail, were analysed, at baseline and after 2 years of treatment with placebo (n=11; age: 68 ± 6 years) or raloxifene (60-120mg/d, n=15; age 67 ± 7 years). All subjects received calcium and vitamin D3 suppletion. Standardized bone histomorphometry was performed and in addition we quantified bone marrow fat content.
Results At baseline bone marrow fat was associated with bone volume (R = -0.382; p= 0.03), but not with bone formation rate or osteoclast number. Bone marrow fat increased in the raloxifene treated group, however this increase was not significantly different to the change in the placebo treated group, (mean difference: +7.7 ± 7.6% and +5.0 ± 12.9%; p= 0.54). In the raloxifene treated group the adipocyte density increased compared to the placebo treated group (mean difference: + 34.1 ± 28.9 cells/mm2 and -3.5 ± 29.5 cells/mm2; p= 0.003), while the adipocyte diameter did not change in the raloxifene group and increased in the placebo treated group (mean difference -0.5 ± 2.8 µm and 3.1 ± 5.1 µm; p= 0.03).
Conclusions Bone marrow fat is associated with bone volume, but not with bone turnover in postmenopausal osteoporotic women. Raloxifene increases the number but not the size of bone marrow adipocytes.

15. Predictors of need for insulin therapy in gestational diabetes mellitus

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Background and aims: The incidence of Gestational Diabetes Mellitus (GDM) is increasing, due to an increase in obesity in pregnant women and more stringent guidelines for screening and detection. Pregnancies complicated by GDM are at risk of neonatal and obstetrical complications. This risk may be reduced by achieving adequate glycaemic control with dietary intervention and/or insulin therapy. In the current high influx of GDM patients, it might be helpful for health care planning to recognize a ''complex care'' group requiring additional insulin therapy. The aim of this study was to identify relevant factors which predict the necessity of additional insulin therapy during pregnancy.
Material and methods: Retrospective cohort study of singleton pregnancies of all women with GDM between January 2011 and September 2014 in a teaching and an academic hospital. Patients were tested for GDM if they had risk factors for GDM or symptoms of GDM (e.g. polyhydramnion). GDM was diagnosed if fasting plasma glucose was ≥7.0 mmol/l and/or 2-h glucose value ≥7.8 mmol after a 75-gram OGTT. After GDM diagnosis, self-monitoring of blood glucose (SMBG) instruction and dietary advice was provided. Insulin therapy was started if SMBG fasting glucose was ≥5.3 mmol/l or if 1-h SMBG glucose values were ≥7.8 mmol/l, despite dietary intervention.
Results: A total of 820 women with GDM were referred for treatment. Their mean (±SD) age was 32±5 yrs, BMI was 27.7 [IQR 24.0-31.9] kg/m2. Of them, 460 women (56%) were able to maintain adequate glycaemic control with dietary restrictions only, while 360 (44%) required additional insulin. Of the women who required additional insulin, 142 women (39%) received thrice daily pre-prandial rapid-acting insulin, 164 women (46%) received basal-bolus insulin therapy and 39 women (11%) received NPH insulin once daily at bedtime. Insulin dose varied from 2 to 80U (median 22 [IQR 12-42]U). Logistic regression analysis showed the following significant predictors for the need of insulin therapy: history of GDM, a previous newborn with birthweight > 4500 gram or > P95, first-degree relative with type 2 diabetes, multiparity, Mediterranean ethnicity, pre-pregnancy BMI ≥ 30, and both increased fasting and 2-hour blood glucose during OGTT. The strongest predictor for insulin therapy was a fasting blood glucose of ≥ 5.5 mmol/l (OR 6.03 (CI 3.56–10.22, P= < 0.001). Maternal age, smoking during pregnancy, history of IUFD, history of PCOS, chronic hypertension and other ethnic groups did not predict insulin need
Conclusion: This study developed a prediction model to identify GDM patients with an increased likelihood for the need for additional insulin therapy. In GDM, fasting glucose ≥ 5.5 mmol/l is the strongest predictor for the need of insulin therapy.

16. Clinical and lifestyle factors associated with skin autofluorescence in a population-based cohort study

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Background Skin autofluorescence (SAF) is a non-invasive marker of advanced glycation end products (AGEs). In diabetes, higher SAF levels has been positively associated with long-term complications, cardiovascular morbidity and mortality. Because little is known about the factors that influence SAF in non-diabetic individuals, we assessed the association of SAF with clinical and lifestyle parameters in a large-scale, non-diabetic population and performed the same analyses in a type 2 diabetic subgroup.
Methods In a cross-sectional study in participants from the LifeLines Cohort Study, extensive clinical and biochemical phenotyping, including SAF measurement, was assessed in 9009 subjects, including 314 (3.5%) subjects with type 2 diabetes.
Results Mean SAF was 2.04 ± 0.44 arbitrary units (AU) in non-diabetic individuals and 2.44 ± 0.55 AU in type 2 diabetic subjects ($p<0.0001$). Multivariable backward regression analysis showed that in the non-diabetic population, SAF was significantly and independently associated with age, BMI, HbA1c, creatinine clearance, genetic polymorphism in NAT2 (rs4921914), current smoking, pack-years of smoking and coffee consumption. In the type 2 diabetic group, a similar set of factors was associated with SAF, except for coffee consumption. In the non-diabetic population, pack-years of smoking and coffee consumption explained 4.0% respectively 3.6% of the variance in SAF whereas current smoking (8.9%) had the largest effect on SAF in the type 2 diabetic group.
Conclusions In addition to the established literature on type 2 diabetes, we have demonstrated that SAF levels are associated with several clinical and lifestyle factors in the non-diabetic population. We need to take these parameters into account when using SAF as a screening or prediction tool for populations at risk for cardiovascular disease and diabetes.

17. Accuracy of continuous glucose monitoring measurements in normo-glycemic individuals

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Background: The validity of continuous glucose monitoring (CGM) is well established in diabetic patients. CGM is also increasingly used for research purposes in normo-glycemic individuals, but the CGM validity in such individuals is unknown. We studied the accuracy of CGM measurements in normo-glycemic individuals by comparing CGM-derived versus venous blood-derived glucose levels and measures of glycemia and glycemic variability.
Methods: In 34 healthy participants (mean age 65.7 years), glucose was simultaneously measured every 10 minutes, via both an Enlite® CGM sensor, and in venous blood sampled over a 24-hour period. The CGM glucose recordings were calibrated using capillary glucose (fingersticks) values from a self- monitored blood glucose meter (Contour®).
Validity of CGM-derived individual glucose measurements, calculated measures of glycemia over daytime (09:00h-23:00h) and nighttime (23:00h-09:00h), and calculated measures of glycemic variability (e.g. 24h standard deviation [SD]) were assessed by Bland-Altman plots, Pearson correlation coefficients, mean absolute relative difference (MARD), and paired t-tests.
Results: Compared to venous glucose measurements, glucose levels derived with CGM were on average 0.10 mmol/L higher during the whole study period. The median correlation coefficient between CGM and venous glucose measurements per participant was 0.68 (interquartile range: 0.40–0.78), and the MARD was 17.6% (SD = 17%). The calculated measure of glycemia during daytime was 0.22 mmol/L higher when derived from CGM, but no difference was observed during nighttime. Measures of glycemic variability were lower with CGM than with venous blood sampling (e.g., 24h SD: 1.07 with CGM and 1.26 with venous blood; p-value = 0.004).
Conclusion: In normo-glycemic individuals, CGM-derived glucose measurements had good agreement with venous glucose levels. A novel finding is that the measure of glycemia was higher during the day and measures of glycemic variability were lower when derived from CGM. This would have implications for the use of the CGM in epidemiological studies.

18. Sleep efficiency as a determinant of insulin sensitivity in overweight and obese adolescents

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Background: Transient insulin resistance is a physiologic phenomenon during puberty. Overweight and obese adolescents may be at risk for persistent insulin resistance during puberty, and therefore have increased risk of developing non-insulin dependent diabetes mellitus. In this study possible associations of anthropometric and lifestyle characteristics with insulin sensitivity (HOMA-IR) were determined.
Methods: 137 overweight and obese adolescents (42m/95f, age 14.4±2.3y, BMI z-score +3.3±0.7, HOMA-IR 3.4±1.8) from the Centre for Overweight Adolescent and Children's Healthcare (COACH, MUMC+) were included. Anthropometrics, puberty stages (Tanner), sleep characteristics (polysomnography), food intake behaviour (Three Factor Eating Questionnaire), and physical activity (Baecke questionnaire) were determined. Associations with HOMA-IR were tested using multiple regression analysis.
Results: HOMA-IR was significantly higher in peripubertal compared to prepubertal overweight and obese adolescents, and did not decrease at the end of puberty. Associations with HOMA-IR differed significantly for gender and puberty stage. BMI z-score (r^2=0.07, p=0.018) was a significant contributor of HOMA-IR in girls. In boys, age (r^2=0.33, p=0.000) and puberty stage (r^2=0.32, p=0.000) were contributors of HOMA-IR. A model wherein age, BMI z-score and puberty stage were positively, and Baecke score was inversely associated with HOMA-IR, explained 55% of the variance of HOMA-IR in boys (r^2=0.55, p=0.002). In prepubertal girls, total sleeping time (r^2=0.59, p=0.02) and sleep efficiency (r^2=0.58, p=0.028) were negatively associated with HOMA-IR.
Conclusion: Overweight and obese adolescents showed a persistently high HOMA-IR instead of transiently higher HOMA-IR during puberty. HOMA-IR was associated with gender, puberty stage, BMI z-score and age, and in prepubertal girls with less total sleeping time and sleep efficiency.

19. Hypoglycemia unawareness in an otherwise healthy 71 year old men.

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We diagnosed diffuse nesidioblastosis a 71- year old men with biochemical hypoglycemia, despite a negative Whipple's triad.

Case presentation

A 71 year-old-men presented with episodes of malaise, vertigo and headaches that had been present for more than 15 years. His medical history was unremarkable, in particular he had no diabetes mellitus nor had he undergone gastrointestinal surgery other than appendectomy. He used no medication. His weight had been stable for years. The laboratory results of the general practitioner included a low fasting glucose of 2.6 mmol/L.

Because of spontaneous biochemical hypoglycemia we performed a 72 hour fasting test. While fasting he developed biochemical hypoglycemia of 2.7 mmol/L, during which he complained about slight dizziness, but no specific adrenergic or neuroglycopenic symptoms were objectified. The complaints did resolve after infusion of glucose. During the hypoglycemic episode, serum c-peptide and serum insulin were high (respectively 6.7 mE/L and 0.81 mmol/L). Anti-insulin antibodies were negative. Pharmacological analyses were negative for the use of sulfonylureas. The results of the 72 hr fasting test did rise the suspicion of an insulinoma or a noninsulinoma pancreatogenous hypoglycemia. A MRI pancreas did not reveal any lesions. A 18F-DOPA-PET scan showed a scintigraphic image of diffuse hyperinsulinism, but no evidence of focal insulinoma. The diagnosis noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS or nesidioblastosis) was made based on the patients complaints, the biochemical tests and the 18F-DOPA-PET-scan.

In the absence of gastrointestinal surgery, nesidioblastosis is very rare in adults. In addition, nesidioblastosis is usually characterized by post-prandial hypoglycemia, whereas this patient presented with fasting hypoglycemia. Presence of Whipple's triad (biochemical hypoglycemia, symptoms of hypoglycemia and resolution of symptoms after glucose has raised) is usually required for initiating evaluation of spontaneous hypoglycemia[1]. We present a case in which symptoms of hypoglycemia were very discrete, probably because of hypoglycemia unawareness caused by longstanding hypoglycemia due to nesidioblastosis. By eating several small meals spread throughout the day our patient is symptom free.

[1.] Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2009. pp. 709–28.

20. The effect of sitagliptin on brown adipose tissue and whole-body metabolism in overweight pre-diabetic men

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Objectives: Brown adipose tissue (BAT) recently emerged as a novel player in energy expenditure in humans as it combusts fatty acids towards heat. Therefore, activation of BAT is considered a promising novel target to reduce obesity and associated disorders. We have recently shown that central agonism of the receptor for the incretin hormone GLP-1 results in activation of BAT in mice. One of the currently used anti-diabetic drugs that enhances GLP-1 availability is sitagliptin (STG). Interestingly, STG also reduces body weight and plasma triglyceride (TG) levels in type 2 diabetes mellitus patients. The mechanism underlying these beneficial metabolic effects is currently unknown. The aim of the present study is to investigate the effect of sitagliptin treatment on BAT volume and activity and whole-body metabolism in overweight pre-diabetic men.
Methods & Results: We are currently performing a randomized double-blinded placebo-controlled study in 30 male Dutch Caucasian adults aged 35-55 years with moderate obesity (BMI 25-32 kg/m2) and pre-diabetes. Subjects will be treated for 12 weeks with STG or placebo. Before and after treatment, we will determine BAT volume and total BAT activity via cold-induced 18F-FDG PET-CT scans, resting energy expenditure via indirect calorimetry using ventilated hoods, body weight, and body composition via DEXA scan. Furthermore, before and after treatment, blood samples will be taken to measure plasma lipids, glucose and insulin levels. In addition, skeletal muscle biopsies will be performed to analyse expression and/or biomarkers for insulin signalling and glucose and lipid metabolism.
Conclusion: As the clinical trial is still ongoing we do not have results yet. However, during this meeting we will discuss the experimental design to brainstorm about additional measurements and research questions. In the end, this study will offer valuable novel insight in the effects of pharmacological activation of BAT in human obese subjects and might provide novel treatment strategies to combat obesity and type 2 diabetes.

21. Fecal microbiota transplantation used to improve postprandial bacterial translocation; the RALSTONIA study

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Background: There is a world-wide epidemic of obesity, which is a major risk factor for type 2 diabetes (T2D) and cardiovascular disease. A key factor in the development of T2D in obese humans is inflammation of mesenteric visceral adipose tissue with a subsequent excess of plasma free fatty acids and insulin resistance. However, the triggering factor for this inflammatory response is still unknown. In the past decade, the intestinal microbiota has been studied with increasing interest and it is currently thought to be involved in the development of T2D in obesity, possibly via translocation of intestinal bacteria to plasma and mesenteric visceral adipose tissue. Our group showed improvement of microbial diversity and insulin sensitivity in patients with metabolic syndrome after fecal microbiota transplantation from lean donors. The enhancement in microbial diversity may result in decreased bacterial translocation of specific Gram-negative bacterial pathogens, which improves inflammatory tone and insulin sensitivity. Thus, we hypothesize that translocation of Gram-negative intestinal bacteria can be improved by lean donor fecal microbiota transplantation.
Methods:
Study design We are performing a randomized controlled trial in 24 metabolic syndrome patients who are scheduled for cholecystectomy. Patients will be randomized to one of two treatment arms: single allogenic fecal microbiota transplantation from lean healthy donors vs. single autologous fecal microbiota transplantation. Before and 3-4 weeks after the transplantation, subjects will receive an oral fat load with postprandial blood sampling at different time points and subjects will undergo a gastroduodenoscopy with sampling of the duodenal mucosa to determine small intestinal bacterial composition. Subsequently, patients will undergo cholecystectomy, allowing us to harvest mesenteric visceral adipose tissue.
Objectives We will investigate whether postprandial translocation of specific intestinal bacteria into plasma and mesenteric visceral adipose tissue is associated with (small) intestinal bacterial composition and whether this is altered upon lean donor fecal transplantation. Moreover, we will study whether changes in (small) intestinal bacterial composition are related to altered bacterial metabolite concentrations in subcutaneous and visceral adipose tissue.
Endpoints The primary outcome measures are a) amount of bacterial 16S DNA in plasma at different time points after a fat load; and b) change in bacterial 16S DNA load in mesenteric visceral adipose tissue. Plasma and adipose tissue samples will also be sequenced using 16S techniques to determine bacterial composition. Secondary outcome measures include bacterial composition in duodenal mucosal samples and fecal samples and expression of inflammatory markers in plasma and adipose tissue.

22. Children with morbid obesity benefit equally as children with overweight and obesity from an ongoing care program

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Context: Despite stabilization of childhood overweight and obesity prevalence, there is a shift towards more severe degrees of obesity, which results in an increasing prevalence of children with morbid obesity. Prior studies demonstrated that lifestyle modification without on-going treatment has only a modest and not sustainable effect in children with morbid obesity. This suggests that a chronic care model is necessary for long-term effects on weight management and health.
Objective: To study the effect of an on-going lifestyle intervention in children with morbid obesity in comparison to children with overweight and obesity.
Design: Non-randomized prospective intervention study with 12 and 24 months follow-up.
Setting: Centre for Overweight Adolescent and Children's Healthcare (COACH).
Patients: 100(F)/72(M) children and adolescents with overweight, obesity, or morbid obesity.
Intervention: Long-term, outpatient, tailored lifestyle intervention.
Main Outcome Measure: BMI z-score.
Results: In children with morbid obesity 12 and 24 months intervention resulted in a decrease of BMI z-score of -0.13 _ 0.25 (p_0.001) and -0.23 _ 0.32 (p_0.01) respectively, while weight status category improved to obese in 21% and 25% of the children. Cardiovascular risk parameters including serum total cholesterol, LDL-cholesterol, HbA1c, and diastolic blood pressure significantly improved after 1-year intervention in the complete group. Most important, BMI z-score as well as cardiovascular risk parameters improved to a similar degree in children with overweight, obesity and morbid obesity.
Conclusions: Children with overweight, obesity, and morbid obesity benefit equally from an ongoing, outpatient, tailored lifestyle intervention, and demonstrate significant weight loss and improvement of cardiovascular risk parameters.

23. Brown fat activation enhances the lipid-lowering effect of statin treatment in APOE*3-Leiden.CETP mice

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Introduction: Statins are currently the most widely used lipid-lowering drugs, but prevent only 15-30% of all cardiovascular events, urging the need for new treatment strategies. Brown fat produces heat by burning triglyceride-derived fatty acids and contributes largely to triglyceride clearance. As a result brown fat activation accelerates the hepatic clearance of cholesterol-enriched lipoprotein remnants via the apoE-LDL receptor (LDLR) pathway, thereby alleviating hypercholesterolemia and atherosclerosis development in hyperlipidemic APOE*3-Leiden.CETP mice (Nat Commun 2015). Since statins upregulate the hepatic LDLR expression, the aim of this study was to investigate whether brown fat activation and statin treatment cooperate in lowering hyperlipidemia.
Methods and Results: Female APOE*3-Leiden.CETP mice were fed a Western-type diet (0.15% cholesterol) and treated with the selective β3-adrenergic receptor agonist CL316243 that activates brown fat (20 µg/day; subcutaneous), atorvastatin (0.0036% w/w, supplemented through the diet), or both, for 2 weeks. CL316243, alone and combined with atorvastatin, induced brown fat activation and beiging of white fat, evident from increased mRNA expression of uncoupling protein-1 in brown adipose tissue (both treatments approx. +180%; $P<0.005$) and white adipose tissue (both approx. +2000%; $P<0.01$). Furthermore, brown fat activation alone and in combination with atorvastatin treatment markedly lowered plasma triglyceride levels (both approx. -60%; $P<0.01$), whereas atorvastatin treatment did not affect triglyceride levels. Total cholesterol levels were lowered by both brown fat activation (-29%; $P<0.005$) and atorvastatin treatment (-31%; $P<0.001$), and were more drastically lowered by the combination (-44%; $P<0.0001$). In order to study triglyceride and cholesterol kinetics we intravenously injected glycerol tri[3H]oleate and [14C]cholesteryl oleate double-labeled 45 nm-sized VLDL-mimicking particles. The selective uptake of triglyceride-derived fatty acids into brown adipose tissue was markedly enhanced by brown fat activation alone (+234%; $P<0.0001$) and on top of atorvastatin (+220%; $P<0.0001$), but not by atorvastatin alone. Moreover, the hepatic uptake of cholesterol-enriched remnants only tended to be increased by brown fat activation (+18%; n.s.) or atorvastatin (+22%; n.s.), and was clearly further increased when brown fat activation was combined with atorvastatin treatment (+70%; $P<0.005$).
Conclusions: Brown fat activation enhances the lipid-lowering effect of statin treatment, via accelerating the formation and subsequent hepatic uptake of cholesterol-enriched lipoprotein remnants. We, therefore, postulate that combining statin treatment with brown fat activation is a promising new avenue to combat hyperlipidemia and likely also cardiovascular diseases.

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24. The role of mitochondrial quality control in brown adipose tissue metabolism

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Mitochondrial dysfunction is at the core of various inherited metabolic disorders. It is also implicated with the onset and progression of age-and obesity-related conditions, such as type 2 diabetes and cardiovascular disease. Brown adipose tissue (BAT) has a high mitochondrial density and contributes to energy expenditure through energy dissipation as heat. Imaging techniques show 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. Interestingly, increased BAT activity improves metabolic risk factors such as insulin sensitivity and body mass index, while lipid accumulation due to inherited fatty acid oxidation deficiency or obesity impair BAT function. Improving BAT capacity is therefore an attractive target for combating metabolic disorders. In this project, we aim to improve mitochondrial function in BAT through regulatory pathways of mitochondrial quality control (mitoQC), such as mitochondrial proteostasis and mitochondrial fusion/fission. These processes might not only play a role in maintaining mitochondrial integrity, but may also contribute to BAT bioenergetics. As such, we seek to elucidate BAT substrate preference, how metabolic pathways converge in BAT, and how mitoQC pathways affect BAT functionality. Gaining insight in BAT metabolism holds potential to accelerate the quest for pharmacological targets that collectively expand BAT volume as well as its mitochondrial function.

25. BCG vaccine lowers plasma cholesterol levels and atherosclerosis development in mice

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Introduction: Bacille-Calmette-Guérin (BCG), prepared from attenuated live Mycobacterium bovis and the only licensed vaccine to tuberculosis, affects atherosclerosis as currently explained by immunomodulatory mechanisms. However, whether BCG is pro- or anti-atherogenic remains inconclusive and seems to depend on the model used. Besides inflammation, hypercholesterolemia is a main risk factor for atherosclerosis development. Therefore, we aimed to elucidate the effect of BCG on cholesterol metabolism and atherosclerosis in APOE*3-Leiden.CETP (E3L.CETP) mice, a well-established mouse model of human-like lipoprotein metabolism.
Methods and results: Hyperlipidemic E3L.CETP mice were fed a Western-type diet with 0.1% cholesterol and injected intravenously with BCG (0.75 mg; 5x106 CFU). Mice were terminated 6 weeks after the injection. BCG reduced plasma total cholesterol (-34%, $P<0.01$) and free fatty acid levels (-48%, $P<0.001$). In accordance with the reduced cholesterol levels, plasma clearance of cholesterol from intravenously injected [14C]cholesteryl oleate (CO)-labelled VLDL-like particles was accelerated as a result of elevated hepatic uptake of [14C]CO (+25%, $P=0.05$) in BCG-treated mice. BCG markedly increased liver weight (+53%, $P<0.001$), but liver total cholesterol content was not affected. Instead, severe leucocyte infiltration and accumulation of F4/80+ macrophages (+280%, $P<0.001$) was observed in the liver upon BCG treatment. Systemically, BCG elevated CD4+ T-cells (within CD3+ cell population, +11%, $P<0.01$), as well as activation (+203%, $P<0.05$) and memory (+84%, $P<0.01$) of CD8+ T-cells within the CD8+ population in the blood. Ultimately, BCG tended to decrease atherosclerotic lesion area in the aortic root of the heart (-59%, $P=0.08$).
Conclusion: BCG induces immune activation, but more importantly, also lowers circulating cholesterol levels, ultimately explaining the reduction in atherosclerosis development. Future studies should investigate the fate of cholesterol that is taken up by the liver; whether it is excreted into the bile via hepatocytes or whether host immune cells or the still present M. bovis use it.

26. The effect of short term high fat diet on mitochondrial function in brown adipose tissue

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Background and aims: Brown adipose tissue (BAT) combusts fatty acids to heat by uncoupled respiration in mitochondria, thereby contributing to total energy expenditure. Long term high fat diet (HFD) feeding of mice results in less and dysfunctional mitochondria in BAT, accompanied by reduced body temperature. This may be due to apoptosis of BAT since genes involved in apoptosis are upregulated in brown adipocytes of obese rats. The aim of the current project is to unravel whether apoptosis forms the link between high fat diet and mitochondrial dysfunction in BAT.
Methods and results: 12-week-old C57Bl/6J mice were fed a HFD (45% of calories derived from fat) for 0, 1, 3 or 7 days (n = 10/group). The HFD increased fat mass after 3 days and 7 days (+48% and +99%,respectively, $P<0.05$) as well as gonadal white adipose tissue weight after 7 days (+76%, $P<0.01$). Of note, BAT weight was increased after 3 and 7 days of HFD (+39% and +31%, respectively, $P<0.05$). This was accompanied by reduced uptake of [^{3}H]oleate derived from glycerol tri[^{3}H]oleate-labeled VLDL-like particles by BAT after 1, 3 and 7 days of HFD (-59%/g, -44%/g and -63%/g, respectively, $P<0.001$), pointing to reduced BAT function.
Conclusion: Based on preliminary data we show that short term HFD markedly reduces the uptake of fatty acids by BAT and increases its weight. Whether apoptosis plays a role in this effect remains to be determined. Therefore, apoptosis assays, histology and general functionality measurements will be performed shortly.

27. Leptin resistance in high-fat high-sucrose diet induced obesity: is there a role for hypothalamic inflammation?

Kathy C.G. de Git, M.C.M. Luijendijk, I. Wolterink, S. Beerens, S.E. la Fleur, R.A.H. Adan

In animals offered forced high-fat diets (HFD), the development of leptin resistance has been shown to be mediated by an inflammatory response in the hypothalamus. However, a forced HFD might not best represent the human situation. Therefore, we have previously developed a free-choice diet consisting of chow, saturated fat, and liquid sugar (fcHFHS). Animals on this diet show persistent hyperphagia and become resistant to the anorectic effects of leptin, but the role of hypothalamic inflammation is still unknown. Besides, it appears that the development of leptin resistance in diet-induced obesity is often not stable, showing varying effect sizes at inconsistent time points.

Here, we studied the development of leptin resistance at the level of food intake, glucose tolerance, and pSTAT3 levels in the arcuate nucleus of animals offered a fcHFHS diet to determine whether this is a robust animal model to interfere with hypothalamic inflammation.

Although we observed high variability in individual leptin sensitivity and between different cohorts, at the group level animals showed a stable response of leptin at 12-24h food intake. From week 4 onwards the fcHFHS group was become leptin resistant at the level of food intake, whereas the effects of leptin on glucose tolerance were less consistent. At week 9 of diet exposure, fcHFHS animals convincingly showed cellular leptin resistance in the arcuate nucleus.

Thus, exposure to a fcHFHS diet results in the development of leptin resistance, which is most evident at the cellular level. Currently, we are studying whether fcHFHS diet fed animals also show upregulation of inflammatory markers in the hypothalamus.

28. Silencing of Tumor Suppressor Genes by gene promoter methylation – search for biomarkers differentiating follicular-cell derived thyroid tumors

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Introduction: Thyroid cancer is the most frequent endocrine neoplasia. Malignant transformation of nodular goiter (NG) can lead to the development of follicular adenoma (FA) that can progress into: papillary thyroid carcinoma (PTC) or thyroid follicular cancer (FTC). There is a need for new biomarkers differentiating follicular-cell derived thyroid tumors (FCDT), especially in case of FNAB with underdetermined cytology or with „*neoplasma folliculare*" diagnosis. Tumor suppressor genes with alterated expression are biomarkers candidates. Expression silencing of TSGs can be caused by gene promoter hypermethylation. In this study we analysed the possible impact epigenetic silencing on expression of recognized TSGs in thyroid neoplasia: *ARHI, CDH1, p16INK4A, TFF3* and *TIMP3.*

Aim of the study: Evaluation of *ARHI, CDH1, p16INK4A, TFF3* and *TIMP3* promoter hypermethylation and gene expression.

The aim of the study: Analysis of expression levels and methylation status of selected tumor suppressor genes (TSG): *ARHI, CDH1, p16INK4A, TFF3* and *TIMP3* in follicular tumor lesions FA, FTC and FVPTC (in tumor tissue examined postoperatively), and thyroid nodule. Assessment of usefulness of examination the expressional and epigenetic changes of selected TSGs in patients with differentiating follicular-cell derived thyroid tumors.

Materials and methods: Biological material was obtained from 56 patients (aged 16 to 76 years) with preoperative diagnosis PTC / „neoplasma folliculare". Histopathological classification: NG, n=23; FA, n=6; PTC, n=22; FTC, n=5. RNA and DNA isolation from thyroid cancer tissue and macroscopically unchanged tissue (both collected during total thyroidectomy). Qualitative and quantitative analysis of RNA and DNA performed spectrophotometrically (BioPhotometer, Eppendorf). DNA bisulfite conversion, followed by promoter methylation level evaluation in methylation specific PCR, Methylation Index (MI) calculation. mRNA expression level (RQ) measured by real-time PCR using Taq Man Low Density Arrays, TaqMan Array Micro Fluidic Card in 7900 HT Fast Real-Time PCR System (Applied Biosystems, USA). Relative gene expression (RQ) level of each TSG was assessed using $\Delta\Delta C_T$ method. Statistical analysis was performed using Statistica for Windows 10.0.

Results: Gene expression level of *ARHI, CDH1, p16INK4A, TIMP3* and *TFF3* genes was at comparable level among analysed histopathological groups (PTC, FA, FTC, NG), with frequent reduced expression. Presence of methylation in promoter region observed for all genes, most frequent in *ARHI*. RQ values of *p16INK4A* were statistically higher for PTC group. No statistical differences were found for *CDH1, ARHI* genes. There was no statistically significant relationship between RQ and MI values within for *ARHI, CDH1, p16INK4A. TIMP3* MI value correlates with RQ (p=0.029). In PTC *TFF3* MI correlates with RQ level (p=0.01).

Conclusions: Molecular analysis of *ARHI, CDH1, p16INK4A, TIMP3* and *TFF3* revealed that expression decrease was present not only in cancer samples. The observed expression reduction in most genes/groups was independent from methylation. However the results for *TIMP3* and TFF3 may have diagnostic significance and in the future may contribute to the improvement of differential diagnosis of thyroid lesions. Simultaneous analysis of methylation profile and expression level of *TIMP3* and *TFF3* may be diagnostically useful. Further studies are needed.

29. THYROID CARCINOMA IN FAMILIES WITH DICER1 GERMLINE MUTATIONS

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Background: The DICER1 syndrome is an autosomal dominant inherited disorder that increases the risk of a variety of cancerous and noncancerous tumors. Features most commonly reported are pleuropulmonary blastoma (PPB), cystic nephroma, ovarian sex cord-stromal tumors (OSCST) and multinodular goiter. Thyroid cancer has occasionally been reported as part of the DICER1 cancer spectrum. Here we report four additional DICER1 related thyroid cancers.
Methods: We performed histopathological investigation of four differentiated thyroid carcinomas and three benign thyroid tumours of patients from three *DICER1* families. Moreover, we performed somatic *DICER1* 'hotspot' mutation analysis in these four thyroid carcinomas and the Ampliseq Cancer Hotspot Panel to investigate other somatic mutations involved in tumorigenesis.
Results: The histopathological characteristics of the thyroid tissue in the seven patients with DICER1 syndrome are different from sporadic thyroid carcinoma and hyperplasia. The most predominant finding was a distinct variant of hyperplasia in all samples. The four malignant samples comprised of multiple foci of classical and/or follicular variant papillary thyroid carcinoma. Analysis of the RNAse IIIB domains in thyroid carcinoma tissue confirmed an additional somatic *DICER1* mutation in three thyroid carcinoma patients. Notably, one somatic *TP53* mutation was revealed in addition to a somatic DICER1 mutation. No other additional cancer hotspot mutations were detected.
Conclusion: Our families with DICER1 syndrome confirm that mutation carriers are at increased risk to develop differentiated thyroid carcinoma, especially during childhood and adolescence. Specific histopathological characteristics can be used to select patients suspect for DICER1 syndrome. The causal role of DICER1 in these thyroid cancers is displayed by the somatic RNAse IIIB domain mutations, which are similar to mutations found in DICER1 related PPB and OSCST. Regarding the increased risk of thyroid carcinoma, yearly thyroid ultrasound during childhood and adolescence, together with appropriate endocrine referral, may be warranted for young *DICER1* mutation carriers.

30. CHARACTERIZATION OF THE HYPERPARATHYROID-JAW TUMOUR (HPT-JT) SYNDROME IN EIGHT LARGE DUTCH KINDREDS

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Background Approximately 5-10% of the primary hyperparathyroidism (PHPT) patients has a genetic predisposition syndrome, including multiple endocrine neoplasia type 1 and 2 [MEN1 and MEN2], hyperparathyroid-jaw syndrome [HPT-JT] and familiar isolated hyperparathyroidism [FIHP]. While parathyroid carcinomas (PC) are very rare tumours, accounting for less than 1% of patients with PHPT, at least 20% of patients with PC have a genetic predisposition. HPT-JT syndrome is an autosomal dominant inherited disorder caused by germline *CDC73* mutations, which increase the risk of a variety of cancerous and noncancerous tumours. Features most commonly reported are parathyroid adenoma and/or carcinoma, ossifying fibroma of the jaw bones, Wilms' tumors, papillary renal cell carcinoma and uterine myoma. In HPT-JT syndrome the prevalence of parathyroid carcinoma is 10-15%. Therefore, these patients could benefit from surveillance to prevent cancer or at least get PC diagnosed at an early stage. Here we report eight Dutch large kindreds with HPT-JT syndrome.
Methods: Since 2004 germline *CDC73* mutation analysis is centralized in the laboratory of diagnostic genome analysis, Leiden, the Netherlands. Clinical and histopathological characteristics were collected from probands and affected family members. Parafibromin expression was evaluated by immunohistochemical staining of formalin fixed and paraffin embedded resection material.
Results: We identified eight patients with germline *CDC73* mutations, in a total of 64 patients send in for mutation analysis. Notably, beside nonsense and frameshift mutations we detected two deletions of the entire *CDC73* gene. Subsequently 37 family members underwent clinical examination and/or genetic assessment. At least 80% of the mutation carriers has PHPT, some probands were diagnosed at a strikingly young age, i.e. before the age of 16. Additionally, two ossifying fibromas, two PCs and two Wilms' tumours were present in six separate families. Extended family history showed individuals with other rare malignant tumours i.e. cholangiocarcinoma, pancreatic cancer, and myxiod liposarcoma, which are not known to be a part of HPT-JT. Lastly, the examined parathyroid carcinomas had loss of parafibromin staining, while the parathyroid adenomas had normal parafibromin expression profiles.
Conclusion: Our results suggest that germline *CDC73* mutation screening in patients with HPT-JT syndrome, familial isolated primary hyperparathyroidism and non-familiar parathyroid carcinoma should be considered. Patients with germline *CDC73* mutation should participate in a surveillance protocol focussing on hyperparathyroidism, jaw-, renal- and uterine tumours.

31. New CgA assay of Thermo Fischer in the monitoring of NET patient

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Background: Chromogranin A (CgA) is used as an aid in the diagnosis and follow-up of neuroendocrine tumors. We evaluated the ThermoFisher Brahms CgA Kryptor assay against the CisBio assay. Recently, we evaluated also the new generation CgA assay (CgA II).
Methods: Analytical validation of the CgA II assay was performed and included precision, linearity and recovery studies as well as a comparison study with the current CgA assay and a stability study. For the stability study, individual serum samples were aliquoted and stored at different storage temperatures (room temperature, 4^{o}C and -20^{o}C) until assayed.
Results: The CgA II assay showed a good correlation with the current CgA assay. Although the current assay was not stable at 4°C, the CgA II assay is stable at 4°C for at least 48 hr and therewith comparable to the stability we used to with the CisBio assay.
Conclusion: Our study showed that the CisBio assay can conveniently be replaced by the Kryptor assay which is a robust assay with good performance. The CgA II assay uses antibodies directed against different epitopes and the results have demonstrated that this epitope is stable over time and at different temperatures, including 4°C.

32. Multiple Endocrine Neoplasia type 1 redefined: a clinical comparison of mutation positive and mutation negative patients

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Multiple Endocrine Neoplasia type 1 (MEN1) is diagnosed if at least two out of three main MEN1 manifestations hyperparathyroidism (pHPT), duodenopancreatic neuroendocrine tumors (dpNET) and pituitary tumors (PIT) occur in a patient. However, in up to 10% of patients with MEN1 phenotype no mutation is found in the *MEN1* gene.(1) It is unclear if the clinical course of the disease of mutation negative patients is comparable with mutation positive patients. Some recent studies suggest that some mutation negative patients with MEN1-like phenotype might have a distinct syndrome such as MEN4 (caused by mutation in the CDKN1B gene). (2)
The aim of our study was to compare age-related penetrance of MEN1 related manifestations and age of death between mutation positive and mutation negative MEN1 patients. In addition, mutation negative patients were asked to participate in a genetic screening program for familial neuroendocrine tumors (including CDKN1B and AIP).
A cohort study was performed using the Dutch MEN1 database, including >90% of the Dutch MEN1 population >16 years (n=323).(3) Two-hundred-ninety-three (91 %) mutation positive and 30 (9 %) mutation negative MEN1 patients were identified. Median follow-up from moment of MEN1 diagnosis was 8 years (range 0 – 44 years). The median age for developing the first main MEN1 manifestation was higher in mutation negative patients (46 vs. 36 years) (P = 0.010). In mutation negative patients, a third primary MEN1 manifestation did not occur in the course of follow-up compared to the occurrence in 42% in mutation positive patients (P = 0.001). Only one non-main MEN1 associated manifestation developed in a mutation negative patient (an adrenal tumor; 3%), while mutation positive patients developed such manifestations in 57.0% at a median age of 58 years (54 – 62). Median survival in mutation positive patients was 73.0 years to 84.0 years in mutation negative patients (P = 0.013). Only two mutation negative patients died, due to non-MEN1-related causes at the age of 64 and 84 years. Genetic analysis revealed a CDKN1B mutation in one patient.
In conclusion, mutation positive and mutation negative MEN1 patients have a different clinical phenotype and clinical course of the disease. MEN1 manifestations occur at a higher age in MEN1 negative patients and they have a better life expectancy than mutation positive patients. The differences in clinical course suggest that *MEN1* mutation negative patients do not have true MEN1, but another MEN1 like syndrome or sporadic co-incidence of two neuro-endocrine tumors.
(1) Lemos et al. Hum Mutat. 2008 Jan;29(1):22-32.
(2) Thakker. Mol Cell Encocrinol. 2014 Apr;386(1-2):2-15.
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33. No Association of Blood Type O with Neuroendocrine Tumors in Multiple Endocrine Neoplasia Type 1.

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CONTEXT: An association between ABO blood type and the development of cancer, in particular, pancreatic cancer, has been reported in the literature. An association between blood type O and neuroendocrine tumors in multiple endocrine neoplasia type 1 (MEN1) patients was recently suggested. Therefore, blood type O was proposed as an additional factor to personalize screening criteria for neuroendocrine tumors in MEN1 patients.
OBJECTIVE: The aim of this study was to assess the association between blood type O and the occurrence of neuroendocrine tumors in the national Dutch MEN1 cohort.
DESIGN: Cohort study using the Dutch National MEN1 database, which includes >90% of the Dutch MEN1 population. Demographic and clinical data were analyzed by blood type. Chi-square tests and Fisher exact tests were used to determine the association between blood type O and occurrence of neuroendocrine tumors. A cumulative incidence analysis (Gray's test) was performed to assess the equality of cumulative incidence of neuroendocrine tumors in blood type groups, taking death as a competing risk into account.
RESULTS: ABO blood type of 200 of 322 MEN1 patients was known. Demographic and clinical characteristics were similar amongst blood type O and non-O type cohorts. The occurrence of neuroendocrine tumors of the lung, thymus, pancreas and the gastrointestinal tract was equally distributed across the blood type O and non-O type cohorts (Grays's test for equality; P = 0.72). Furthermore, we found no association between blood type O and the occurrence of metastatic disease or survival.
CONCLUSIONS: An association between blood type O and the occurrence of neuroendocrine tumors in MEN1 patients was not confirmed. Addition of the blood type to screening and surveillance practice seems for this reason not of additional value for identifying MEN1 patients at risk for the development of neuroendocrine tumors, metastatic disease or a shortened survival.

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