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Endocrinology Research Unit (EFE) is a venture of all research efforts around endocrinology concentrated at department of Endocrinology M, Odense University Hospital.

EFE has its own research lab – clinic for Molecular Endocrinology and Endocrinology Treatment (KMEB) and presently, 22 Ph.d. students are trained in EFE. EFE also embraces two PhD Schools in which a close collaboration between the faculty of Natural Science and Technology of University of Southern Denmark and the Panum Institute of University of Copenhagen is taking place.

The goal is diagnostic treatment based on scientific knowledge, and therefore it is my pleasure to ascertain the high number of medical publications - around 70 this year. The research stories presented here are showing that department M is producing interesting results which can be implemented in the clinical work for the benefit of patients.

Congratulations to this year’s scientific award winners, where specifically professor Laszlo Hegedüs has been active. His international awards are very prestigious and important for EFE.

Indeed, we want to thank everybody who has contributed to our research, specifically all the patients who agreed to be at the disposal for science. The same gratefulness applies to all the health control subjects. I hope that this annual report shows all of you that it was worth it. I also want to extend my thanks to all staff, both those who are directly involved in research and those who in their clinical work enables research to thrive.

A special thank to our research secretaries, who through their daily work secure a smooth work flow, and who have produced this annual report: Nanett Mosumgaard, Tine Hylle, Tina Barbisan Hansen and Kristine Michailidis.

Also a warm thank to the Faculty of Health Science, the hospital management and to the foundations for their support to the research activities in endocrinological research.
ENDOCRINE RESEARCH UNIT COMMITTEE

Marianne Andersen
Consultant, PhD
The Pituitary Gland Resarch Group

Henning Beck-Nielsen
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Chairman
The Diabetes Research Group

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The Thyroid Research Group

Moustapha Kassem
Professor, consultant, DMSc
The Molecular Endocrinology Unit (KMEB)

Dorthe Nielsen
Nurse, RN, MHS, PhD-student
The Bone and Calcium Research Group

Nanett Mosumgaard
Research Secretary
FIVE SELECTED RESEARCH STORIES FROM 2008
- examples of EFE research areas

Adiponectin levels in polycystic ovary syndrome and hypogonadal men.

by consultant Dorte Glintborg, and PhD student Louise Frederiksen, the Pituitary research group

Introduction
Obesity is associated with changed secretion of several adipose tissue-derived peptide hormones, commonly named adipokines (1). Low circulating levels of the adipokine adiponectin have been linked to increased risk for insulin resistance and type 2 diabetes. The mechanisms by which adiponectin increases insulin sensitivity are not fully clarified, but low adiponectin levels were associated with impaired insulin-stimulated oxidative and non-oxidative glucose metabolism, suggesting that low plasma adiponectin levels contribute to impaired glucose transport and glycogen synthesis (2). Adiponectin circulates in different polymer-complexes classified as high-molecular weight (HMW) multimers, medium-molecular weight hexamers (MMW), and low-molecular weight (LMW) trimers (3). The effect of adiponectin on insulin-stimulated glucose metabolism may be mediated primarily by the HMW form (4).

Polycystic ovary syndrome (PCOS) is a frequent, heterogeneous disease characterized by insulin resistance, anovulation, hyperandrogenaemia and/or polycystic ovaries (5). Adiponectin levels were decreased in insulin resistant PCOS patients, suggesting that low adiponectin levels in PCOS could be a possible mediator of insulin resistance in PCOS and may be associated with hyperandrogenemia (6).

In men adiponectin has been shown to correlate inversely with waist circumference and DXA measurements of central adiposity. These findings have been interpreted as negative consequences of visceral adiposity.

Study protocols
1. PCOS
Method: Thirty PCOS patients randomized to pioglitazone, 30 mg/day or placebo for 16 weeks and fourteen weight-matched healthy females were included. All participants had measurements of total and HMW adiponectin, euglycemic hyperinsulimemic clamps, and indirect calorimetry performed. Results: Pre-treatment adiponectin levels were decreased in PCOS patients vs. controls, whereas no significant differences were found in HMW adiponectin levels. Following pioglitazone treatment, total and HMW adiponectin increased and changes in total adiponectin levels correlated positively with changes in insulin-stimulated Rd, changes in oxidative glucose metabolism and inversely with changes in fasting FFA levels and lipid oxidation during insulin stimulation. Measures of HMW adiponectin did not add further information. Conclusion: A close correlation between increased total adiponectin levels and increased insulin-stimulated glucose metabolism during pioglitzone treatment supports that the insulin sensitizing effect of pioglitazone in PCOS is, at least in part, mediated by adiponectin.

2. Young men
Methods: Population-based, cross-sectional study of 783 men recruited from a sample of 3,000 men aged 20-29 years, randomly drawn from the Danish Central Personal Registry. Serum analysis and whole body DXA scan were performed in all men, while abdominal MRI was performed in 406 consecutively included men. Results: Central fat mass and subcutaneous adipose tissues on the abdomen was significantly negatively associated with adiponectin, while fat on the lower correlated positively with adiponectin. Conclusion: Adiponectin is reduced in abdominally obese men. In young men, this reduction is accounted for by increasing SAT, while VAT did not modify adiponectin levels independently. In medical literature, there’s a tendency to report relationships with CFM (or waist girth) as relationships with visceral adiposity. Our data suggest that appropriate modalities like MRI in conjunction with multivariate analyses are required to confirm or disprove a hypothesis in this area of research.
Enhanced differentiation of human embryonic stem cells into multipotential mesenchymal progenitors by inhibition of TGF-β/Activin/Nodal signaling using SB-431542

by Amer Mahmood, Cand. Scient, Ph.D. student-The Molecular Endocrinology Unit (KMEB)

Human embryonic stem cells (hESCs) are established from the inner cell mass of pre-implantation embryos. HESCs hold the ability to differentiate into every known tissue in the body, and self-renewable capacity makes them an unlimited source of cells for therapy in many degenerative diseases, such as diabetes, myocardial infarction, muscle dystrophy, osteoporosis and traumatic spinal cord injuries. In this context, we are interested in studying the differentiation of hESC into mesenchymal/osteogenic lineage in order to understand the early human bone development and to provide an unlimited supply of osteogenic cells for cell therapy in many bone diseases. In general, differentiation of hESC in currently available protocols is always random and uncontrolled due to less knowledge of their differentiation regulatory mechanisms and due to cellular heterogeneity. Different research groups have derived osteogenic population from both human and mouse ESCs, however these studies are based on co-culture with mouse cells, or animal serum has been used. These protocols cannot be used in the clinic though they are contaminated with xenograft animal products.

Aim and Methods
The aim of this study was to develop a serum free protocol for directing the differentiation of hESC into muscle progenitor cells and then further into mesenchymal progenitor cells. For this propose we differentiated hESCs as human embryoid bodies (hEBs) for 10 days in 3D culture in presence of TGF-β inhibitor (10µM of SB-431542 (SB)) or control, hEBs were plated onto fibronectin coated plates in Chemically Defined Medium (CDM) and allowed for outgrowth in presence of TGF-β inhibitor (1µM of SB). The outgrowth cells were passaged (up to 5 times) and analysed for the expression of specific markers for skeletal muscle and mesenchymal cells by Affymatrix microarray analysis, immunohistochemical analysis and Real-time PCR. In addition, cells were examined for their in vitro differentiation capacity into mesoderm-derived lineage including; skeletal muscle, adipocytes and osteoblasts. Cells were also tested for their in vivo differentiation capacity via implantation with or without the osteoconductive scaffold Hydroxyapatite/tricalcium phosphate (HA/TCP) in SCID/NOD mice, histological analysis of implants revealed the formation of, skeletal muscle, bone and cartilage in SB treated cells.

Results
Our results including, immunohistochemical, FACS and microarray analysis demonstrated clearly that continuous inhibition of TGF-β signaling in hEBs outgrowth cultures (SB-OG) led to enrich myocyte progenitor cells (figure 1) that can form myofibres when implanted intramuscularly in vivo. Shifting these SB-treated myocyte progenitors into 10% fetal bovine serum (FBS), developed into a homogeneous population of mesenchymal progenitors (MSC) that expressed characteristic MSC CD markers: CD44 (100%), CD73 (98%), CD146 (96%) and CD166 (88%) with the ability to differentiate into osteoblasts, adipocytes and chondrocytes in vitro and in vivo (Figure 2).
Slow-growing craniopharyngioma masquerading as early-onset eating disorder: Two cases.

By Vad Winkler L, Andersen M, Hørder K, Schumann T, Støving RK

Background
Craniopharyngiomas are slow-growing tumors, which can either be asymptomatic or present themselves with visual, neuropsychiatric or endocrine disturbances. Eating disorders (EDs) are syndromes with unknown etiology, associated with multiple endocrine abnormalities. In pediatric cases the presentation of EDs may differ markedly from those of adults.

Objective
We report on two pediatric patients with craniopharyngioma misinterpreted as ED.

Method
Available patient records, psychiatric examinations, neuro-radiographic imaging, and biochemical data were evaluated. CONCLUSION: The reported cases illustrate the importance to consider slow-growing craniopharyngioma in ED. Especially in atypical ED, neuro-radiographic, ophthalmologic and endocrine examination should be carried out. Furthermore, structural hypothalamic lesions in these cases mimicking features of ED, suggesting the possibility of an as yet unidentified structural hypothalamic disorder to be implicated in the etiopathogeny of ED.

Pictures from the Annual EFE-meeting in December
Adverse effects of recombinant human TSH (rhTSH) in the context of effect amplification of radioiodine therapy for nontoxic multinodular goiter.

By MD, Ph.D student Søren Fast, the Thyroid research group

Context
During the last decade recombinant human TSH (rhTSH) has been introduced for amplifying the effect of radioiodine therapy for nontoxic multinodular goiter (NMG). We have previously demonstrated that pre-treatment with rhTSH increases the thyroid volume (TV) reduction by 35-56% compared to conventional (without rhTSH) ¹³¹I-therapy at one year follow-up.

Before rhTSH gains widespread use in this context, safety issues remain to be clarified. RhTSH-augmented ¹³¹I-therapy is challenged by both acute and long-term side effects related to alterations in thyroid function and size. Thus the thyroid research group has previously demonstrated that rhTSH causes a dose dependent transient increase in thyroid hormones in both healthy and goitrous individuals. Reassuringly, the potential risks of thyroid hormone excess, especially in the elderly and patients with cardiovascular disease, have been demonstrated to be negligible with low doses of rhTSH.

While the thyroid hormone response to rhTSH is well documented, less is known about the dose dependency of the other major acute adverse-effect, which is the swelling of thyroid tissue. Potentially, a TV increase may be fatal in patients with intra-thoracic goitre extension and/or tracheal compression. We have previously demonstrated that 0.9 mg of rhTSH resulted in a mean 35% increase of TV in healthy individuals, while 0.3 mg rhTSH resulted in a mean 24% TV increase in NMG patients.

Aim and Methods
To clarify whether the acute TV response to rhTSH is dose dependent we evaluated the effect of three doses of rhTSH. In a paired design including four consecutive study rounds, nine healthy male volunteers received placebo, 0.1, 0.3 and 0.9 mg of rhTSH intramuscularly. TV was measured by ultrasound, at baseline, 24h, 48h, 96h, 7 days and 28 days after rhTSH.

Results
In the placebo round and the 0.1 mg rhTSH-round the TV did not change significantly from baseline at any time during the 28 day follow-up. In the 0.3 mg rhTSH-round the mean TV increased by 37% and 45% at 24h and 48h, respectively. Following 0.9 mg rhTSH the mean TV increased by 23% and 36% at 24h and 48h, respectively.

In all rounds of the study, the maximum thyroid enlargement in each individual was observed between day 1 and 4. In the placebo and the 0.1 mg rhTSH-round the mean maximum TV increase was not significantly different from baseline. In contrast, maximum TV was increased by 53% and 42% in the 0.3 and 0.9 mg rhTSH-round, respectively. The maximum TV increases after 0.3 and 0.9 mg rhTSH were comparable, but both significantly greater than placebo.

The maximum relative TV increase from baseline showed considerable inter-individual variation indicating that some individuals are more prone to develop thyroid enlargement than others. After 0.9 mg rhTSH one individual developed profound thyroid enlargement (TV increased from 21 to 90 ml 30 hours after rhTSH), which responded promptly to a non-steroidal anti-inflammatory drug (NSAID).

Conclusion
Variation in the individual sensitivity to rhTSH underlines that an occasional case of clinically relevant thyroid swelling cannot be ruled out. However, it is reassuring that our study indicates that this risk can be reduced by lowering the rhTSH dose. Fortunately the beneficial effect of rhTSH on thyroid radiiodine uptake is maintained with even very low doses of rhTSH. If valid also in NMG patients, our findings indicate that rhTSH doses of 0.1 mg or lower can be used as a safe pretreatment before radioiodine therapy for NMG. Future studies should address the mechanism behind the TV increase and the treatment and prevention of this worrisome side-effect.

By Birgitte Falbe Vind, MD, PhD student, the Diabetes Research Group

Type 2 diabetes (T2D) is a common endocrine disease, and the prevalence is increasing steadily. T2D is a well-established risk factor for cardiovascular disease as well as microvascular complications, and thus is associated with substantial morbidity and premature mortality. Insulin resistance is a major component in type 2 diabetes, and the pathogenesis has not been fully elucidated. The effect of insulin on glucose metabolism in skeletal muscle is mediated through the activation of intracellular signaling pathways leading to glucose uptake and storage (figure). In type 2 diabetes, glucose uptake and glycogen synthesis are impaired, and defects in the signaling cascade may be partly responsible.

Physical activity is considered an important tool for diabetes prevention and control, although the exact mechanism is unknown. Part of the effect is believed to be achieved through modulation of insulin resistance, and thus a direct effect of training on signaling pathways may be part of the mechanism.

This study was performed in order to investigate the effect of endurance training on insulin signaling with emphasis on changes in the phosphorylation of glycogen synthase (GS), Akt and AS160.

Material and methods
13 type 2 diabetic patients and 13 healthy controls matched with regard to age and BMI completed 10 weeks of endurance training on stationary bikes. Training was a mixture of interval training and continuous cycling, and intensities ranged between 50 and 85% of VO2-peak. Training sessions lasted 25-30 min and were conducted 4-5 times per week. Before and after the training period, VO2-peak, anthropometric data and insulin sensitivity assessed by a hyperinsulinemic, euglycemic clamp were measured. Muscle biopsies were obtained at the clamp procedures before and after insulin stimulation.

Main results
Both before and after training, insulin-stimulated glucose disposal was lower in the diabetic group compared with the control group, primarily due to impaired insulin-induced glucose storage; however, in both groups training induced an increase insulin-stimulated glucose disposal. Furthermore, protein levels of Akt, AS160 and GS increased due to the training. Before training, insulin-stimulated phosphorylation of AS160 at Ser318, Ser588 and Ser751 were lower in diabetic subjects compared with controls. Training increased insulin-mediated AS160 phosphorylation at Ser588, and at Ser751 in the diabetic group when related to protein content, and these effects normalized the insulin responses on these sites. Insulin-stimulated Akt Thr308 phosphorylation was lower in the diabetic subjects both before and after training, whereas no differences in insulin-induced Akt Ser473 phosphorylation were observed. Training did not induce effects on either basal or insulin-stimulated Akt Thr308 or Ser473 phosphorylation in either group, when normalized to protein content. Insulin-stimulated GS activity (% I-form and % FV) was reduced in the diabetic group before and after training compared with the control group, and training did not affect insulin action on these activities in either group.

Conclusions
Defect phosphorylation of AS160 at sites Ser318, at Ser588, and at Ser751 may contribute to insulin resistance in T2D. The defects at sites Ser588 and Ser751 may be improved by endurance training; however, the effect of training on insulin sensitivity is mainly achieved through increments in protein content of AS160, Akt and GS.
PHD SCHOOL OF ENDOCRINOLOGY

About the school
www.sdu.dk/health/endocrinologyphd

Background
The Danish PhD School of Endocrinology is a new PhD school established on 1 August 2008 and financed by the University of Southern Denmark and Nordic Bioscience A/S, Center for Clinical and Basic Research. The school is financed by the above-mentioned parties for a three-year period from 1 August 2008 to 31 July 2011 and may be extended following that period.

The main research areas of the school are application-oriented clinical endocrinology. The disease areas include calcium metabolic diseases, diabetes mellitus, metabolic syndrome, obesity, cardiovascular risk factors and fibrosis.

The aims of the PhD School of Endocrinology are:

- To establish a high-standard, international PhD programme in medical endocrinology with special emphasis on metabolic diseases with increased tissue turnover, such as osteoporosis, osteoarthritis, cardiovascular diseases and fibrosis.
- To intensify the efforts of the involved institutions (Nordic Bioscience and the University of Southern Denmark) to educate PhD candidates with a solid background in medical endocrinology.
- To build up a national and international network between leading researchers in the field of medical endocrinology.

PhD students
The school has now enrolled the first 8 PhD students primo 2009 and will autumn 2009 again applied for PhD students for PhD studies proposed by the coordinating committee.

Organisation
The head of the PhD school is Professor Henning Beck-Nielsen, Dept. of Endocrinology, Odense University Hospital.

A coordination committee is responsible for proposal and approval of PhD projects and PhD students. The committee members are the head of the school Henning Beck-Nielsen, the daily leader of the PhD school Associate Professor Jan Erik Henriksen, Claus Christiansen, Nordic Bioscience, Rector Jens Oddershede, University of Southern Denmark, Dean Ole Skøtt, The Faculty of Health Science, University of Southern Denmark. An international advisory board will be established during 2009.

PhD Courses and Curriculum
The curriculum of the school consists of two basic courses one on clinical endocrinology and one in molecular endocrinology. Other courses to be followed by the PhD students registered at the school will be offered by the Faculty of Health Sciences, University of Southern Denmark, and the PhD School of Molecular Metabolism.
THE DANISH PHD SCHOOL OF MOLECULAR METABOLISM

About the school
http://www.metabolism-phd.dk/

Background
The Danish PhD School of Molecular Metabolism is a joint venture between the Faculty of Science and the Faculty of Health Sciences at the University of Southern Denmark and the Faculty of Health Sciences, University of Copenhagen. It was established in 2003 (under the name the PhD Graduate School of Metabolism) in collaboration with the Faculty of Health Sciences and the Faculty of Science at the University of Southern Denmark. As per 1 January 2007, the school changed its name to the PhD School of Molecular Metabolism due to a new collaboration with the Cluster for Endocrinology and Metabolism at the Faculty of Health Sciences, University of Copenhagen.

The School is interdisciplinary and research-focused and employs new cellular methods for the study of molecular metabolism, especially in connection with diseases in glucose and fat metabolism, i.e. obesity and type 2 diabetes. The school integrates the entire metabolic spectrum from genome through transcriptome and proteome to metabolome. The techniques are applied to large, well-defined cohorts (epidemiology) and clinically representative patient groups (diseases). The PhD school covers subject areas such as genetics, physiology, biochemistry, molecular biology, endocrinology and diabetology. The PhD school collaborates with a large number of well-estimated researchers and private companies both nationally and internationally.

Aims
• To train PhD students in theoretic and practical methods in the field of molecular metabolism, i.e. genomics, transcriptomics, proteomics, lipidomics, metabolomics and bioinformatics.
• To train PhD students in using these methods in the field of cell biology and for clinical application, especially in relation to type 2 diabetes, obesity and the metabolic syndrome.
• To stimulate “translational research” by assuring that more PhD projects stretch from “molecule to sick bed”
• To organise a core curriculum covering the above spectrum.
• To strengthen the cooperation between university research, public sector research and industrial research.
• To build up a national and international network between leading researchers in the field of molecular metabolism.

PhD students
In total the Danish PhD School of Molecular Metabolism has 53 PhD students enrolled pr. 1 June 2009. 5 more PhD students are under enrolment.

Management structure and organization
The Danish PhD School of Molecular Metabolism is organised with a head, a board, an international Advisory Board (approx. 20 members) and a national Faculty (approx. 45 members). The members of the board, the Advisory Board and the Faculty function as supervisors, teachers and examiners for the PhD students. In addition, the members of the Advisory Board also function as external supervisors for the PhD students.

Chief consultant, professor, MD, DMSc Henning Beck-Nielsen is head of the Danish PhD School of Molecular Metabolism.

Curriculum
In order to receive a PhD certificate from the PhD School of Molecular Metabolism the PhD students are required to complete a number of basic, compulsory courses provided by the School corresponding to at least 12 ECTS points and a number of optional courses, either proposed by the PhD School or offered by other national or international universities. In all, the PhD student must have course activities equal to at least 30 ECTS points. Furthermore, the PhD programme must be completed in accordance with the Danish PhD Regulations.

The two compulsory courses to be followed are PhD course A1: “Basal metabolism and molecular mechanisms in the metabolic syndrome” and the annual A2: “Summer School on molecular metabolism”.
CURRENT PHD PROJECTS CARRIED OUT IN EFE

The Bone and Calcium Research Group

**Jesper Ryg, MD, PhD-student**

*Hip fractures and osteoporosis*
A study evaluating the prevalence of osteoporosis and vertebral fractures in patients with recent hip fracture and the incidents of future fractures.

**Dorthe Nielsen, RN, MHS, PhD-student**

*The Importance of Patient Education in Groups in Patients with Osteoporosis*
- A qualitative and quantitative project.
  In this study, we aim to understand the influence of patient education in relation to patients’ way of coping with osteoporosis in the conduct of every day life. The first part of the study is a randomised controlled trial, here we hypothesise that multi-disciplinary education of patients with osteoporosis increases compliance, physical activity and knowledge on osteoporosis. In part two focus groups are being completed with 4x6 men diagnosed with osteoporosis, to get the gender perspective. In the last part of the study 20 qualitative interviews are being performed in Odense University Hospital, DK and Nottingham University Hospital, UK. Focus is held on *similarities and differences* for patients’ possibilities to act in concrete contexts.

**Morten Frost Nielsen, MD, PhD-student**

*SOMA – Study on Male Osteoporosis and Aging.*
Osteoporosis and fractures in men are a significant public health problem. The disease is a very heterogeneous clinical entity: some develop osteoporosis in a relatively young age for idiopathic reasons while others may have secondary causes.
Some 10.000 men aged 60-75 received a questionnaire by mail in 2004. The questionnaire was designed to capture potential factors that could be related to the development of osteoporosis or increase fracture risk. Respondent were all enrolled in a nested case-control study of causes of fragility fractures in men. In cases as well as controls, BMD, hormonal status etc are evaluated.
The study is expected to improve our knowledge on male osteoporosis including in particular risk factors for fragility fractures.

The Diabetes Research Group

**Iben Brock Jacobsen, MD, PhD-student**

*New Aspects of insulin treatment in type 1 diabetes*
The Ph.D. thesis includes a meta-analysis and two original papers describing insulin treatment of type 1 diabetestes. The meta-analysis demonstrated that best metabolic control in the treatment of type 1 diabetes is achieved by insulin pump treatment, and further that the use of rapid-acting insulin analogues is not superior to human regular insulin regarding metabolic control, but results in a reduced frequency of hypoglycaemia. Subsequently, we performed a study in which we showed that the rapid-acting insulin analogues insulin aspart produced a different counter regulatory hormone response during and after hypoglycaemia compared to human regular insulin. Aiming at reducing the cardiovascular risk factors in type 1 diabetic subjects, we investigated the effect of Metformin as an adjunct to insulin treatment in patients with poor metabolic control.

**Lisbeth Minet, Physiotherapist, MHS, PhD-Student**

*Self-care behaviour treatment in patients with type 2 diabetes*
The aim of the project is to study the long-term effect of a motivational intervention program based on cognitive-behavioural strategies in patients with diabetes. A randomized controlled trial with 400 patients with type 2 diabetes is carried out at an endocrinology unit. The sample size was determined by a power calculation based on a standard deviation of 1.15 in the HbA1c-value and a 5% two-sided significance level. The power is set to 90%. Assessments are made at baseline, follow-up 1 year and follow-up 2 year. Statistical analysis will be used to compare end points between the two groups. The effect will be evaluated on both physiological and psychosocial parameters.

**Martin Mogensen, MSc, PhD-student**

*Mitochondrial function in patients with type 2 diabetes*
The purpose of this research project is to investigate the mitochondrial function in skeletal muscles of patients with T2D. Mitochondrial function is assessed by respiratory measurements in isolated mitochondria. The project includes a basal investigation (T2D patients vs. matched control subjects) and an intervention study (effect of hyperinsulimic clamp and effect of physical training).

**Mikael Kjær Poulsen, MD, PhD-student**

*Prevalence of macrovascular disease in Type 2 Diabetes Mellitus: Left ventricular diastolic dysfunction, myocardial ischemia and peripheral vascular insufficiency*
The study aim is to establish a consecutive population of newly referred type 2 diabetes mellitus patients and among these to describe the degree of macrovascular di-
includes weekly aerobic lessons in a fitness centre and coaching in small groups. Both groups will be examined during pregnancy with extra ultrasound scanning of the fetus and measuring of weight, blood pressure, and metabolic markers.

Xiaolu Zhao, MSc, PhD-student

**Mass spectrometry-based quantitative proteomics in the study of mitochondrial dysfunction in muscle in type 2 diabetes**

Mitochondria are the primary energy-generating systems in eukaryotes. They play a crucial role in oxidative metabolism including carbohydrate metabolism, fatty acid oxidation and urea cycle, as well as in calcium signaling and apoptosis. Mitochondria dysfunction is centrally involved in a number of human pathologies, such as type 2 diabetes (T2D). Abnormalities in mitochondrial oxidative phosphorylation (OXPHOS) activity are believed to contribute to fat accumulation and insulin resistance in skeletal muscle of patients with T2D. To which extent this is caused by post-translational modifications (PTMs) of OXPHOS proteins remains undetermined. Mass spectrometry-based proteomic analysis is a powerful tool for global profiling and quantification of proteins and their PTMs. Therefore, the aim of this project is to establish methods for application of mass spectrometry-based quantitative proteomics and phosphoproteomics in mitochondria isolated from human skeletal muscle biopsies, and apply these methods in identifying OXPHOS proteins with altered expression and/or phosphorylation in muscle mitochondria from patients with T2D.

The Molecular Endocrinology Unit (KMEB)

Lasse Kjær, MSc, PhD student

**The potential for the generation of nerve cells from human embryonic stem cells**

Human embryonic stem cells (hESCs) have the ability for unlimited self-renewal and the potential for generating all cell types of the human adult body. By these virtues they present a prospective source of cells for treatment of diseases resulting from cellular, such as Parkinson’s disease. In this thesis it was shown that the hESC lines OD3 and HUES9 had a normal karyotype and were maintained in the undifferentiated state. Differentiation of the cell lines revealed that OD3 had a predisposition for generation of the mesodermal germlayer, and HUES9 for the ectodermal germlayer. Inhibition of the TGF-b/Activin/Nodal and BMP signalling pathways during differentiation was found to promote generation of neuroectoderm. It was furthermore found that exogenous Retinoic acid resulted in a concentration dependent acquisition of neural rostrocaudal identity and was found to increase the number of tyrosine hydroxylase positive neurons for certain concentrations of RA.
Malth Kristiansen, MD, PhD student
Isolation and characterisation of human mesenchymal stem cells from bone marrow and their potential use in therapy
The discovery of stem cells, which have clonogenic and self-renewing capabilities and are able to differentiate into multiple cell lineages both in vivo and in vitro, have shown great promise in tissue regeneration therapy. This has lead to clinical investigations attempting to use implantation of bone marrow derived stem cells to repair damaged tissues. The results have been encouraging with improvement in both subjective and objective measures of tissue function. The PhD project involves two parts: Isolation and characterisation of stem cells from bone marrow in an attempt to identify subpopulations of stem cells with the greatest therapeutic abilities; and 2) Clinical application of bone marrow derived stem cells in two clinical settings: a) Myocardial infarction; and b) Severe limb ischaemia due to atherosclerosis.

Amer Mahmood, MSc, PhD student
Differentiation of human embryonic stem cells into osteogenic precursor cells through mesodermal induction
The general aim of the project is to establish a new strategy for directing human embryonic stem cell differentiation into homogenous osteogenic cell lineage and to understand the developmental signalling cascade controlling osteogenic cell differentiation. Also, the ability of these cells to function in vivo will be tested in a cell-based replacement therapy protocol of bone defect.

Ann Dorte Storm Pørneki, MSc, PhD student
Derivation of pancreatic progenitor cells from human embryonic stem cells
A transplantable source of β-cells for the treatment of Diabetes type I could be obtained from the directed in vitro differentiation of human embryonic stem cells (hESC). To achieve the goal of functional cells for therapy it is necessary to recapitulate the embryonic development and to direct the hESC through the various developmental stages along the path to mature β-cells. The first step in this directed in vitro differentiation is the induction of definitive endoderm. This is accomplished by a combination of high TGF-β/Activin A signalling and low PI3K-signalling. The aim is to differentiate these endodermal progenitor cells further towards cells with markers of foregut and ultimately to pancreas progenitor cells.

Hamid Saeed, Msc, PhD Student
The Physiological role of telomerase enzyme in osteoblast differentiation in vitro and bone homeostasis in vivo
Osteoporosis is a multifactorial and multifaceted disorder leading to an increased risk for osteoporotic fractures. Senescent micro-environment due to aging coupled with telomerase deficiency resulted in accumulation of senescent osteogenic stem cells that implicate the differentiation of mesenchymal stem cells. It is well documented that telomerase activity is one of the limiting factors that accelerated cellular senescence and decreased organ regeneration. We have demonstrated that ectopic over-expression of telomerase gene (hTERT) in hMSC leads to rescuing the senescent phenotype of hMSC and also enhanced their differentiation capacity. The biological function of telomerase activity regarding mMSC diff & bone formation is not known. Therefore, the aim of this project is to understand the physiological role of telomerase in osteogenic stem cell proliferation and differentiation and also its effects on bone homeostasis in vivo by using TERC knockout mouse model that lacks telomerase activity due to null mutation in RNA component (TERC).

Lars Peter Kristiansen, MSc, PhD student
Using quantitative proteomics methods for studying the secreteome of human mesenchymal stem cells during osteoblast differentiation
We employed stable isotope labeling by amino acids in cell culture (SILAC) to determine quantitative profiles of proteins from medium conditioned by human mesenchymal stem cells (hMSC) during the course of osteoblast differentiation. Furthermore, the fraction of labeling was measured by pulsed SILAC labeling to selectively enrich for genuine secreted proteins and to study the global protein turnover suggest a global correlated transient increase in protein turnover at day 4 and a modulated state of protein turnover at day 14 during osteogenic differentiation. We identified 466 potentially secreted proteins that were quantified at 5 time-points during osteoblast differentiation and were able to pick secreted candidate proteins with a potential novel role in osteoblast differentiation based on the pulsed SILAC approach.

Maria E. O. Nielsen, MSc, Phd student
Identification and characterization of membrane marker proteins selective for adipogenesis, chondrogenesis, and osteogenesis in human mesenchymal stem cells using quantitative proteomics and live-cell imaging in combination with RNA interference
We have identified and quantified proteins from plasma membrane selective for adipogenesis and osteogenesis using a high-through put proteomics strategy. By comparing the protein profiles for each differentiation pathway
it is possible to distinguish which proteins are selectively up- or down-regulated in each of the two differentiation processes. These proteins will be validated with fluorescence microscopy among others methods.

The Pituitary – and Clinical Nutrition Research Group

Kristian Wraae, MD, PhD student

*Odense Androgen and Growth Factor Study in Elderly Men*

The project is a clinical investigation of 800 men between 60 and 75 years of age. Primarily, we investigated the relationship between androgens, growth factors and other hormones, on one hand, and body composition (muscle, fat and bone) and physical ability (strength and physical performance) on the other hand. Moreover, we investigate the importance of various genes and lifestyle factors for the correlation between hormones and body composition/physical ability. In addition, the study will answer questions regarding aging and the causes of decreasing androgen levels and growth factor levels in the blood.

Louise Frederiksen, MD, PhD student

*Odense Androgen Study - The effect of Testim and training in a population based, randomized, placebo controlled, double blinded study on hypogonadal men*

Testosterone is widely used to treat relative hypogonadism in elderly males, but the indication for treatment and safety of treatment is still being debated. This study will examine the effect of testosterone (gel) on body composition, insulin sensitivity, physical performance, muscle strength, sexual function and quality of life.

The study duration is 6 months. 60 males (60-78 years) are randomized into groups (testosterone, placebo and strength training) and the strength training group is further randomized to placebo or testosterone after 3 months. Examination program, including physical testing, MRI spectroscopy, DXA and euglyceamic clamp will be conducted every three months.

The Thyroid Research Group

Daniel El-Fassi, MD, PhD student

*Clinical and immunological aspects of B-lymphocyte depletion in Grave’s disease*

We have conducted a controlled, clinical study evaluating the effect of B lymphocyte depletion with rituximab on autoantibody levels, autoantibody specificity, thyroid function, and ophthalmic findings in Graves’ disease. Further studies addressing changes in the immune functions of the patients are ongoing.

Søren Fast, MD, PhD student

*The effect of pretreatment with 0.1 mg of recombinant human TSH (rhTSH) on thyroid $^{131}$I uptake and goitre volume reduction, following $^{131}$I-therapy for non-toxic benign nodular goitre. A randomized double blinded, placebo-controlled trial*

The main objective of this study is to examine whether the amplification of $^{131}$I-therapy with 0.1 mg of rhTSH allows a $^{131}$I-dose reduction, from 100 Gy to 50 Gy, in non-toxic nodular goitre patients. In addition the optimal time interval between rhTSH administration and $^{131}$I-therapy is studied.

![Thyroid examination](image-url)
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COMPLETED SCIENTIFIC THESES 2008
PHD THESES

Helle Christiansen
Mass spectrometry based identification and quantitation of membrane proteins of un-differentiated and osteogenically differentiated human mesenchymal stem cells.

Nis Nissen
Hip geometry in relation to bone strength and risk of fracture in the proximal femur.

Anne Christine Bay Jensen
The type II collagen fragments Helix-II and CTX-II reveal different enzymatic pathways of human cartilage collagen degradation.

Tine Engberg Tingholm
Phosphoproteomic analysis of human cells by mass spectrometry - setting up strategies.

Søren Feddersen
Fatty acid homeostasis in eukaryotes; consequences of cellular excess or deficiency of fatty acids.
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SCIENTIFIC AWARDS

Frontiers in Science Award

Haines Lecturer and visiting professor
September 2008. Mayo Clinic, Rochester, Minnesota, USA. (L. Hegedüs)

The Bagger-Sørensen Foundation’s Honoury Award

FINANCIAL SUPPORT TO EFE FROM INSTITUTIONS AND FOUNDATIONS

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