# Index

<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Running Program</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Abstracts Speakers</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Cancer and Aging: Rival Demons?</td>
<td>Judith Campisi</td>
</tr>
<tr>
<td>12</td>
<td>How stressful are “normal” laboratory conditions and how can it affect your research?</td>
<td>Steven Austad</td>
</tr>
<tr>
<td>14</td>
<td>Lipofuscin in cellular aging: cytotoxic properties and the role of autophagy</td>
<td>Annika Höhn</td>
</tr>
<tr>
<td>16</td>
<td>Sources of and responses to stress in old age – a clinical perspective</td>
<td>Kaisu Pitkälä</td>
</tr>
<tr>
<td>18</td>
<td>Stress, ROS, Immunity and Lifespan: Observations and Predictability</td>
<td>Georg Fuellen</td>
</tr>
<tr>
<td>20</td>
<td>Membrane protein isolation coupled to 2-dimensional electrophoresis and mass spectrometry for ageing biomarker discovery</td>
<td>Helen Griffiths</td>
</tr>
<tr>
<td>22</td>
<td>Effects of aging on regulation of muscle contraction from the motor unit to the motor protein level</td>
<td>Lars Larsson</td>
</tr>
<tr>
<td>24</td>
<td>Lipid peroxidation and protein modification in pancreatic β-cells in diabetes: the sweet road from hormesis to disaster</td>
<td>Shlomo Sasson</td>
</tr>
<tr>
<td>26</td>
<td>Integrating lipidomics and proteomics for oxidative stress research</td>
<td>Maria Fedorova</td>
</tr>
<tr>
<td>28</td>
<td>The Ageing vasculature: Mechanisms and therapy</td>
<td>Grant N. Pierce</td>
</tr>
<tr>
<td>30</td>
<td>Physiological and pathophysiological stress in lung – importance for lung aging</td>
<td>Babett Bartling</td>
</tr>
</tbody>
</table>
32 Is Geroprotector an option to treat an accelerated ageing lung disease? 
Kazuhiro Ito

34 Cellular stress and aging in the lung epithelium 
Andreas Günther

36 Stress, Resilience and Health in Later Life 
Carolyn Aldwin

38 Self-perceptions of aging and how they affect the health of older adults 
Susanne Wurm

40 Stress of Sensory Impairment in Later Life: Psychological Perspectives 
Hans-Werner Wahl

42 Psychological and psychobiological contributors to well-being in old age 
Karl-Heinz Ladwig

44 Alterations of the cardiac pacemaker and conduction system in the elderly 
Udo Klöckner

46 The stress of heart failure for the old individual 
Karl Werdan

48 Atrial fibrillation and bradyarrhythmias: the stressors of the old heart 
Michael Näbauer

50 Ventricular Assist Devices used for permanent support “destination therapy” 
Juliane Vierecke

52 Stress Resistance in Ageing Mesenchymal Stem Cell 
Günter Lepperdinger

54 Functional Genomics of Stress Resistance and Longevity in Mice 
Thomas E. Johnson

56 Metabolic stress and nutritional behavior in geriatric patients 
Ralf-Joachim Schulz

58 Living at Home until the End of Life- Social Support for Coping and reducing the Stress in formal and informal Networks of Elderly 
Astrid Hedtke-Becker
<table>
<thead>
<tr>
<th>Page</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Posters (in alphabetical order)</td>
</tr>
<tr>
<td>103</td>
<td>List of speakers</td>
</tr>
<tr>
<td>107</td>
<td>Organizers</td>
</tr>
<tr>
<td>108</td>
<td>Involved Societies</td>
</tr>
<tr>
<td>110</td>
<td>Authors Index</td>
</tr>
<tr>
<td>115</td>
<td>List of Sponsors</td>
</tr>
<tr>
<td>116</td>
<td>Map of Halle (Saale)</td>
</tr>
</tbody>
</table>
Running Program

Stress and Ageing:
from Molecular Biology to Clinical Perspectives

- Meeting language English -

Friday September 6th 2013

14:00 – 16:00 Protein-Modification (ProMoAge-Session)

Chair: Tilman Grune, Andreas Simm

Analytical method for the quantitative evaluation of α-dicarbonyl compounds in vivo
Christian Henning

Premature senescence and proteolytic activities in endothelial cells
Odeta Meçe

Identification of gliadin as an advanced glycation end product-modified compound in bread crust extract and their effect on mouse macrophage activation
Sandy Pötzsch

Influence of different macronutrients and feeding exposure time on intestinal tight junction proteins and liver status: Studies in mice
Stefanie Gärttner

Metabolic stress in the diabetic rabbit blastocyst
Elisa Haucke

Oxidative regulation of voltage-gated sodium channels in dorsal root ganglia neurons
Martin Schink
Friday September 6th 2013

18:00 Opening

Andreas Simm

Address
M. Gekle, Dean of the Medical Faculty
T. Klöss, Medical Director of the University Hospital
R.-J. Schulz, German Society of Geriatrics
M. Gogol, German Society of Gerontology and Geriatrics
N. Dhalla, European Section of the International Academy of Cardiovascular Sciences
R.-E. Silber, Heart Centre University Hospital Halle (Saale)

Keynote lecture and Schober award

Laudation Judith Campisi
by Kaisu Pitkälä, Helsinki, FI

Keynote lecture:

Judith Campisi
Buck Institute, Novato, US

“Cancer and Aging: Rival Demons?”

20:00 Come Together
Saturday September 7th 2013

08:00 – 10:00 Session 1

What is beneficial/harmful stress?

Chair: Judith Campisi, Manfred Gogol

How stressful are “normal” laboratory conditions and how can it affect your research? Steven Austad

Lipofuscin in cellular aging: cytotoxic properties and the role of autophagy Annika Höhn

Sources of and responses to stress in old age – a clinical perspective Kaisu Pitkälä

Stress, ROS, Immunity and Lifespan: Observation and Predictability Georg Fuellen

10:00 – 10:30 Poster Session / Coffee Break

10:30 – 12:30 Session 2

Protein Modification (EU-COST Session)

Chair: Bertrand Friguet, Günter Lepperdinger

Membrane protein isolation coupled to 2-dimensional electrophoresis and mass spectrometry for ageing biomarker discovery Helen Griffiths

Effects of aging on regulation of muscle contraction from the motor unit to the motor protein level Lars Larsson

Lipid peroxidation and protein modification in pancreatic ß-cells in diabetes: the sweet road from hormesis to disaster. Shlomo Sasson

Integrating lipidomics and proteomics for oxidative stress research Maria Fedorova

12:30 – 13:30 Lunch Break
Saturday September 7th 2013

13:30 – 15:30 Session 3

Stress in Vessels and the Respiratory System

Chair: Naranjan S. Dhalla, Rolf-Edgar Silber

The ageing vasculature: Mechanisms and Therapy
Grant N. Pierce

Physiological and pathophysiological stress in lung – importance for lung aging
Babett Bartling

Is Geroprotector an option to treat an accelerated ageing lung disease?
Kazuhiro Ito

Cellular stress and aging in the lung epithelium
Andreas Günther

15:30 – 16:30 Poster Session / Coffee Break

16:30 – 18:30 Session 4

Psychosocial Stress

Chair: Astrid Hedtke-Becker, Ursula Müller-Werdan

Stress, Resilience and Health in Later Life
Carolyn Aldwin

Self-perception of aging and how they affect the Health of older adults
Susanne Wurm

Stress of Sensory Impairment in Later Life: Psychological Perspectives
Hans-Werner Wahl

Psychological and psychobiological contributors to well-being in old age
Karl-Heinz Ladwig

20:00 Gala-Dinner (Moritzburg)
**Sunday September 8th 2013**

**08:30 – 10:30 Session 5**

**Arrhythmias and heart failure – the stressors for the aged population**

*Chair: Ján Slezák, Bohuslav Ostadal*

- Alterations of the cardiac pacemaker and conduction system in the elderly  
  Udo Klöckner
- The stress of heart failure for the old individual  
  Karl Werdan
- Atrial fibrillation and bradyarrhythmias: the stressors of the old heart  
  Michael Näbauer
- Ventricular Assist Devices used for permanent support “destination therapy”  
  Juliane Vierecke

**10:30 – 11:30 Poster Session / Coffee Break**

**11:30 – 11:45 Posterprice**

**11:45 – 13:45 Session 6**

**Stress and Repair/Regeneration/Coping**

*Chair: Kaisu Pitkälä, Steven Austad*

- Stress Resistance in Ageing Mesenchymal Stem Cell  
  Günter Lepperdinger
- Functional Genomics of Stress Resistance and Longevity in Mice  
  Thomas E. Johnson
- Metabolic stress and nutritional behaviour in geriatric patients  
  Ralf-Joachim Schulz
- Living at Home until the End of Life- Social Support for Coping and reducing the Stress in formal and informal Networks of Elderly  
  Astrid Hdtke-Becker

**13:45 – 14:15 Farewell**
Cancer and Aging: Rival Demons?

Judith Campisi

Cellular senescence is a cell fate, distinct from apoptosis, and a potent anti-cancer mechanism that arrests the proliferation of cells at risk for malignant transformation. Paradoxically, senescent cells are also thought to contribute to aging and many age-related pathologies. How and why does this cell fate have such seemingly disparate effects? The senescence growth arrest is stable and essentially irreversible, which can explain why senescent cells accumulate with age. Senescent cells also adopt a complex phenotype, including the expression and secretion of numerous inflammatory cytokines, chemokines, growth factors and proteases. This feature is termed the senescence-associated secretory phenotype (SASP). The SASP is likely responsible for driving aging phenotypes. By why did the SASP evolve? We recently found that among the SASP components is PDGF-A, a growth factor that induces fibroblasts to differentiate into myofibroblasts. Using a novel mouse model that enables us to eliminate senescent cells, we found that cutaneous wounding induces senescence at the site of the wound. The wound-induced senescent cells secrete PDGF-A and are present only transiently – they appear to be eliminated upon resolution of the granulation tissue. Optimal wound closure required the induction of senescence and secretion of PDGF-A by senescent cells – in the absence of senescence, wound healing was significantly delayed. Our findings suggest the complex senescent phenotype evolved for a dual purpose – to suppress cancer by preventing cell proliferation and to promote tissue repair by virtue of the SASP. In the case of their role in tissue repair, senescent cells are present transiently. In the case of aging, however, they are present chronically, where they can fuel both age-related degeneration, as well as, ironically, cancer.
How stressful are “normal” laboratory conditions and how can it affect your research?

Steven Austad

Laboratory mice, the mammalian workhorses of biomedical research, are the domesticated descendants of the house mouse (*Mus musculus*), a nocturnal rodent species that has been living in close association with humans for at least 8,000 years. To be most useful and informative for ageing and other types of medical research, mice should be maintained under environmental conditions that keep them healthy, comfortable, and with minimum chronic stress. Surprisingly, common laboratory conditions are not designed with consideration of a mouse’s evolved biology in mind, something that could have a significant impact on what we think we know about ageing and health in mice. Four particular features of common laboratory conditions need to be addressed to improve this situation – the thermal environment, the social environment, the physical environment, and the acoustic environment. How each of these environmental features can be improved to reduce mouse stress will be discussed as will the possible effects on research findings relevant to ageing.
Lipofuscin in cellular aging: cytotoxic properties and the role of autophagy

Annika Höhn

One of the life limiting factors in postmitotic aging cells is the intracellular accumulation of lipofuscin, a highly oxidized aggregate of proteins and covalently cross-linked lipids, influencing the metabolism of a senescent cell. The contribution of proteasomal inhibition as a cause of increasing protein oxidation and consequently elevated lipofuscin formation is well established; further the lysosomal system is considered to be involved. We could show that both, macroautophagy and the lysosomal system, are not mandatory for the formation of lipofuscin, since that material accumulates in the cytosol if autophagy or lysosomal activity is inhibited. Thus, autophagosomes/lysosomes are not required for lipofuscin formation, but represent a storage for protein aggregates/lipofuscin and reduce, but not prevent, aggregate toxicity. Proteasomal inhibition and the resulting decrease of proteolytic degradation of (oxidatively) damaged proteins is one of the main aspects of the cytotoxic effects of lipofuscin. Besides this, it was proposed that lipofuscin is cytotoxic due to its ability to incorporate transition metals, resulting in a redox active surface. We could demonstrate a lipofuscin-mediated formation of oxidants and the role of iron in this process in a model of senescent fibroblasts, as well as with artificial lipofuscin in vitro. Furthermore we are able to present a biochemical lipofuscin-model explaining the mechanisms of proteasomal inhibition, derived from the altered characteristics of lipofuscin caused by partial proteolytic degradation by protease K. These results display a strategy of strongly reducing the cytotoxic effects of lipofuscin that result in particular from proteasomal inhibition.
Sources of and responses to stress in old age:  
a clinical perspective

Kaisu Pitkälä

Of people 75+, 97% suffer from diseases – some leading to sudden, catastrophic disability and some to slowly progressing frailty and further to disability. Depending on physical and cognitive reserves the onset of these diseases represents a major stress and a challenge for adjustment for an older person. Older people often have to face with a cascade of losses. Older people have to face with abandonment of significant roles they have had during their life span. This is a significant stress factor depending on the coping skills and life situation. At some time point of old age individuals experience a loss of their partner and, in addition, successive losses of friends of their age. Having to take a role of a caregiver for a disabled or demented partner or a proxy predisposes often an older person to major stress.

Older people are often masters in readjusting and coping with such sources of stress. In a long run readjustment takes place in a form of developmental tasks. According to Erikson in old age people look back on their lives. They contemplate their accomplishments, develop feelings of contentment and integrity if they believe that they have led a happy, productive life. They may instead develop a sense of despair and hopelessness if they look back on a life of disappointments and unachieved goals. With longer perspective and contentment it is easier to accept losses and approaching death.

However, stress may also create undesirable responses. Losses of partner, friends and meaningful roles may lead to bitterness, depression, anxiety and inappropriate means of adjustment such as social isolation, loneliness and poor self-management. It is well-known that depression and loneliness may further lead to cognitive decline and disability. With low cognitive reserves any acute illness may lead to delirium – a syndrome that has extremely poor prognosis.

In this presentation typical sources of stress, such as diseases and losses will be presented as well as how older people cope with stress. It will also be presented how health and social care can support older people's coping skills.
Stress, ROS, Immunity and Lifespan: Observations and Predictability

Georg Fuellen

Identifying reliable biomarkers to describe the differences between biological and chronological age in human is a challenging task. It is also an important task, since such biomarkers would not only allow predicting the health status of individuals, but may also show possible points to intervene in the aging process.

Here, the interpretation of data collected from other mammals may yield transferable ideas. We used longitudinal datasets of the Mouse Phenome Database, combining them with data of life expectancy from the same database. We used regression analyses to characterize longitudinal trends, and correlation analyses to find predictors of life expectancy.

We find straightforward correlations like body mass and life expectancy and more illuminative ones concerning the immune system. We describe preliminary insights obtained by applying the same methods on data describing the impact of genetic manipulations of the respiratory chain on the immune system. These data are currently generated by the ROSAge consortium, on "Reactive Oxygen Species and the Dynamics of Ageing -- a Mitochondrial Multi-gene, Multi-organ Approach".

Because we are able to replicate a number of results of individual longitudinal studies in our combined analyses, we conclude that connecting aging studies can be meaningful, and reveal useful hints for further analyses.
Membrane protein isolation coupled to 2-dimensional electrophoresis and mass spectrometry for ageing biomarker discovery

Helen R. Griffiths

There has been a lack of robust markers that define biological aging. Such markers may improve understanding of mechanisms of accelerated aging and age-associated disease. To address this, we have examined the CD4$^+$ T cell membrane proteome from young and mid-life adult male groups for novel biomarkers of healthy aging. Briefly, CD4$^+$ T cell membrane proteins (purified by cell surface labelling using Sulfo-NHS-SS-Biotin reagent) from young (n=9, 20-25y) and mid-life male (n=10; 50-70y) groups were separated by 2-dimensional gel electrophoresis using 3-10 non-linear IPG strips and 8-16% polyacrylamide gels. Protein spots with age-dependent density differences ($p < 0.05$ and > 1.4 fold change) were identified by LC-MS/MS. 2-DE analysis revealed seventeen protein spot density differences (ten increased and seven decreased in the mid-life adult group) between young and mid-life adults. Four discrete and more abundant protein spots were excised and analyzed by LC-MS/MS for protein identification. One protein spot, which decreased in expression in the mid-life age group, was identified as $\alpha$-enolase. CD4$^+$ T cell surface expression of $\alpha$-enolase was confirmed as being significantly lower in mid-life adults ($p<0.05$, independent samples t-test) by flow cytometry. In conclusion, T cell surface levels of $\alpha$-enolase decrease with age in healthy adults and may be a biomarker of healthy aging. $\alpha$-enolase is present on the T cell surface and is one of several plasminogen receptors involved in the plasminogen/plasmin pathway. The consequences of altered surface expression of $\alpha$-enolase on the CD4$^+$ T cell surface merit further investigation for T cell function during aging.
Effects of aging on regulation of muscle contraction from the motor unit to the motor protein level

Lars Larsson

Profound impairments of motor functions, such as slowing of movement and muscle weakness, are prominent features of old age. In attempt to improve our understanding of the mechanisms underlying the loss of motor units and the altered contractile properties in old age we have combined experimental animal models to characterize the spatial organization of motor unit fibers, contractile properties at the motor unit, muscle cell and motor protein levels, myonuclear organization in individual muscle fibers and post-translational myosin modifications. The aging-related loss of muscle fibers has been shown to be secondary to loss of alpha-motoneurones, an incomplete reinnervation of denervated muscle fibers and reorganization of muscle fibers within the motor unit. A decreased speed of contraction and loss of force normalized to the cross-sectional area (specific force) are observed at the single muscle fiber level in both humans and rodents. According to the analyses at the motor protein level, the slowing of contractile speed is caused by altered catalytic properties of the motor protein myosin, while the force generation capacity of the motor protein appears to be maintained in old age. Post-translational modifications of myosin are forwarded as a possible mechanism underlying the decreased contractile speed in old age, while quantitative changes in the content of contractile proteins appears to play a more important role for the decreased specific force in old age and related to an altered spatial 3D organization of myonuclei in old age.
Lipid peroxidation and protein modification in pancreatic β-cells in diabetes: the sweet road from hormesis to disaster

Shlomo Sasson

Hyperglycemia and high concentrations of fatty acids (i.e., palmitic acid, 16:0) are detrimental to pancreatic β-cells. The combination of both deteriorates β-cell function in a phenomenon termed glucolipotoxicity. Our recent analyses of membrane phospholipids in INS-1E cells (a rat pancreatic-derived β-cell line) revealed marked high glucose (11 and 25 mM)-induced remodelling of saturated (SFA)-, mono-unsaturated (MUFA)- and poly-unsaturated fatty acids (PUFA). Of a particular interest was the release of arachidonic- (20:4, AA) and linoleic acids (18:2, LA) to the cell interior. Both fatty acids readily underwent free radical-propagated peroxidation to generate 4-hydroxynonenal (4-HNE), which under these conditions augmented glucose-stimulated insulin secretion (GSIS) by activating the nuclear receptor PPARδ. The content of 4-HNE adducts with proteins remained at these glucose levels below the cytotoxic threshold. Furthermore, exposure of these cells to palmitic acid (up to 200 µM) significantly and dose-dependently liberated AA and LA from phospholipids, while the viability of the cells was not compromised. Similarly, peroxidation of these fatty acids produced 4-HNE that also stimulated GSIS in a PPARδ-dependent manner. The cytotoxic effects of the combination of high glucose and palmitic acid levels were apparent only in cells that were maintained at 11 or 25 mM glucose with 250-500 µM of palmitic acid for an overnight incubation: cell number and total cell insulin content were reduced by 30-50%, parallel to a marked increase in the content of stable protein-4-HNE adducts. Interestingly, no such detrimental effects of palmitic acid were observed in cells that were exposed to 5 mM glucose, in which 4-HNE and adducts levels remained low. These findings point to the key role of phospholipid remodelling and PUFA peroxidation in mediating regulatory and cytotoxic interactions induced by nutrient overload in β-cells. Specifically, we highlight the changing role of 4-HNE from a signalling molecule that mediates hormetic/adaptive responses to a potent cytotoxic agent that causes β-cell failure and demise.
Integrating lipidomics and proteomics for oxidative stress research

Maria Fedorova

Oxidative stress and protein oxidation are considered major hallmarks of biological aging. Among the different types of protein oxidation carbonylation, e.g. formation of aldehyde and keto groups in proteins, is widely known and well accepted as biomarker of oxidative stress. Carbonylated proteins irreversibly lose their functions and tend to aggregate. Carbonylation of proteins can occur in vivo via several different pathways, such as direct oxidation, glycoxidation or the reaction with lipid peroxidation products (LPP). These LPP modifications attract special attention due to the high susceptibility of lipids to oxidants and the huge variety of possible LPP formed. However, the chemical structures of reactive LPP have been poorly characterized with only a dozen being intensively studied in recent years.

We developed a combined mass spectrometry (MS) based approach to assess changes of both lipidomes and proteomes caused by oxidative stress in different in vitro and in vivo models. A new highly specific method to detect and identify reactive LPP by shotgun and LC-MS/MS based lipidomics allowed us to identify over 200 various reactive LPP generated from different lipid classes in a single analysis. In order to reveal the damaging potential of LPP as secondary oxidants we studied their reactivity towards proteins using a library of reactive LPP prepared in house. In a bottom-up LC-MS/MS proteomics approach, we could identify many modifications at nucleophilic amino acid residues. We will present our recent results obtained by this integrated lipidomics and proteomics strategy for oxidative lipid and protein modifications in plasma samples from patients with type 2 diabetes and obesity, as well as in cellular models of oxidative and nitrosative stress. Using in vitro model of oxidative stress we identified 210 carbonylated proteins in HeLa cells treated with hydrogen peroxide. In plasma samples from patients with type 2 diabetes and obesity integrated MS approach allowed us to detect and relatively quantify over hundred of LPP resulted from oxidation of free fatty acids, phospholipids and cholesterol. Wherever proteomics data provided knowledge about 150 carbonylated proteins.
Oxylipins are endogenously produced from polyunsaturated fatty acids and play a key role in preventing or accelerating chronic disease. It is possible, but currently unknown, if oxylipin concentrations may be influenced by dietary modification with functional foods. Furthermore, despite the influence age has on metabolism, the effects of subject age on the plasma oxylipin profile is unknown. Twenty healthy individuals were recruited into a younger (19-28 years) or older (45-54 years) age group (n=10/group). They ingested one muffin/day containing 30 g of milled flaxseed (6 g alpha-linolenic acid (ALA)) for four weeks. Plasma oxylipins were extracted using solid phase extraction and analyzed using HPLC-MS/MS. The method scanned for 81 oxylipins and 40 were quantified according to the quantification limits. At baseline, the older group had 10 oxylipins derived from linoleic acid, arachidonic acid, eicosapentanoic acid, and docosahexanoic acid that were significantly greater than the younger group (p<0.05). Pro-inflammatory oxylipins such as 5-hydroxyeicosatetraenoic acid (5-HETE), 9,10,13-trihydroxyoctadecenoic acid (TriHOME), and 9,12,13-TriHOME were significantly higher in the older group at baseline. After 4 weeks of flaxseed consumption, the oxylipin profiles of the older group declined significantly to concentrations equivalent to the younger group at 4 weeks. At 4 weeks, 9-HEPE was the only oxylipin significantly greater in the older versus the younger group. Surprisingly, ALA-derived oxylipins did not change in response to the flaxseed intervention. In summary, higher concentrations of pro-inflammatory oxylipins were observed in the older age group. Flaxseed consumption improved the oxylipin profiles of both age groups by significantly reducing n6 derived pro-inflammatory oxylipins. These data emphasize the involvement of oxylipins in the aging process and how nutritional interventions like flaxseed can beneficially disrupt these biological changes associated with inflammation and aging. These changes have important implications for hypertension in view of the potent effects of flaxseed on hypertension.

(Supported by CIHR, Flax2015, ARDI and St Boniface Hospital Foundation)
Physiological and pathophysiological stress in lung: importance for lung aging

Babett Bartling

Lung tissue is subjected to different kinds of physiological and pathophysiological stress situations. Physiologically this includes biomechanical forces (strain, shear stress) which influence cellular signaling and gene expression in lung epithelium, endothelium and probably other types of cells. Pathophysiologically this includes environmental factors (bacterial infections, cigarette smoke, air pollutions) which cause a more or less pronounced chronic disorder (inflammation, endoplasmic reticulum stress, cellular senescence). Normal aging of the lung diminishes its capacity to respond adaptively to these extrinsic factors, but less is still known about age-related changes at molecular level. In this regard, we identified the down-regulation of the essential transcription factor CREB (cAMP response element-binding protein) that subsequently influences the expression of other genes (IGFBP3, Rab27A). This has been demonstrated for aging lung tissue, senescent lung cells and lung cells subjected to oxidative stress. Oxidative stress was mediated by reactive oxygen species, but it can also be mediated by neighboured senescent cells. To what extent biomechanical changes of the aging lung, which might be predominantly caused by an altered quality of the extracellular matrix (elastin, collagens) with higher age, also contribute to such cellular changes is still unknown. Therefore, we developed a cyclic multi-axial Strain Cell Culture Device which applies biomechanical forces on lung epithelial cells in vitro. The application of multi-axial strain influences the expression of a high number of genes in lung epithelial cells but at moderate level. Among these genes we identified genes coding for transcription factors and enzymes of the metabolic pathway. The identification of strain-regulated genes that are simultaneously influenced by age-related factors will be a matter of future studies.
Is Geroprotector an option to treat an accelerated ageing lung disease?

Kazuhiro Ito

During ageing, pulmonary function progressively deteriorates and pulmonary inflammation increases, accompanied by structural changes, which are described as senile emphysema. Chronic obstructive pulmonary disease (COPD), used to be called as smoker's lung, is a major and increasing global health problem with enormous amount of expenditure of indirect/direct health care costs and now affects over 10% of the world population over the age of 40 years. There is still a fundamental lack of knowledge about the cellular, molecular and genetic causes of COPD and current therapies are inadequate as no treatments reduce disease progression or mortality. COPD is a chronic inflammatory disease of the lungs, which progresses very slowly and the majority of patients are therefore elderly. There is increasing evidences for a close relationship between ageing and chronic inflammatory diseases. We here hypothesize that accelerating aging of lung in response to oxidative stress from environmental gases, such as cigarette smoke or other pollutants, may accelerate the aging of lung or worsen aging-related events in lung by defective resolution of inflammation, for example by reducing anti-aging molecules, such as histone deacetylase 2 and sirtuis or anti-oxidative stress transcription factor, such as Nrf2 and FOXO, and this consequently induces accelerated progression of COPD. Recent studies of the signal transduction mechanisms in ageing have identified novel anti-aging molecules that may provide a new therapeutic approach to COPD, such as a strategy to increase anti-oxidant capacity and geroprotectors (AMPK activator, sirtuin activator and PI3K inhibitor).
Interstitial Lung Diseases (ILD) are a heterogenous group of more than 100 parenchymal lung diseases being unified by the (usually) progressive deposition of collagen-rich extracellular matrix in and the destruction of the delicate structure of the distal lung parenchyma. Some of these disease are extremely rare, (e.g. Hermansky-Pudlack-Syndrome Interstitial Pneumonia), some are in the upper prevalence rate of orphan diseases. One disease being in the focus is Idiopathic Pulmonary Fibrosis (IPF), a dreadful and fatal disease typically affecting elder people beyond the 60ties. Patients usually present with exertional dyspnea and/or dry coughing, not uncommonly after experiencing a respiratory infection. In ~ 15% of all cases a familiar background has been described. In these families and - also infrequently - in sporadic IPF, younger affected individuals have been observed, usually in an age range of 40-50. The pathomechanism of IPF is still not fully settled. For quite some time it was thought that IPF is caused by chronic inflammation. Indeed, lymph node enlargement, plasmacellular infiltration of septae and neutrophilic alveolitis are usually found in IPF. Unfortunately, steroids and immunosuppressants have been applied without obvious clinical success and, indeed, a randomized controlled phase III trial has recently been finished and forwarded extended mortality in this patient group in the steroid / azathioprin arm. Instead, important insights into the pathomechanism of IPF stem from genetic analyses of familial cases. In here, mutations of the surfactant protein (SP)-A and SP-C, as well as of the telomerase complex, were found to underlie the evolution of the disease. In the case of SP-A and SP-C, these mutations were found to cause misfolding, ER-stress and apoptosis of the cell being primarily responsible for the production, namely the alveolar type II cells (AECII). In case of the telomerase mutations, the mutations were found to result in a loss of function, hence causing accelerated telomer shortening, DNA damage and - again - AECII apoptosis. Of note, a similar ER stress reaction and extensive apoptosis were also encountered in AECII in sporadic IPF, despite the obvious lack of any of the above mentioned mutations. As underlying reason we were able to disclose defective intracellular processing of the hydrophobic surfactant proteins SP-B and SP-C, which is due to down-regulation of the respective aspartyl proteases Napsin A and Cathepsin H and which seems to cause i) intracellular accumulation of the pro-forms in AECII, alongside with ER-stress and apoptosis and ii) loss of surface tension reducing properties in the alveolus, which itself seems to cause repetitive alveolar collapse and re-opening and serves as a possible explanation for a biomechanical link in IPF. In my talk I am going to focus on the available evidence to understand IPF as a consequence of accelerated alveolar epithelial aging and I will review underlying reasons, consecutive cellular stress patterns and survival strategies and pathways involved in epithelial regeneration and fibroblast proliferation.
Stress, Resilience and Health in Later Life

Carolyn M. Aldwin

Stress is rapidly becoming a central construct in both psychosocial and biological aging research. However, surprisingly little is known about the course of different types of psychosocial stressors in adulthood, and controversies remain over the effects of different types of stressors on health in later life. We review research from the Normative Aging Study, a longitudinal panel study which has been following 2,280 men for 45 years, with stress data on a subsample of nearly 1500 men over 18 years. We have identified different patterns of stress trajectories in both life events and hassles. Only patterns indicative of chronic stress were related to mortality outcomes, with HRs ranging from 1.44 - 5.77. However, how one appraises stress may be more important than the simple occurrence of stressors for health outcomes. Resilience in late life may reflect the maintenance of positive affect in the face of negative events.
Self-perceptions of aging and how they affect the health of older adults

Susanne Wurm

Aging is intrinsically tied to physiological changes, which include a worsening of health. Can this process be accelerated or decelerated by how we perceive our own aging process (self-perceptions of aging)? In recent years, a growing body of research has examined this intriguing question, and the presentation will give a short overview over these findings. The studies suggest that positive self-perceptions of aging promote health, well-being and longevity, while negative self-perceptions of aging seem to have detrimental effects. However, we still only know little about the pathways that link self-perceptions of aging to health and other indicators of successful aging, or in other words: How do self-perceptions of aging become a self-fulfilling prophecy? Based on recent empirical studies, this presentation discusses possible explanations and goes into the potential meaning of stress, psychological resources, and health behavior in the context of positive and negative self-perceptions of aging. Together the findings suggest that taking into consideration how people view their own aging process is a promising approach that is worth being further examined in the context of stress and aging. This could expand our understanding of how self-perceptions of aging can affect different health outcomes, not only by psychological and behavioral pathways but by biological ones as well.
Stress and sensory impairment in later life: Psychological perspectives

Hans-Werner Wahl

Is the experience of vision and hearing impairment a major stress factor in advanced old age due to its immediate negative impact on day-to-day functioning in dealing with spatial and social environmental interactions? In this study, we compared older adults with vision impairment, hearing impairment, and without sensory impairment based on a broad range of developmental outcomes. Data came from samples of severely visually-impaired (VI; N=121; mean age: 82.6 years), severely hearing impaired (HI; N=116; mean age: 82.7 years), and sensory-unimpaired older adults (UI; N=150; mean age: 82.3 years). Participants were assessed according to their everyday competence, cognitive functioning, social resources, self-regulative strategies, as well as cognitive and affective well-being. The most pronounced difference between groups appeared in the area of everyday competence (lowest in VI). Multi-group comparisons in latent space revealed similar but also different strengths in relations between health, everyday competence, social resources, self-regulation and overall well-being, depending on sensory status. Additional findings point to specific adaptation-relevant strategies when confronted with sensory impairment, e.g., regarding the interplay of cognitive resources and everyday competence. In sum, a multi-dimensional approach to the understanding of stress related to the experience of sensory impairment in old age reveals a complex picture of loss and maintenance in psychosocial adaptation.
Healthy aging is increasingly defined not only by the absence of illness and disability but by the presence of subjective well-being (SWB). SWB is a broad construct embracing positive mood (good spirits and relaxation), vitality, maintaining a general interest in things and an optimistic cognitive evaluation of one’s future life. Indicators of SWB predict favorable life outcomes including somatic health and longevity.

KORA-Age study including a postal health questionnaire administered to all 4,565 participants with a mean age of 72.8 years (SD 5.78; range 65-90 years); a telephone interview to determine the physical and mental health status (n= 4,127), and a face-to-face medical examination of an age and gender stratified sub-sample of the cohort (n=1,079).

Almost 80% of the elderly population had high levels of SWB. Mean scores decreased with the passage of time – however, remained far above the suggested cut-off for poor/fair SWB. Participants with low SWB were more likely to be female, to live alone and to have a low income. Risk constellations surrounding low SWB were suffering from somatic disease conditions, from sleep problems, from self-perceived memory decline and from depression which was confirmed in the multivariate model.

We then looked at the biological underpinning of SWB and first focused on the insulin like growth factor 1 (IGF-I) and its primary binding protein 3 (IGFBP3). We found no association between IGF-1 and health related quality of life – however, confirmed a robust association between IGF-BP3 (a prominent IGF-I binding protein) and SWB (with a 3-fold increased odds of having high levels of BP3 despite adjustment for important covariates in men) apparently reflecting the bioavailability of IGF-I. While physical activity was protective against dangerously low levels of IGFBP3, high well-being further improved these levels in both sexes. The association of well-being with high IGFBP3 levels suggests that this binding protein may be critical in the neuroprotective role associated with IGF-I in aging.
The cardiac conduction system (CCS) enables human hearts to beat about 3 billion times during an average lifespan. This system is a specialized tissue network of cardiac muscle cells that is responsible for initiation and propagation of action potentials throughout the heart. Roughly, CCS can be divided into the impulse generating sinoatrial node (SAN), the heartbeat coordinating atrioventricular node and the His-Purkinje system that enables fast conduction of the electrical impulses to the working myocardium.

In healthy humans the heartbeat is initiated in the myocytes of the SAN due to the presence of the fastest spontaneous diastolic depolarizations. Though the mechanisms underlying these essential diastolic depolarizations in SAN cells are elusive, recent literature suggests that it is the interplay of two competing oscillators, the “membrane voltage clock” and the “Ca²⁺ clock” which controls the timekeeping mechanisms of cardiac pacemaker cells. Although SAN malfunction is accompanied by structural remodeling it is most likely that in addition changes in the electrical properties of pacemaker cells contribute to heart rhythm disturbances.

From the SAN cardiac impulses are propagated through broad bands of atrial myocardium to the atrioventricular node. At this structure impulse conduction is delayed for a brief period of time to give atrial contraction the time to fully fill the ventricles with blood. After crossing the insulating fibrous plane provided by the artroventricular junctions the stimulus is propagated to the ventricular conduction network consisting of the bundle of His, the bundle branches, and the subendocardial Purkinje fibers that rapidly conduct the sinus impulse to ventricular myocardium thereby triggering cardiac contraction. Conduction velocity in the peripheral CCS is mostly determined by the activity of voltage dependent sodium channels, the electrical coupling between myocytes mediated by gap junctions, and the amount of intercellular fibrosis.

In developed countries the proportion of the population that is over the age of 65 continues to increase. The elderly tend to be at risk for SAN dysfunction. As a result the incidence of sick sinus syndrome rises with age and the regulation of the heart rate becomes erratic. When the heart ages functional and structural abnormalities within the CCS may lead to a delayed conduction or cause ectopic sites of activation thereby generating irregular heartbeats. Although the molecular pathways underlying the increased incidence of arrhythmias in the elderly are far from fully understood, some putative mechanisms will be presented in this overview.
The stress of heart failure for the old individual

Karl Werdan

Heart failure (HF) is one of the most important medical concerns in industrialized countries, with about 50,000 annual deaths and annual hospital costs of about 3 billions € in Germany. Heart failure with reduced left ventricular ejection fraction (HFREF) has to be discerned from heart failure with preserved left ventricular ejection fraction (HFPEF). In a 45 – 83 years-old population\(^1\), the prevalence of symptomatic HF is 7.7 % for men and 9.0 % for women, with a strong age dependency: 45-54 years: 3.0 %, 75-83 years: 22.0 %; HFPEF (prevalence 3.8 %) is as frequently as HFREF (5.1 %) in this population, and hypertension (HR 3.4) and previous myocardial infarction (HR 2.5) are the main risk factors for HF. While HFREF can be treated effectively with ACE-inhibitors/ARBs, beta blockers, the If-channel blocker ivabradine, diuretics, and digitalis, no effective treatment is available for HFPEF, with the exception of adequate antihypertensive treatment and normalization of an inadequately high heart rate. Medication in the elderly must take into account the reduced drug elimination by the renal and extrarenal route.

The very old patient (87±7 years)\(^2\) with HFREF and HFPEF have similar cardiovascular mortality of 18 % and 19 % resp. per year.

The problem in chronic stable HF is acute decompensation („acute heart failure“, AHF), with 335.000 hospitalizations per year in Germany. In people > 65 years, AHF is the most frequent reason for hospitalization. Hospital mortality is about 12 % and 1-year mortality about 30 – 40 %. Treatment of AHF is mainly symptomatic. Only recently, for he first time, encouraging results of reducing 180-day-mortality by one third have been reported with the hormone relaxin\(^3\).

References:


Atrial fibrillation and bradyarrhythmias –
the stressors of the old heart?

Michael Näbauer

Older age with associated structural and functional changes of the heart is a major contributor in the development of atrial fibrillation and bradyarrhythmias. Conversely, the older heart is less tolerant to the more extreme ranges of the heart rate which related to the age dependent deterioration of the diastolic function. Additional changes such as limited coronary perfusion due to coronary atherosclerosis make the ageing heart even more sensitive to rapid ventricular rates often observed in atrial fibrillation. Thus, a variety of factors contribute to stress in the ageing heart during arrhythmias.
In recent years, ventricular assist devices (VAD) have rapidly emerged as the standard of care for advanced heart failure patients. Though initially evaluated as a bridge to transplant strategy to support patients with unstable heart failure who could not survive on the waiting list for a heart transplantation, left ventricular assist devices (LVAD) are increasingly being used for permanent support (“destination therapy”).

Large studies have clearly demonstrated markedly improved survival and quality of life with ventricular assist devices as compared with optimal medical therapy. With an aging population, the incidence of heart failure is expected to dramatically increase. Furthermore, there will be a very large number of elderly patients presenting with heart failure either failing medical therapy or temporarily stabilized medically. Given a severely limited supply of donor hearts, transplant is not a viable option for the aged population with end-stage heart failure.

Data on outcomes after LVAD implantation in the elderly patient cohort are promising. While some studies have identified advanced age as a risk factor for poor outcomes after VAD implant, other showed marked benefit in survival and quality of live after LVAD implantation.

**Conclusion**

The progress in medical therapy has led to increased aging of the population, the number of donor hearts available remains limited. For this reason, long-term mechanical support has become a reality for patients of advanced age.
Stress Resistance in Ageing Mesenchymal Stem Cells

Günter Lepperdinger

Stem cells play an important role during development and regeneration, and their dysfunction is associated with a variety of diseases, such as sarcopenia, osteopenia, or several classes of cancer. Also, the clinical use of adult stem cells in regenerative medicine is an emerging field, and elderly patients are thought to be the main target population.

Progenitors derived from mesenchymal stem cells (MSC) maintain the musculoskeletal system in adults, yet also spawn many types of interstitial stroma in various organs. Notably, MSC when residing in a vascular niche promote vessel integrity through remodeling of the adventitial layer. The potential of MSC to regenerate tissues is declining with age and it is generally believed that deviations such as the accumulation of fat deposits in bone, impaired wound healing, or deregulated hematopoiesis are consequences thereof.

We investigated primary human stem cells from systemically healthy individuals, firstly studying their interindividual age-associated phenotypes, in particular the potential of the MSC to cope with various stressors such as oxygenation, inflammation and intoxication. In order to unravel underlying mechanisms, we have established a technology that allows us to investigate the translatome of MSC. In this context, polysomes are isolated rather than naked mRNA. These are subsequently fractionated by sucrose gradient sedimentation, which separates mRNAs according to the number of ribosomes bound to individual RNA molecules, which corresponds to the level of translation. RNA from each fraction is isolated and analysed by microarray technology. This approach allows a genome-wide characterisation of altered protein translation-expression profiles. In this way, we could recently obtain the array raw data for an aging-associated translation signature in MSC. We could further show that MSC when exposed to low stress levels respond in an anticipated instructive, or developmental mode (= eustress), while high or chronic levels are discerned as an imperatively coping, or accelerated aging mode (=disstress).
The link between stress resistance and extended life span was first developed in the nematode and has been confirmed in other species, including the mouse. In many cases, selecting for stress-resistance mutants concomitantly yields long-life as well. We have successfully selected for stress-resistant mutants and are testing for longevity effects. Although this forward genetic is a very powerful genetic tool, it has largely been limited to lower organisms and invertebrates. Since one cannot effectively screen millions of mice for novel mutations, we have moved the selection into mouse embryonic stem (ES) cells, and have developed strategies allowing these cells to retain their ability to develop into an intact mouse. Mice developed from paraquat-resistant ES cells have retained the stress-resistant trait in fibroblasts. Using this ES cells screening strategy to derive long-lived mouse strains, we might be able to discover the mammal-specific causes of aging. We have gone well beyond our initial studies (Chick et al., 2009), and now use a transposon mutagenesis approach to easily identify the gene(s) mutated. Our initial screens have identified several genes not previously known to modulate stress resistance or aging in any species, as well as mutational hits previously reported to modulate aging in the nematode. Mutations, for example, in Gltp, Tiam1, and Rfl1 confer oxidative-stress resistance. The genes we have identified so far have roles in diverse physiological functions, such as nutrient transport, cell surface anchoring, tumor suppression, and inflammatory regulation. The genes thus identified are immediate targets for therapeutic intervention. Overall, this is a highly efficient system to generate novel stress-resistant mouse mutants for functional genomic analysis of longevity and extended health span.

(Supported by the USPHS, RO1 AGO41801)

Metabolic stress and nutritional behaviour in geriatric patients

Ralf-Joachim Schulz

A variety of diseases influence the metabolic situation of multimorbid elderly patients. Beside the fact that individuals above 70 years need only 70 % of the energy consumption of younger agers, 65% of the geriatric patients indicate a risk or an already existing malnutrition. It will be a main issue in this session to indicate the risk factors for metabolic stress situations and the consequences resulting from malnutrition.
“Living at home until the end” – this is what most elderly persons wish for themselves even when being increasingly in need of assistance. In contrast to this, reality proves that most elderly people die in institutions and that devoted private care and ambulant services become less and less sufficient. In view of the advancing social change and the demographic development the politically wanted and economically demanded care of elderly persons at their home gains increasing significance for research and practice. The intensive support of elderly persons (also suffering from senile dementia) in need of changing or also extremely extensive care makes particular demands on the field of ambulant care and assistance. It is a moot question whether future corresponding informal and formal settings of life and support measures can be created, accompanied and ensured. The self-determination and autonomy of the elderly in need of care as well as the preservation of their and their helping persons’ well-being and health should preferably and sustainably be assured and supported. The orientations within families concerning social, family and care cultures, which become increasingly differentiated, have a complicating impact as well. In practice, the general and expert opinion can still be heard that, “…at some point of time, you can’t stay at home any more…” There are only few services with innovative concepts that reply: “But in a lot of cases it is definitely possible!”, and they illustrate the necessary conditions for staying at home and the preservation of the quality of life for all persons involved. Within the generations of a family, considerable efforts in private care are made when assisting their elderly who are restricted in terms of life conduct. Senior citizens’ networks beyond families have proved to be a stable and efficient support, too. Longstanding and close friendships belong to the most stable relationships in the course of lives. The network perspective combines the internal and external resources available which can be analyzed, as well as the netting of positive and maybe also negative support that the network entails. Network relationships can have a negative impact if, e.g., an oversupply of care leads to dependency. The linking of the informal social networks with professional formal networks “from caring assistance to helpful arrangements” (Evers & Olk 1996) aims at the social and individual handling of care dependency as well as at a balanced and more adjusted assistance. The social support can only be handled if care experts and all other persons involved participate in flexible and durably tailored support measures. However, the demand to cooperate may not mean a further strain on already overburdened female supporters.
Posters
(in alphabetical order)

Sunday 8\textsuperscript{th} of September
of 11:30 to 11:45

The Poster Award Ceremony
The "Isolated perfused mouse lung" as a suitable ex vivo system for age-dependent studies of the lung

Samiya Al-Robaiy, Andreas Simm, Rolf-Edgar Silber und Babett Bartling

Klinik und Poliklinik für Herz- und Thoraxchirurgie, Universitätsklinikum Halle (Saale), Ernst-Grube-Str. 40, D-06120 Halle (Saale)

Background: The lung undergoes age-dependent morphological and functional changes. At high age these changes are often similar to those of lung emphysema (senile lung emphysema). Some functional parameter can be measured using the "Isolated perfused mouse lung". As mice are increasingly used for age studies, this ex vivo-system should be tested for its use in functional lung studies of old mice.

Material: Lung physiological parameters of female C57BL/6 mice were studied ex vivo at a weight-adapted tidal volume and a constant minimal intra-thoracic (pleural) pressure. Using staining methods for lung sections elastin and collagen amount was assessed.

Results: A) The lungs from aging mice showed thicker alveolar wall without changes in elastin and collagen content in comparison to that from the young. B) The dynamic lung compliance increased with higher age. Correspondingly, the maximal intra-thoracic pressure and the maximal expiratory flow were reduced. The airway resistance remained nearly unchanged. C) Aging had no influence on the pulmonary ex vivo artery pressure and the permeability of the endothelial-epithelial barrier. D) While some inflammatory factors remained unchanged, the sRAGE content was increased in aging mice.

Conclusion: The ex vivo study system of the "Isolated perfused mouse lung" is a suitable method for mouse lung age-dependent studies. Current studies using this experimental system indicated that the aging process of the mouse lung starts from month 7 after birth.
(2) Compromised cellular metabolism during senescence is associated with the oxidative modification of a restricted set of enzymes

Martín A Baraibar1, Janek Hyzewicz1, Adelina Rogowska-Wrzesinska2, Anne-Laure Bulteau3, Carina Prip-Buus3, Gillian Butler-Browne4, Bertrand Friguet1

1  Laboratoire de Biologie Cellulaire du Vieillissement, UR4, Université Pierre et Marie Curie-Paris 6, Paris, France;  
2  Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark;  
3  Institut Cochin, UMRS INSERM U1016, CNRS UMR8104, Paris, France;  
4  Institut de Myologie, UMRS INSERM U974, CNRS UMR 7215, CHU Pitié-Salpêtrière, Université Pierre et Marie Curie-Paris 6, Paris, France

Senescent cells are causally implicated in generating age-related phenotypes and their removal prevents or delay tissue dysfunction. Adult human skeletal muscle stem cells (satellite cells) are a valuable cellular model of aging because they experience both chronological and replicative aging. In addition, their replication and differentiation is compromised with age, contributing to the development of sarcopenia. However, the molecular events related to myoblasts dysfunction during ageing are not completely understood. In this study, we provide evidence for the accumulation of oxidatively damaged proteins, as well as proteins modified by glycation and conjugated with lipid peroxidation products during replicative senescence of human myoblasts. These modified proteins are involved in key cellular functions such as cellular bioenergetics, cellular morphology, as well as in lipid and aminoacid metabolism. To provide mechanistic insights into the role of oxidized proteins in the development of the senescent phenotype, untargeted metabolomic profiling was performed between young and senescent myoblasts. Major metabolic differences are related to energy and lipid metabolism. Hence, a strong correlation was found between protein modifications and impairment of the related cellular metabolic pathways. Due to the fact that 5 enzymes of the glycolytic pathway were specifically affected by oxidative damage, functional proteomics analyses were performed. Glucose oxidation was decreased more than two-fold in senescent myoblasts; however, the basal respiration and the respiratory capacity of senescent cells were not affected. This study establishes a new concept in relation with the impact of oxidative protein modifications on the impairment of cellular metabolism and the development of the senescent phenotype.
(3) Germline genetics of the p53 pathway affect longevity in a gender specific manner

Sebastian Groß1, Uta-Dorothee Immel2, Michael Klintschar3, Frank Bartel1

1 Institute of Pathology, Medical Faculty, Martin-Luther-University of Halle-Wittenberg, Magdeburger Str. 14, 06097 Halle/S., Germany
2 Institute for Forensic Medicine, Medical Faculty, Martin-Luther-University of Halle-Wittenberg, Franzosenweg 1, 06097 Halle/Saale, Germany
3 Institute for Forensic Medicine, Medical Faculty, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

Aging is thought to occur through the accumulation of molecular and cellular damage. A key regulator of the cell’s stress response is p53. In mice, the activity of p53 associates with lifespan. We were therefore interested whether SNPs in members of the p53-pathway are associated with longevity in humans. We genotyped the following SNPs: p53 – rs1042522 (Arg72Pro), MDM2 – rs2279744 (SNP309), MDM4 – rs4245739 (SNP34091), rs1563828 (SNP31826), PPP2R2B - rs319217 in 155 long-lived individuals (LLIs) who died at the age of 91 and over and in 171 ethnically-matched control subjects. Kaplan-Meier survival curves and log-Rank-test were used to determine the mean and median survival times. In female LLIs, the Pro-allele of rs1042522 (Arg72Pro) and the G-allele of rs2279744 (SNP309) were significantly associated with an increased survival time (P =0.026, P <0.001, respectively, log-Rank-test). In contrast, there was no difference regarding the survival time in male LLIs (rs1042522: P=0.58, rs2279744: P =0.503, log-Rank-test). There was no difference regarding the average age of death for the genotypes of the respective SNPs in the MDM4 gene (rs1563828: P =0.99; rs4245739: P =0.179, respectively). Here we show for the first time that the G-allele of rs2279744 (SNP309) is associated with increased lifespan. Importantly, this effect is gender-specific. Our data support the hypothesis that genetic variants that are associated with lower activity of p53 - and therefore increased tumor risk – are associated with prolonged lifespan in a gender-specific manner.
Polyphenolic compounds are discussed to contribute to the health beneficial effects of a diet rich of vegetables and fruits. Distinct substances display interesting pharmacological properties, e.g. antioxidative activity as well as modulation of redox-active signaling pathways. We investigated effects of the dietary flavonol myricetin on ROS production, stress resistance and lifespan in the model organism Caenorhabditis elegans. Myricetin was rapidly assimilated by C. elegans and caused an increase in mean adult lifespan by 32.9%. This longevity effect was associated with a decrease in the formation of the aging marker lipofuscin, a reduction in Nile Red stainable lipid stores as well as a decrease in the heat-induced generation of ROS (DCF assay). However, myricetin failed to improve resistance against thermal stress (SYTOX assay). In order to elucidate the molecular mechanisms of myricetin-mediated lifespan extension in C. elegans, we investigated the influence of myricetin on factors known to have important functions in stress response and the regulation of aging: the transcription factors DAF-16 (FoxO homologue) and SKN-1 (Nrf2 homologue), the heat-shock transcription factor HSF-1 and the protein deacetylase SIR-2.1. Myricetin induced a nuclear translocation of DAF-16; however, SKN-1 localization was not affected. Lifespan extension by myricetin disappeared in daf-16 and sir-2.1 loss of function (lof) mutant strains, showing the effect is at least partially dependent on these signaling molecules. The decrease in ROS generation in wildtype nematodes was also abolished in the daf-16 (lof) strain. By using a hsf-1 (lof) strain, it was further shown that the life prolonging effect of myricetin was independent of hsf-1. In conclusion, our results strongly indicate that myricetin exerts biological effects by modulating specific signaling pathways involved in stress response and aging.

e-mail: christian.buechter@landw.uni-halle.de
(5) Comparison between various biomarkers of senescence in bone marrow-derived stromal cells *in vitro* and *ex-vivo*

**Charles Edward Frary**¹, Jan Nehlin² and Moustapha Kassem¹

¹ Clinic for Molecular Endocrinology Treatment (KMEB), Department of Endocrinology,  
² Department of Clinical Immunology, Clinical Institute, Odense University Hospital &  
University of Southern Denmark, Odense, Denmark

Senescent stem cells are classified as non-quiescent, irreversibly growth-arrested, non-terminally differentiated, apoptosis resistant multipotent stem cells that maintain an altered gene expression from their juvenescent precursors. Established markers of senescence such as senescent-associated β-galactosidase, p16, and senescent-associated heterochromatic foci (SAHF) can only be analyzed through the use of cell toxic stains or fixatives while BOCS, biomarker of cellular senescence, along with certain morphological qualities can be visualized and quantified without inflicting any damage to cellular structures.

Bone marrow-derived stromal cells were isolated from young and old healthy subjects and cultured to senescence. The senescent cells were compared to their passage 1 counterparts through fluorescent high-throughput examination of C12FDG, SAHF, p16, BOCS stainings and morphology. This analysis was then repeated on passage 1 alone from both young and old healthy donors to examine the effect of donor age on biomarkers *ex-vivo*.

Cellular C12FDG staining, morphology, SAHF and nuclear p16 expression were increased similarly to BOCS from early to late passages. When bone marrow-derived stromal cells from young and old healthy subjects were compared *ex-vivo*, BOCS was the only biomarker found to be significantly up-regulated showing that BOCS correlates with the senescent phenotype at least as well as C12FDG and nuclear p16 stainings but without fixation or permeabilization.
Sarcopenia is associated with skeletal muscle atrophy (loss in muscle mass) and weakness (loss in muscle force) during ageing, leading to a progressive loss of mobility and quality of life. However, the cellular and molecular mechanisms involved in this process are not well understood. A hallmark of ageing is the accumulation of oxidatively modified (carbonylated) proteins, leading to a decreased quality of the cellular proteome that could directly affect normal cellular functions. Although increased oxidative stress has been reported during skeletal muscle ageing, the proteins targeted by oxidation have not been characterized yet. Herein, the occurrence and characterization of carbonylated proteins was studied in human biopsies obtained from young and old healthy donors. Although no significant differences in total protein carbonylation was observed between young and old donors at the total proteome level, when the resolution of individual proteins was improved after 2D electrophoresis separation, 39 protein spots were evidenced either as increasingly carbonylated or decreased when comparing biopsies from old donors to their young counterparts. These results indicate that only a restricted set of proteins are target of carbonylation in skeletal muscle, suggesting that proteins have some intrinsic characteristics that make them prone to oxidation. Eighteen of the proteins spots that were differentially carbonylated in between the two groups of samples were further identified by mass spectrometry approaches. Of note, the proteins identified as increasingly carbonylated during skeletal muscle ageing are involved in key cellular functions such as cellular morphology and transport, muscle contraction and energy metabolism. Importantly, the impairment of these pathways has been described in skeletal muscle during ageing. A possible functional impairment of these proteins due to oxidation may impact directly on the above-mentioned pathways contributing to the generation of the sarcopenic phenotype.
(7) Influence of different macronutrients and feeding exposure time on intestinal tight junction proteins and liver status: Studies in mice

S. Gärttner¹, C. Sellmann¹, A. Spruss¹, O. Huber², I. Bergheim¹

¹ Dept. of Nutritional Sciences, SD Model Systems of Molecular Nutrition, Friedrich-Schiller-University Jena, Jena;
² Dept. of Biochemistry II, University Hospital Jena, Friedrich-Schiller-University Jena, Jena

Background and aims: Epidemiological studies have shown that the relative risk to develop metabolic diseases is positively associated with overnutrition (e.g. a diet rich in fat and/or sugar), increased body weight but also age. Recent studies further suggest that alterations of the intestinal barrier function and subsequently an enhanced translocation of bacterial endotoxins may be an important factor in the development of metabolic diseases (e.g. type 2 diabetes; non-alcoholic fatty liver disease). Starting from this background, the aim of the present study was to investigate the effect of different macronutrient rich diets (e.g. fructose, fat and the combination of both) on tight junction proteins in the small intestine and on liver status in mice exposed 8, 16 and 24 weeks to the different diets. Methods: C57BL/6J mice (n=6) per group were fed either with plain water, 30% fructose solution, a high fat diet (30% of the total energy from fat) or a combination of 30% fructose solution and a high fat diet for 8, 16 or 24 weeks ad libitum. Portal endotoxin levels as well as tight junction protein concentrations (occludin) were measured in the duodenum. In addition, indices of liver damage were assessed. Results: As expected, chronic intake of diets rich in fat, fructose or a combination of both resulted in a marked accumulation of fat in the liver after 8 weeks, which progressed with time to steatohepatitis in the fructose+fat group. Alanine-aminotransferase (ALT) levels in plasma and body weight were only found to be significantly increased in mice fed fructose+fat and also increased with time in comparison to water controls. After 8 weeks, protein levels of the tight junction protein occludin were found to be markedly lower in the duodenum of all treated groups, whereas after 16 and 24 weeks a similar effect was only found in the fat and fat+fructose treated groups. In line with these findings bacterial endotoxin in portal plasma was also found to increase in all treatment groups in comparison to the control. Conclusion: Taken together these data suggest that the markedly more pronounced damaging effect of the combined chronic intake of fat and fructose on the liver, is associated with an impaired intestinal barrier function.

(Funded, in part, by a grant from the BMBF FKZ: 01EA1305)
(8) Hypoxia Mediated Differential Expression of polysialic acid and NCAM in Neuroblastoma cancer

Gnanapragassam, V.S1, Horstkorte, R.1, Bache, M.2, Vordermark, D.2

1 Institut für Physiologische Chemie, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg
2 Universitätsklinik und Poliklinik für Strahlentherapie, Universitätsklinikum Halle

Neuroblastoma is a heterogeneous and highly metastatic solid malignant tumor in infants. Neuroblasma cancer cells utilize multiple mechanisms to suppress host immune defense and escape [1, 2]. There is an urgency to investigate the novel mechanism implemented by tumor cells during this process. Tumor hypoxia strongly favors the tumor cells to proliferate, metastasize and afforded the resistance to various therapies [3]. Our aim is to investigate whether hypoxia can induce the differential expression of polysialic acid, and their major carrier neuronal cell adhesion molecule (NCAM). Their functional implication on neuroblastoma cancer will be examined. Neuronal Cell Adhesion Molecule (NCAM) is the major cell adhesion molecule in neuronal cell type. They are expressed as three isoforms, NCAM 120, 140 and 180. NCAM 140 and 180 were polysialylated by Polysialyltransferases (PST2 and PST4) [4].

In our investigation SHSY5Y cells were cultured under the hypoxic condition, and the expression profiling of polysialic acid and NCAM were carried out. The real time cell analyzer (xCELLigence RTCA) was used to examine the impact of hypoxia on migration, adhesion and invasion. Further sialic acid metabolic engineering (SME), was applied, as a tool for sensitizing the tumor cells under hypoxic condition. Cytotoxicity assay for anticancer agent and radiation treatment were examined under this condition, and all of these results will be reported on this meeting.

References:
Background: The accumulation of Advanced Glycation Endproducts (AGEs) in skin and blood is associated to diabetes mellitus, cardiovascular diseases and the loss of cognitive function. In general AGEs are seen as a biomarker of ageing. So far there is no equivalent data concerning geriatric inpatients. Question: Is there an association between the level of AGEs in skin and blood and the functional outcome in geriatric inpatients? Setting: Geriatric department for acute, subacute and rehabilitation care. Methods: Examination of all patients, admitted within a period of 10 weeks, who were willing to participate; excluding all medically instable or isolated patients and those, who were otherwise incapable. AGEs were measured using an AGE-Reader via autofluorescence in the skin. A number of standard assessment instruments are used for functional description. Results: 196 patients were included, due to partial patient data, we included 56 M und 108 F in this analysis. The average age of M was 78.3±8.5, compared to F 81.7±7.0 years (p=0.007). The Mini Mental State Examination were ranking from 23.2±4.9 (M) to 23.1±4.6 (F, p=0.902) points. The handgrip measurements showed for M 25.3±10.2/23.8±9.8 kg and for F 15.0±6.6/13.3±6.4 kg (p=0.000/0.000). AGEs levels were 3.2±0.6 for M and 2.8±0.7 for F (p=0.0019). Mean Barthel Index (BI) at admission/discharge were 48.1±20.4/77.8±21.1 (M), and 51.0± 18.2/74.4±20.4 (F, p for BI gain 0.003). In an univariant analysis the Pearson correlation r showed a medium correlation of AGEs and BI at admission (M 0.42, F 0.40) and discharge (M 0.59, F 0.53). For handgrip (taking handedness into account): M 0.34/0.40, F 0.33/0.29. Discussion: Significantly higher AGEs levels were found in male patients. This may be the result of a higher risk exposition of men. Results showed that the linear correlation of AGEs and age was no longer significantly measurable in our population. Possibly this is caused by multimorbidity. A modest correlation for functional status (measured through BI) and handgrip was proven. We observed the strongest correlation between BI improvement and AGEs.
Mineralocorticoid (MR) and glucocorticoid receptor (GR) are corticosteroid receptors that act as ligand-dependent transcription factors. The MR physiologically regulates salt and water homeostasis but is also capable of inducing pathological effects in the cardiovascular system, including inflammation, fibrosis and remodeling processes. The GR, on the other hand, exerts protective effects in the cardiovascular system and is involved in metabolism and immune response. In their inactive form, both receptors are located in the cytosol bound to chaperone molecules. After binding their endogenous ligands both receptors translocates into the nucleus and acts as transcription factors at glucocorticoid receptor response elements (GRE). The molecular mechanisms responsible for differential actions of MR and GR are still unclear. There are, however, indications in literature that the pathological MR effects occur preferentially in the presence of a proinflammatory micromilieu containing ROS and RNS as has been associated with advanced age or preexisting disease. To investigate the effects of nitrosative stress on MR and GR activity, we measured GRE activation after stimulation of HEK cells with the NO donor SNAP or the peroxynitrite donor Sin-1. In the presence of corticosteroids, NO led to a reduction in MR and GR transactivation. Further analyses revealed that NO impairs binding of MR and GR to DNA without affecting nuclear translocation. In contrast, peroxynitrite enhanced MR transactivation activity without affecting GR genomic activity. Mechanistically, peroxynitrite led to a ligand-independent translocation of the MR into the nucleus while GR distribution remained unchanged. NO or peroxynitrite stimulation of truncated MR without regulatory N-terminal domain led to the same effects as stimulation of complete MR, suggesting that these changes are mediated either by the DNA-binding domain, the hinge region or the ligand-binding domain of the MR. An attractive hypothesis for the underlying mechanism of nitrosative stress-induced changes in MR activity are posttranslational modifications of MR like s-nitrosylation, tyrosine nitration or carbonylation, which will be investigated in the future. Overall, we found that nitrosative stress associated with advanced age or disease can differentially modulate corticosteroid receptor activity and thereby can potentially mediate the shift of physiological to pathophysiological MR effects.
**Adaptation of embryonic amino acid metabolism to a diabetic environment**

Jacqueline Gürke ¹, Frank Hirche ², Elisa Haucke ¹, Maria Schindler ¹, Bernd Fischer ¹, Anne Navarrete Santos ¹

¹ Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Faculty of Medicine, Halle (Saale), Germany
² Department of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

During pregnancy an adequate amino acid supply is essential for embryo development and fetal growth. Amino acids are precursor molecules of protein synthesis, energy metabolism and cell signalling. As the embryo is highly sensitive to its surrounding milieu and vulnerable to dysregulations by external stimuli, we have investigated the influence of a maternal diabetes mellitus type 1 on embryonic amino acid metabolism.

In female rabbits an experimental diabetes mellitus type 1 was established by alloxan treatment. The amino acid concentrations were measured in maternal plasma and in embryos during early pregnancy. In plasma of diabetic mothers the concentrations of 12 amino acids were altered. Alanine and branched chain amino acids (BCAA) were increased 2 fold in maternal plasma and also in blastocyst cavity fluid. The expression of BCAA oxidising enzymes (Bcat2, Bckdha, Dbt and Dld) was determined by real time PCR. Embryos grown in a diabetic environment revealed an increased expression of Bcat2 and Dbt, whereas the expression of BCAA transporters was not affected. They also had an increased phosphorylation of mTOR and its downstream target ribosomal S6 kinase 1 (S6K1).

To analyse the effects of L-leucine stimulation on mTOR signalling, we cultured day 6 blastocysts with 800µM L-leucine in vitro. L-leucine supplementation led to a 1.8 fold increase in mTOR phosphorylation after 30 min, indicating that the blastocyst is sensitive to L-leucine. Rapamycin, an allosteric inhibitor of mTOR, reversed the L-leucine effect and, accordingly, the phosphorylation of S6K1 was inhibited.

Our findings demonstrate that maternal diabetes leads to enhanced BCAA concentrations and a disturbed BCAA metabolism in embryos. It affects embryonic mTOR activation with likely changes in mTOR signalling.

(Supported by EU FP 7 EpiHealth (N°278418) and Cost Action TD 1101: RGB-Net)
Decreased regenerative capacity and alteration of mitochondrial activity with age in the short-lived fish Nothobranchius furzeri

Nils Hartmann, Sebastian Wendler, Beate Seliger and Christoph Englert

Molecular Genetics Group, Leibniz Institute for Age Research – Fritz Lipmann Institute, Beutenbergstr. 11, 07745 Jena, Germany

Among vertebrates that can be kept in captivity the annual fish Nothobranchius furzeri has the shortest known lifespan. It also shows typical signs of ageing and is therefore an ideal model to assess the role of different physiological and environmental parameters on ageing and lifespan determination. We used N. furzeri to study whether the regenerative capacity decreases with ageing. As other teleost fishes, N. furzeri has the ability to regenerate its caudal fin after amputation. Analysing fin regeneration in animals of different age (young, middle aged, and aged) revealed that young fish were able to nearly completely (95%) regenerate their amputated caudal fins within 27 days, whereas middle-aged fish only reached 74% and aged fish 57% of their original fin size. The difference between the groups was already significant at 3 days post amputation (dpa) and increased with time. Staining of early regenerated fin tissue (at 2, 3, and 4 dpa) with proliferative markers revealed a higher percentage of proliferating cells in young fish than in aged fish. In addition, we observed that several genes involved in wnt/β-catenin signalling were highly up-regulated in early regeneration as it has been shown in zebrafish. Interestingly, the young fish showed highest up-regulation of most analyzed genes at an earlier time point than the aged fish assuming regenerative processes are initiated earlier and faster in young fish. We are also interested in the role of mitochondria during ageing and have recently shown that mitochondrial DNA copy number, expression of mitochondria-related genes, and mitochondrial respiration significantly decreases with age in N. furzeri. To further investigate the impact of mitochondria on ageing, we want to manipulate mitochondrial gene expression. We therefore developed a protocol how to inject DNA transgenes into the 1-cell embryo of N. furzeri. We generated several transgenic lines that over-express the mitochondrial transcription factor A (Tfam) and the peroxisome proliferator-activated receptor γ coactivator-1α (Pgc-1α) under different promoters. The amount of Tfam has been described to regulate mitochondrial DNA copy number and Pgc-1α has been shown to increase mitochondrial biogenesis in mammals. The effect of elevated Tfam and Pgc-1α levels on mitochondrial function and whether this will delay ageing and extend lifespan is currently under investigation.

Email: hartmann@fli-leibniz.de
About 7% of pregnancies are complicated by diabetes mellitus (DM). Although our understanding and management of DM have improved over the last decades, diabetic pregnancies are still at risk for congenital malformations. The underlying mechanisms are unclear. Advanced Glycation End products (AGEs) are known to play a critical role in the development of diabetic complications. AGEs are formed non-enzymatically via a reaction between reducing sugars and amine residues. They are known to alter biological properties of proteins and to cause oxidative stress. We have investigated whether a poorly controlled maternal DM induces metabolic stress in the preimplantation embryo, employing a rabbit model.

Blastocysts developed under diabetic conditions showed an increased CML and Arg-pyrimidine concentration in embryonic cells; pentosidine was not affected by slot blot assay. The blastocyst cavity fluid (BFC) showed enhanced AGE-fluorescence with excitation and emission 330/405nm, 360/440nm and 440/535nm, respectively, in blastocysts. Besides fluorescent AGEs soluble CML was detectable in the BCF by high-performance liquid chromatography. Various AGE inductors (glucose, methylglyoxal) were used to simulate the AGE accumulation in the blastocyst in vitro. As AGE formation is closely related to oxidative stress we determined first the oxidative status in embryos. Oxyblot analysis revealed a higher rate of oxidized proteins in diabetic blastocysts. The 20s proteasome takes a major part for elimination of damaged proteins in vivo. Measuring their activity via peptide substrate suc-LLVY-MCA showed a noticeable activity in blastocysts.

Taken together poorly controlled maternal diabetes during the preimplantation period leads to metabolic stress in the embryo. Although these results do not provide strict causation between congenital malformation and diabetes, it is likely that AGEs play an important role as stimuli for activating intracellular stress pathways.

(Supported by EU FP7-EpiHealth 278418 and the Wilhelm Roux Programme, MLU)
(14) Analytical method for the quantitative evaluation of α-dicarbonyl compounds \textit{in vivo}  

Christian Henning, Kristin Liehr, Marcus A. Glomb

Institute of Chemistry - Food Chemistry, Martin-Luther-University Halle-Wittenberg, 06120 Halle/Saale, Germany

Regardless of their origin (Maillard-reaction, triose phosphate catabolism, lipid peroxidation) α-dicarbonyls are highly potent precursor structures, which lead to formation of Advanced Glycation Endproducts (AGEs). Reliable quantitation of α-dicarbonyl compounds is prerequisite for a fundamental understanding of the complex reaction pathways of AGE generation \textit{in vivo}. In model systems and various matrices, few structures were already identified \cite{1,2} but a multimethod covering the complete range of relevant dicarbonyl compounds in human blood plasma is lacking. As expected, the α-dicarbonyl intermediates found were related to glucose and L-ascorbic acid degradation.

With the present work we succeeded in the development of an efficient LC-MS/MS multimethod. Derivatisation with \(o\)-phenylenediamine transforms reactive α-dicarbonyl structures into their corresponding quinoxalines. The impact of reaction conditions, particularly of pH, was thoroughly evaluated. A comprehensive validation provided the limit of detection, limit of quantification, intra- and interday coefficients of variation and recovery for all compounds included, i.e. ethanedial (glyoxal), 2-oxopropanal (methylglyoxal), 1-deoxy-\(D\)-erythro-hexo-2,3-diulose (1-deoxyglucosone), 3-deoxy-\(D\)-erythro-hexos-2-ulose (3-deoxyglucosone), \(D\)-arabino-hexos-2-ulose (glucosone), 4,5-dihydroxy-2,3-pentanedione (1-deoxypentosone), 4,5-dihydroxy-2-oxopentanal (3-deoxypentosone), 3,4-dihydroxy-2-oxobutanal (threosone), 1-hydroxy-2,3-butanedione (1-deoxythreosone), 4-hydroxy-2-oxobutanal (3-deoxythreosone), 3,4-dideoxypentos-2-ulose (3,4-dideoxypentosone), dehydroascorbic acid and 2,3-diketogulonic acid. In addition, we compared quantitation results of an external matrix-matched calibration with the standard addition method, which is much more complex.

References:
Sialic acids are widely expressed as terminal monosaccharides on eukaryotic glycoconjugates. They are involved in many cellular functions, such as cell-cell interaction and signal recognition. The key enzyme of sialic acid biosynthesis is the bifunctional UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE), which catalyses the first two steps of sialic acid biosynthesis in the cytosol. In this study we analysed sialylation of muscles in wild type and heterozygous GNE-deficient mice. We found that C57Bl/6 GNE+/− mice showed a significantly lower performance in the initial weeks of a treadmill exercise compared to wild type C57Bl/6 GNE++ animals. When analysing the membrane bound sialylation of C57Bl/6 GNE+/− mice in comparison to C57Bl/6 GNE++ mice, levels of sialic acid were reduced by 33-53% at week 24 and by 12-15% at week 80. Interestingly, membrane bound sialic acid concentration increased with age of the mice by 16-46% in C57Bl/6 GNE++ , but by 87-207% in C57Bl/6 GNE+/−. Furthermore we could identify specific morphological changes in aged muscles.
(16) Effects of Advanced Glycation Endproducts on the proliferation and ageing of human vascular smooth muscle cells

Kathleen Jacobs*, Britt Hofmann, Andreas Simm, Rolf-Edgar Silber, Alexander Navarrete Santos

Department of Cardiothoracic Surgery, Martin-Luther-University, Halle (Saale), Germany

Advanced Glycation Endproducts (AGEs) are posttranslational modifications resulting from non-enzymatic glycation of proteins. AGEs seem to be involved in ageing as well as in the development of cardiovascular diseases. One major cell population of vessels are smooth muscle cells (SMCs). Several studies suggests that binding of AGE-modified proteins modulate intimal SMC properties and is associated with increased proliferative activity. The aim of the study was to assess the effects of AGEs on the cell growth and ageing of primary culture SMCs.

Cultured SMCs were received from media explants isolated from residual bypass graft material (saphenous vein) from patients suffering from coronary heart disease. The proliferative activity was evaluated by alamarBlue® assay and cellular senescence was assessed measuring senescence-associated beta-galactosidase (SA-βgal) activity by cytochemical detection (X-gal). A flow cytometric method using 5-dodecanoylaminofluorescein di-β-D-galactopyranoside (C12FDG), a fluorogenic substrate for SA-βgal activity, was established to quantify the increased lysosomal content of SA-βgal in senescent SMCs.

Primary culture of SMCs yields to a heterogenic population of non-senescent and senescent SMCs. In contrast to the published literature, our preliminary results show that glycolaldehyde induced AGE-BSA (100 – 500 µg/ml) seems to have no effect on the proliferation of the SMCs, on the activation of the pERK/MAP kinase pathway and also on the ageing features of the SMCs. A possible reason for this could be the fact that the SMCs are obtained from veins and not from arteries. Further experiments (e.g. analyzing the AGE receptors) are in progress and should explain the lack of effects.

* Kathleen.Jacobs@student.uni-halle.de
Meanwhile, water-filtered infrared A (wIRA) application has become a widespread approach in medicine, therapy, cosmetics, and even wellness, and thus, the biological effects of its spectrum (780 to 1400 nm) became a focus of experimental research.

Recent publications compared wIRA-induced cellular effects with those induced by UVA/B-irradiation. To name just a few: an induction of the metalloproteinases MMP-1, -2, -3, -9 and -13, of TGF-beta1, activation of the MAPK-pathways was observed, release of cytochrome c and Smac/DIABLO from the mitochondria into the cytosol. Furthermore Bax was translocated from cytosol to nucleus, HSP27 and -70 both increased, as well as ROS formation, while cellular carotenoid concentration decreased and trypase and p53 were induced.

The effects of wIRA-exposure were even assumed to include photooxidation of proteins and long-term effects like premature skin aging as found after chronic UVA/B-irradiation.

Two main effects are currently discussed: Thermal effects that are just induced by wIRA-absorption mainly of the cellular water content, and athermal ones, that may result from a direct interaction of wIRA and non-aqueous cellular structures.

This poster compares both thermal and athermal wIRA effects.

e-mail: tobias.jung@uni-jena.de
Stem cells are vitally involved in tissue regeneration and homeostasis in later life. Mesenchymal stem cells (MSC) are one particular type of tissue specific adult stem cells that can differentiate into mesoderm-type cells, such as osteoblasts, chondrocytes and adipocytes. The progressive loss of differentiation capacity during aging is well acknowledged and is known to cause attenuated regeneration. Various biological stresses are well acknowledged to be drivers of the ageing process. MSC's age-associated altered capabilities, regarding both intrinsic as well as extrinsic parameters in particular appropriately handling and responding to biological stressors is poorly understood.

A common stress response in most eukaryotic cells is to inhibit translation initiation leading to the formation of cytoplasmic RNA-protein complexes referred to as stress granules. Stress granules (SG) contain non-translating mRNAs, translation initiation components, and many additional proteins affecting mRNA function. Stress granules are believed to serve as a decision point for untranslated mRNAs to become stored, degraded or rerouted for translational re-initiation.

We have first investigated how 4-methyl umbelliferrone (4MU), a known inhibitor of the extracellular polysaccharide hyaluronan, impinges on the stemness of MSC. We observed that 4-MU specifically triggers SG formation. Notably, 4-MU induced SG contained distinct mRNA species and thus inferring that through this mechanism stemness and differentiation especially along the osteogenic lineage are being specified. We could further show that 4-MU treatment of MSC mesenspheres leads to 3D essemble closely resembling the in vivo situation of trabecular bone marrow.
(19) Age-dependent effects of saturated and polyunsaturated fatty acids in adult rat cardiomyocytes

L. Li¹, A.-C. Aurich³, D. Stumpp¹, B. Niemann², M. Buerke⁴, S. Rohrbach¹

Institute of Physiology, Justus Liebig University Giessen (1),
Department of Vascular and Cardiac Surgery, Justus Liebig University Giessen (2),
Institute of Pathophysiology, Martin Luther University Halle (3),
Department of Cardiology, Angiology and Intensive Care Medicine, St. Marien Hospital Siegen (4)

Obesity-associated heart disease results in myocardial accumulation of triglycerides and fatty acids leading to lipotoxicity. Among the mediators of lipotoxicity are ceramides, reactive oxygen species or leptin deficiency. However, recent studies are suggestive for protective effects of high fat diets (HFD), also depending on the fat composition. Previous investigations from our group have shown that HFD enriched with the saturated fatty acid palmitic acid (PA) induces structural cardiac changes associated with deterioration in LV diastolic function predominantly in old mice. This was associated with increased myocardial triglyceride, diacylglycerol, NEFA and ceramide content as well as higher caspase 3 activation in old mice. Changes in mitochondrial biogenesis and function suggest a prematurely aged phenotype in young HFD mice without impairing their LV function.

The present study investigated the differential effects of palmitic acid and the polyunsaturated fatty acids docosahexaenoic acid (DHA) on mitochondrial biogenesis, fatty acid oxidation, ceramide formation and cell viability in adult rat cardiomyocytes from young (2 months) and old rats (18-20 months). PA significantly reduced AMPK activation and mitochondrial biogenesis in young cardiomyocytes while DHA showed an opposite effect. Activation of AMPK, an important regulator of cardiac mitochondrial biogenesis, was abolished by simultaneous application of DHA and PA. In old cardiomyocytes, DHA failed to activate AMPK or mitochondrial biogenesis. Cell viability was impaired and caspase 3 activation was increased after PA treatment in young and old cardiomyocytes. DHA, however, increased cell viability without influencing caspase 3 activation in young but not in old cardiomyocytes. Fatty acid oxidation was increased in young cardiomyocytes but not in old cardiomyocytes after treatment with palmitate. This was associated with increasing ceramide content in old cardiomyocytes. Thus, aged but not young cardiomyocytes are unable to respond to higher PA with increased fatty acid oxidation. This inability of old cardiomyocytes to adapt to high fatty acid load may enable channeling of palmitic acid into ceramide synthesis and lead to lipotoxicity.
Endothelial cells (EC) underlie senescence in vivo, which is believed to contribute to endothelial dysfunction and atherosclerosis. Senescence in EC may be triggered by stress-induced pathways and may especially take place in certain inflammatory and oxidative microenvironments. We hypothesize that oxidative stress leads to a decline of proteasomal and lysosomal protein degradation resulting in protein aggregate accumulation, which promotes senescence and cellular dysfunction. The current project aims to establish a model of oxidative stress-induced premature senescence in EC and to characterize protein degradation pathways in these cells.

The study was performed in human umbilical vein EC (HUVEC). To obtain chronic oxidative stress, 100 or 200 µM H₂O₂ were supplemented to growth medium containing 10% fetal calf serum on a daily basis. Several parameters analyzing oxidative stress (protein carbonylation in Western blots) or senescence (staining of senescence-associated-β-galactosidase (SA-β-gal) and the proliferation marker Ki-67) were assessed up to 8 days. To characterize proteolytic capacity of cells, fluorogenic substrates specific for peptidyl-glutamyl-like (β1 subunit), trypsin-like (β2 subunit) and chymotrypsin-like (β5 subunit) activities of the 20S proteasome were employed. The expression of proteasomal subunits was analyzed by Western blotting. Our results show that H₂O₂ treatment induces considerable protein carbonylation. In parallel, a time and dose-dependent increase of SA-β-gal positive cells (45 % of total cell numbers at 8 days after treatment with 200 µM H₂O₂) as well as growth arrest were observed. At the same time proteasomal activities, especially the chymotrypsin-like activity, declined. In contrast, the expression levels of the proteasomal subunits remained unchanged.

Taken together, our data show that chronic oxidative stress leads to premature senescence in EC and a decline of proteolytic activities. Further studies will be required to understand whether and how these changes are linked and to characterize responsible mechanisms.
Introduction: Transition from home care to nursing home is a decisive moment in the trajectory of dementia care. Decision-making in this context is not well understood. We investigated why professional caregivers consider nursing home admission as necessary and whether their judgments differ across countries. The analysis is part of the EC 7th framework project RightTimePlaceCare with England, Estonia, Finland, France, Germany, Spain, Sweden and the Netherlands.

Methods: Professional caregivers of persons with dementia living at home were asked to openly report anticipated reasons for nursing home admission. Answers were translated into English and inductively categorized, after 20% were checked for reliability and validity.

Results: Unstructured risk assessments of 1162 professional caregivers were eligible for analysis. The content analysis of anticipated reasons for admission revealed 25 main categories containing several subcategories. In the participating countries, the following 5 main categories were most often identified: caregiver burden (39%, n=453), caregiver inability to care (20%, n=235), neuropsychiatric symptoms (18%, n=204), overall deterioration (16%, n=185) and ADL dependency (11%, n=128). Professionals in Finland more frequently mentioned patient-related characteristics; caregiver burden was identified in only 26% of all Finnish cases. Risk assessment of Spanish professionals focused primarily on caregiver-related factors: burden was identified in 72% and caregiver inability to care in 15% of all Spanish cases.

Conclusion: Professional risk assessment for institutionalisation considered caregiver and patient-related aspects, whereas caregiver-related aspects were predominantly mentioned. In this regard, our analysis revealed interesting variations between countries. Analysis of subcategories and comparison between formal and informal caregivers is ongoing.
Aortic aneurysms are characterized by weakened and distorted arterial architecture and are relatively common causes of death because of arterial dissection or rupture. The most common causes of aortic aneurysm are congenital genetic defects in the aortic wall occurring in various pathological conditions such as Marfan syndrome and bicuspid aortic valve (BAV).

BAV, the most common congenital cardiac malformation, is associated with abnormalities of the ascending aortic wall. Whether mechanisms related to the existing aneurysmal alterations in BAV are due to genetic heterotopy and/or wall stress induced by flow turbulence requires further investigation.

Endothelial nitric oxide synthase (eNOS) is associated with the development of BAV in mice. A high incidence of aortic aneurysms was detected in eNOS/apolipoprotein E double-knockout mice. Recent studies also reported a significant decrease in the amount of the eNOS protein present in aneurysmal BAV aortic tissue compared with tricuspid aortic valve (TAV) tissue. Local differences in eNOS protein expression were observed in BAV compared with TAV aortic aneurysms, probably due to variations in aortic wall shear stresses.

In this summary, we review our present understanding of aortic aneurysms pathogenesis, particularly in patients with MFS and BAV. We discuss the genetic basis and basic pathology underlying BAV and ascending thoracic aortic aneurysms, and compare these to known mechanisms underlying MFS. In addition, we discuss recent insights into pathophysiology.
Ivabradine in combination with beta-blocker improves symptoms and quality of life in patients with stable angina pectoris: age-related results from the ADDITIONS study

Ursula Müller-Werdan, G. Stöckl*, Henning Ebelt, Sebastian Nuding, Florian Höpfner, Karl Werdan

Several clinical trials have demonstrated the antianginal and anti-ischemic efficacy of ivabradine in combination with beta-blocker in patients with stable angina pectoris. The ADDITIONS (PrActical Daily efficacy anD safety of Procoralan In combinaTION with betablockerS) study evaluated the efficacy, safety, and tolerability of ivabradine added to beta-blocker, and its effect on angina symptoms and quality of life in routine clinical practice.

This non-interventional, multicenter, prospective study included 2,330 patients with stable angina pectoris of different age groups treated with a flexible dose of ivabradine twice daily in addition to beta-blocker for 4 months. The parameters recorded included heart rate, number of angina attacks, nitrate consumption, tolerance, and quality of life.

After 4 months ivabradine (mean dose 12.37 ± 2.95 mg/day) reduced heart rate by 19.4 ± 11.4 to 65.6 ± 8.2 bpm (p<0.0001). The number of angina attacks was reduced by 1.4 ± 1.9 per week (p<0.0001), and nitrate consumption by 1.9 ± 2.9 U per week (p<0.0001). At baseline (i.e., on beta-blocker), half of the patients (51%) were classified as Canadian Cardiovascular Society (CCS) grade II; 29% were CCS grade I. After 4 months’ treatment with ivabradine, most of the patients were CCS grade I (68%). The EQ-5D index improved by 0.17 ± 0.23 (p<0.0001). The overall efficacy of ivabradine was considered by the physicians as “very good” (61%) or “good” (36%) in most patients. Suspected adverse drug reactions were documented in 14 patients; none were severe.

Collagen analysis of the aging aorta and in aortic aneurysms associated with bicuspid aortic valve disease

Alexander Navarrete Santos¹, Peter Lochmann¹, Andreas Simm¹, Rolf-E. Silber¹, Hans H. Sievers², Salah A. Mohamed²

¹Department of cardiothoracic surgery Martin Luther University, Halle-Wittenberg; ²Department of Cardiac and Thoracic Vascular Surgery, UKSH-Campus Lübeck, Germany

Aim: Aneurysm is a common occurrence in the elderly and patients with bicuspid aortic valve (BAV) disease. The aim of the current study was to characterize collagen content in advanced glycation end products (AGEs) of aneurysmal aortic tissue from BAV patients and control tricuspid aortic valve (TAV) patients.

Materials and Methods: Aneurysmal specimens were collected from 11 BAV patients (mean age, 52.5 ± 17.0 years) and 6 TAV patients (mean age, 68.0 ± 6.8 years). Collagen from the aortic tissue was isolated by enzymatic digestion using pepsin (PEF) and the non-digested material was further digested using cyanogen bromide (CNBr; CNBr fraction), whereas insoluble collagen was extracted by HCl hydrolysis (HF). The AGE content in the different collagen fractions was analyzed by measuring AGE fluorescence. The AGE-modified carboxymethyllysine (CML), pentosidine, and argpyrimidine in the CNBr fraction were immunologically analyzed via slot blot analysis. The immunological detection of carbonyl groups introduced into proteins by oxidative reactions was analyzed in the CNBr fraction using the Oxyblot protein detection kit. The collagen content in the different fractions was quantified using the 4-hydroxyproline assay.

Results: Samples of TAV tissue showed diminished fluorescence of the pepsin extracted fraction compared to BAV tissue. CML and pentosidine content in BAV tissue was significantly higher in comparison to that in TAV tissue. Within the BAV group, the amount of pentosidine, CML, and carbonyl groups detected in the CNBr collagen fraction was significantly correlated with AGE-fluorescence. A significant correlation between CML and pentosidine content in the CNBr fraction was also detected. In the BAV tissues, increased pentosidine and carbonyl group content and AGE-fluorescence in the CNBr fraction during aging was observed.

Conclusion: Collagen analysis of AGE content in thoracic aortic aneurysms is critical to understand the nature of early BAV-associated aneurysms.

Corresponding author: salah.mohamed@uk-sh.de
The incidence of proximal humerus fractures is currently approx. 5% and will rise due to demographic developments. This is a fracture that is typically associated with osteoporosis in geriatric patients. While Neer type I and III/2 fractures can often be treated successfully using conservative measures, surgery is usually indicated for multifragmentary fractures of the humeral head. The choice of surgical procedure is influenced by the presence of osteoporosis and the post-traumatic damage to the blood supply to the head of the humerus, as well as the number of fragments and degree of dislocation. In addition, degenerative changes in the rotator cuff and omarthrosis are often also present in a high percentage of elderly patients.

In most cases, x-rays taken in the 3 standard planes, AP, axial and Y views, are sufficient for pre-operative diagnostics. Complex fractures require computer tomographic imaging. Osteosynthesis can be conducted in cases where there are no signs of omarthrosis or superior displacement of the humeral head and where the risk of post-traumatic necrosis of the humeral head is low. Alternatively, a humeral head replacement is planned, especially in cases of head-split fractures.

The implantation of a fracture prosthesis is a pure humeral head replacement is currently regarded as the gold standard. Fourth generation implant models can restore the correct centre of rotation in 3 planes. However, attachment of the rotator cuff tendons to the body of the shoulder implant is crucial to its functional outcome. In spite of correct surgical techniques and consistent restrictive follow-up treatment, migration or lysis of the tubercula often occurs. The subsequent decentralization causes restricted mobility, superior displacement of the replacement and subluxation. For this reason, implantation of a reverse total shoulder prosthesis should be preferred if strong degenerative changes to the rotator cuff, in particular the M. supraspinatus tendon, are detected intra-operatively, or in cases of rupture of the rotator cuff. The reversal of the ball and socket configuration prevents secondary subacromial impingement. The distal shift in the centre of rotation increases the deltoid lever arm, such that this broad muscle facilitates an adequate everyday range of motion (ROM) for anteversion, retroversion and abduction. The attechament of tuberculas is not so important for in these cases. Implantation of a reverse total replacement is recommended above the age of 75 due to the limited lifetime of the glenoid component.
(26) MP-activated protein Kinase and mediate cardio-protection in a model of premature cardiac senescence

Bernd Niemann*, Hassan Issa§, Ling Li$, Rolf-Edgar Silber#, Andreas Böning*, Susanne Rohrbach$

* Department of Adult- and Pediatric- Cardiac and Vascular Surgery, UKGM, Justus Liebig University Giessen
§ Department of Pediatric Cardiology, Martin Luther University Halle-Wittenberg
$ Institute of Physiology, Justus Liebig University Giessen
# Department of Cardiac- and Thoracic Surgery Martin Luther University Halle-Wittenberg

Introduction: Ageing is associated with a loss of AMP-activated protein kinase (AMPK) function, dysbalanced adipocytokine-signalling, cardiac hypertrophy and loss of cardiac function. Here, we investigated the impact of CR and/or antagonism of the cannabinoid type-1 (CB1) receptor, (Rimonabant) in an experimental model of afterload induced cardiac dysfunction (aortic banding) on left ventricular and mitochondrial function.

Hypothesis: Adipokine-mediated AMPK-activation results in improved diastolic and systolic cardiac function. Modulation of AMPK-activation (via CR/CB1-blocker), cell survival and mitochondrial biogenesis participate in this effect. Methods: Male Wistar rats underwent aortic banding or sham operation. After 4 months animals (failing/sham) were matched (cardiac function) and treated with standard chow, CR (-40%) and/or medical therapy (CB-1-blockade, Rimonabant) for 12 weeks. Follow up was performed via echocardiography and Millar-Tip-catheter. Paraformaldehyde-conserved and cryo-conserved samples were stained (cross sectional area, collagen content, TUNEL, COX, SDH). Mitochondrial function (microrespirometry, maximal complex-activity), adipokine expression, markers of apoptosis, afterload and markers of cardiac metabolism were analyzed. Results: Aortic banding induces cardiac systolic and diastolic dysfunction and later on congestive heart failure. These hearts show reduced mitochondrial respiration and AMPK-activation. CR/Rimonabant restored cardiac function and reduced BNP and proapoptotic activation. Cardiac hypertrophy / collagen content are reduced in both interventional groups. Adiponectin release from adipose tissue contributes to an increased AMPK-phosphorylation, induction of mitochondrial biogenesis and thus stabilization of mitochondrial function. CR and CB1 blockade show comparable effects without developing additive potency. Conclusion: Metabolic reoptimization and AMPK activation protect the dysfunctional myocardium. Medical interventions (CR-mimetics), may be an additional therapeutic option for perioperative stabilization and riskreduction of senescent patients, suffering from cardiac diastolic and systolic dysfunction, impaired adipocytokine signaling and impaired AMPK-activation and complex-I dysfunction.
Most age-associated diseases are based on modified proteins, lipids or nucleic acids and their accumulation in cells and tissues. Accumulation of these modified biomolecules occurs over time and is characteristically for normal aging. Elevated levels of these protein modifications are associated with many chronic diseases, such as diabetes, inflammatory disorders, Alzheimer’s disease and renal failure. Accumulation of modified proteins occurs due to an increase in oxidative stress, decrease of antioxidative defense or proteolytic activity. To obtain more information on protein turnover in young and senescent cells we examined interactions of the proteasomal and the lysosomal system using ferritin as a model protein. It is composed of 24 protein subunits, which are divided into two isoforms, ferritin light chains (ferritin L) and ferritin heavy chains (ferritin H). It has been described that the proteasomal and the lysosomal system are both involved in the degradation process of ferritin. The interactions of both proteolytic systems were analyzed and compared in young and in senescent human fibroblasts. While higher basal levels of ferritin H were quantified on protein levels in senescent cells, mRNA expression of ferritin H was higher in young cells. Also the inducibility of ferritin showed a significantly higher increase in young cells. In addition, the degradation rate of ferritin in young and senescent cells was studied, resulting in a reduced degradation rate of ferritin H in senescent cells. By inhibiting both proteolytic systems, we are able to show that especially the lysosomal system plays a crucial role in ferritin turnover. The comparison of young and senescent cells showed that the activity of some lysosomal proteases is up-regulated in senescent fibroblast. The higher activity of the lysosomal system but still the reduced degradation rate of ferritin in senescent cells, maybe due to an impaired protein uptake into the lysosomes. The impaired protein degradation during aging will be examined in further experiments concerning the uptake of ferritin in lysosomes.
(28) Vascular smooth muscle cells amplify the induction of the NF-κB-pathway in monocyte-macrophages

Hannes Petruschke, Kathleen Jacobs, Sandy Pötzsch, Andreas Simm, Rolf-Edgar Silber, Harald Loppnow*, Alexander Navarrete Santos

Department of Cardiothoracic Surgery, University Hospital Halle (Saale)
*Department of Medicine & Heart Centre, University Hospital Halle (Saale)

**Background**: Monocytes are able to communicate with vascular smooth muscle cells (VSMC). In early stage of atherosclerosis monocytes migrate through the blood vessel wall and activate VSMC affecting plaque progression, by a not fully understood mechanism. In order to obtain further details in the interaction between VSMC and monocytes/macrophages a monocyte/macrophage NF-κB reporter cell line was co-cultured with VSMC isolated from graft vein material. We questioned whether VSMCs were able to further activate the NF-κB-pathway in the LPS stimulated monocytes/macrophages. In addition the effect of advanced glycation end products (AGEs) on our cell-culture model was analyzed.

**Methods**: VSMC were obtained from media explants isolated from residual bypass graft material (saphenous veins). As reporter gene under transcriptional control of an NF-κB response element monocytes/macrophages RAW 264.7-cell line expresses the secreted form of alkaline phosphatase (SEAP). The amount of secreted enzyme was analyzed via absorption measurement of p-nitrophenylphosphate, which is transformed after reaction with SEAP. In stimulation experiment LPS was used in a concentration range of 5 – 500 ng/mL and glyoxal (GO) / methylglyoxal (MGO) induced BSA-AGE 10 – 100 µg/ml.

**Results**: A 10-fold increase in the activation of the NFκB-pathway was observed when LPS stimulated monocytes/macrophages were co-cultured with VSMC. In contrast BSA or GO/MGO-BSA-AGE did not show any effect on the NFκB-pathway activation. Further VSMC increased proliferation of monocytes/macrophages.

**Outlook**: Whether the contact of monocytes/macrophages with VSMC or the secretion of an unknown factor is responsible for enhanced NF-κB pathway activation will be elucidated.

Corresponding author: h.petruschke@gmx.net
Glycolysis and gluconeogenesis are reciprocally controlled central metabolic pathways. Addition of glucose to yeast cells growing on a nonfermentable carbon source causes proteolytic breakdown of the gluconeogenetic enzyme fructose-1,6-bisphosphatase (FBPase) via the ubiquitin-proteasome system. Our recent work defines a novel E3 ubiquitin ligase complex (Gid complex) of at least 7 subunits responsible for the metabolic switch from gluconeogenesis to glycolysis. Most ageing studies focus on changes of transcription during lifetime of a cell. These studies imply a correlation between changes in glucose metabolism and ageing with a shift from glycolysis towards gluconeogenesis. However, less focus is put on alterations in protein levels or enzyme activities during ageing. Therefore, we plan to directly study changes of enzymatic activity and posttranslational modifications (PTM), such as ubiquitination or sumoylation of key glycolytic and gluconeogenetic enzymes during ageing with *Saccharomyces cerevisiae* as a model organism. We intend to compare enzymatic activity and protein levels of the key gluconeogenetic enzymes Fructose-1,6-bisphosphatase (FBPase), Pyruvatecarboxykinase (PEPCK) and malate dehydrogenase (Mdh2) between young and aged yeast cells. Changes in PTMs of these enzymes and their effect on activity/stability during ageing will be measured using Western blot or mass spectroscopy analysis with focus on ubiquitination/sumoylation and the involvement of the Gid complex.
(30) Molecular genetic investigation of the LMNA-Gene in Lipodystrophy Patients


*Institute of Human Genetics, University Medical Center Halle,
** Institute for Human Genetics, University Medical Center Leipzig,
***Internal Medicine Clinic, University Medical Center Leipzig

Patients/Methods: 21 patients (17 female and 4 male) from 19 families with suspected diagnosis of lipodystrophy were included. Mutation screening of the LMNA-Gene was performed by Sanger sequencing. The individual clinical characteristics of the mutation carriers were classified in accordance with the criteria of the recent AACE Consensus Statement about detection of lipodystrophy (Handelsman et al. (2013) Endocr Pract.). Results: 6 female patients were identified as LMNA-mutation carriers and showed the following clinical characteristics: Main features: All patients were characterized by a loss of subcutaneous adipose tissue in the extremities, a clinical main feature of FPLD usually developed in puberty. However, only 4 out of the 6 mutation carriers developed this clinical sign at this typical period. Supporting features: All the 4 patients with diabetes did not manifest an increased daily need of insulin about 2 IE/kg. Except 2 patients with a mild hirsutism, as a sign of PCO-like symptoms, there were no more indications of a severe insulin resistance. 3 of 6 patients exhibited a hypertriglyceridemia (>= 250 mg/dl) despite treatment or diet. Moreover, in the case history of no one mutation carrier a pancreatitis was stated. A steatosis hepatitis was known of 2 patients, but only one of them manifested elevated transaminases. 4 patients declared similar symptoms of their family members. Conclusion: 6 of 21 persons included were identified to be LMNA-mutation carriers in association with their clinical diagnosis FPLD2. They also manifested the main feature lipoatrophy in the extremities.

Because of a variable clinical manifestation caused by a variety of supporting features as well as the difficult diagnose of a lipoatrophy (vs healthy phenotype) a genetic analysis should be included in the confirmation of the early diagnosis of FPLD.
Identification of gliadin as an advanced glycation end product-modified compound in bread crust extract and their effect on mouse macrophage activation

S. Pötzsch1*, M. Dalgalarrondo2, B. Bakan2, D. Marion2, V. Somoza3, R.-E. Silber1, A. Simm1, A. Navarrete Santos1

1 Department of Cardiothoracic Surgery, University Hospital Halle (Saale), Germany
2 Unité Biopolymères, Interactions, Assemblages, Institut National de la Recherche Agronomique, Nantes cedex 3, France
3 Department of Nutritional and Physiological Chemistry, University of Vienna, Austria

Advanced glycation end products (AGEs) represent non-enzymatic posttranslational modification-derived products, which are thought to play a role in age-related diseases, like diabetes and Morbus Alzheimer. AGEs accumulate endogenously or exogenously by food intake. The dietary AGEs significantly increase the AGE pool in the body. Therefore nutrition seemed to be a very strong factor to influence the rate of aging. Previous studies reported an oxidative as well as antioxidative capacity for food derived AGEs. Our group used bread crust (BCE) as an AGE-rich dietary extract, which induced a moderate elevation of ROS production causing an activation of p42/p44MAPK, p38MAPK and NF-κB in cardiac fibroblasts. However it is still unclear which protein/peptide is the active compound as well as what kind of modification leads to their formation. The present work focuses on the identification of the bioactive compounds in bread crust extract. The amino acid analysis by RP-HPLC and LC-MS/MS by Orbitrap Velo was used to determine these compounds. We identified some gliadines/secalines in the bread crust extract and by means of boronate affinity chromatography the majority of these proteins in the BCE seemed to be glycated. The soluble BCE was fractionated by use of a reversed Phase-HPLC with a Zorbax 300SB-C18 column. 31 fractions were collected and analyzed regarding their typical AGE fluorescence at 360/440 nm as well as 330/405 nm. The results were checked by immunoblotting with specific antibodies against AGE’s and ω5- and γ-gliadin. Fractions no. 17 till 31 showed a very strong signal for ω5- and γ-gliadin as well as for Carboxymethyllysine (CML), Pentosidine and Imidazolone. Preliminary results showed that gliadin stimulate macrophages due to mechanisms including activation of the MAPKinases (p42/p44MAPK, p38MAPK). Further experiments are still in progress to elucidate the results.

* sandy.poetzsch@student.uni-halle.de
Previously, we showed that calcium decay was slowed and calcium sensitivity of myofilament ATPase activity was reduced in aged wild-type ventricular myocytes. Consequently, kinetics of contraction/relaxation was decelerated. We attributed this contractile dysfunction of aged ventricular myocytes to an increased superoxide formation by upregulated cardiac NADPH oxidases (NOX2/NOX4). Since NOS1 is also upregulated in aged murine hearts, we wondered whether NOS1 could play a role in cardiac aging. Hence, we analyzed isolated ventricular myocytes from young (2-4 months) and aged (24-26 months) wild-type and NOS1^{-/-} mice. Contractions (sarcomere shortening, MyoCam system), calcium transients (Indo-1 fluorescence) and myofilament ATPase activity (blebbistatin-sensitive phosphate generation) were evaluated. Expression of NADPH oxidase subunits and components of the cardiac renin-angiotensin system were quantified (real-time PCR). Here, we report that age-dependent contractile dysfunction does not occur in ventricular myocytes from NOS1^{-/-} mice. Calcium homeostasis and calcium sensitivity of myofilament ATPase activity are unchanged with aging. This can be explained, at least partially, by the finding that cardiac NOX expression and NADPH oxidase activity are not increased in aged NOS1^{-/-} mice. The renin-angiotensin system is a well-known inducer of NADPH oxidase-dependent radical formation. We found that renin, the enzyme that catalyses the rate-limiting step within the cascade, is induced in hearts of aged wild-type mice but not aged NOS1^{-/-} mice.

We conclude that upregulation of NOS1 contributes to oxidative stress in aged myocytes via activation of the intra-cardiac renin-angiotensin system that in turn induces NOX expression. Increased NADPH oxidase-dependent superoxide formation then interferes with the contractile function of aged murine ventricular myocytes.
Mitochondrial production of reactive oxygen species (ROS) is suggested to play a key role in organismal ageing. The specific role of mitochondrial pathways in this process is still unclear. We presumed that a dysfunction of parts of the mitochondrial respiratory chain leads to enhanced oxidative stress, ROS production, DNA damage, cellular senescence and ageing. The identification of relevant respiratory chain molecules and pathways activated by these should provide deeper insights into the process of ageing. We used different mouse strains with defined and stable mutations in mitochondrial genes encoding for respiratory chain proteins of complex I-V and uncoupling protein 2. Primary skin fibroblasts of mutated mice were isolated and exposed to cellular stress exerted by genotoxic agent doxorubicin, UVB irradiation and hydrogen peroxide, respectively. Analysis of expression and activation of different age-related pathways like p53, p38, JNK, and NF-κB, age-related histones (H3K9 and γH2AX) and β-galactosidase was performed. Mice were analyzed at different time points (0, 3, 6, and 12 months). One of six analyzed strains, C57BL/6J-mt^ALR/LTJ, with a single polymorphism in NADH dehydrogenase subunit 2 gene in complex I, shows phenotypically markers of ageing such as hair loss and decreased fibroblast growth in contrast to the control strain C57BL/6J-mt^AKR/J. Interestingly, this strain showed decreased ROS and ATP basal level in contrast to the control strain in 12 month old mice. Immunoblots showed that age-related marker H3K9me3 and stress signaling protein IκBα were differentially expressed/ activated between C57BL/6J-mt^ALR/LTJ and control mice after doxorubicin treatment. Based on these findings, it might be suggested that mitochondrial DNA mutation-associated ageing induce individual reaction pattern in ageing fibroblasts, which points to putative new treatment targets.
(34) Oxidative Regulation of Voltage-gated Sodium Channels in Dorsal Root Ganglia Neurons

Martin Schink, Jana Schirmeyer, Enrico Leipold, Roland Schönherr and Stefan H. Heinemann

Center for Molecular Biomedicine, Department of Biophysics, Friedrich Schiller University Jena & Jena University Hospital, 07745 Jena, Germany

Dorsal root ganglia (DRG) are the main relay stations between the periphery and the central nervous system. Oxidative stress and a decay of the antioxidant system result in aberration of electrical signalling, particularly in aged organisms. This process appears to be linked to an accumulation of oxidatively modified residues in membrane proteins involved in ion transport. Here we studied the impact of oxidative stress (generated via blue-light excitation or by application of the mild oxidant chloramine-T, ChT) on the electrical properties of mouse DRG neurons. While oxidative stress profoundly prolonged the action potential repolarisation time in wild-type animals, the impact was much smaller in animals with deficient Na\textsubscript{v}1.8 sodium channels (Na\textsubscript{v}1.8\textsuperscript{+/-}), which play a pivotal role in the generation of electrical signals in DRG neurons. The underlying ionic currents were measured in the whole-cell voltage-clamp mode. In order to eliminate a confounding contribution of Na\textsubscript{v}1.9 channels, the function of Na\textsubscript{v}1.8 channels was studied in DRG neurons of Na\textsubscript{v}1.9\textsuperscript{+/-} mice. Application of 10 µM ChT decreased the peak current at 0 mV by more than 15% within 150 s. On a slower time scale, channel inactivation was progressively slowed down. Irradiation with blue light did not diminish the peak current but also resulted in progressive loss of inactivation. We also investigated the effect of oxidative stress on recombinant Na\textsubscript{v}1.8 and other Na\textsubscript{v} channel types in a heterologous expression system. Application of 10 µM ChT on Na\textsubscript{v}1.8 channels expressed in N2A cells caused an initial peak current reduction at 0 mV to about 40% (hNa\textsubscript{v}1.8) and 60% (rNa\textsubscript{v}1.8). At longer time scale this effect was compensated by progressive loss of inactivation. In contrast, Na\textsubscript{v}1.4 from skeletal muscle showed only minor changes in current phenotype upon application of 10 µM ChT. In summary, Na\textsubscript{v}1.8 channels are strongly dependent on the oxidative status of the cell and hence undergo diverse functional changes, occurring on different time scales, when exposed to oxidative stress. The molecular mechanisms underlying this regulation remain to be elucidated.
(35) Epidermal growth factor receptor is involved in the protection against age related hypertension and heart hypertrophy

**Barbara Schreier**, Sindy Rabe, Sigrid Mildenberger, Mirja Hünerberg, Daniel Bethmann, Michael Gekle

Julius-Bernstein Institute of Physiology, Martin-Luther University Halle-Wittenberg, Germany

The epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, activates various signaling pathways, thereby regulating e.g. cell proliferation, survival and differentiation. In addition to activation by its classical ligands, EGFR is also subject to activation by cross-talk with non-receptor tyrosine kinase pathways - a mechanism called transactivation. Both mechanisms may induce pathophysiological effects leading for example to vascular dysfunction and remodelling.

To investigate the role of EGFR in the cardiovascular system in aging we generated a mouse model with an inducible deletion of the EGFR in vascular smooth muscle cells (VSMC). In these KO mice the EGFR mRNA was reduced to 25% in the aortas 56 days after induction, while there was no difference in hearts or skeletal muscle compared to WT animals. In 4 month old (young), conscious KO mice systolic blood pressure was reduced compared to WT. This was confirmed by Millar catheter blood pressure measurements, where we were able to detect a reduced diastolic and mean blood pressure. Interestingly, there was no significant difference in blood pressure between the two genotypes in aged (10 month) mice. Upon angiotensin II infusion, we found a reduced duration of increased blood pressure in aged KO mice. Interestingly, we also found a significantly lower heart weight/tibia length ratio in aged KO mice compared to as well aged WT mice or young KO mice.

Taken together the presented data indicate, that the EGFR is necessary for physiological blood pressure homeostasis and that it is involved in age related blood pressure increase. We hypothesize that by decreasing the blood pressure due to EGFR deletion age induced heart hypertrophy can be inhibited. To elucidate the underlying mechanisms, further studies need to be performed.
(36) Adaptation mechanisms to metabolic stress in endothelial cells

Katrin Spengler, Silke Lindenmüller, Doreen Weigel, Regine Heller

Institute for Molecular Cell Biology, Centre for Molecular Biomedicine (CMB), Jena University Hospital, Hans-Knöll-Str. 2, 07745 Jena

Endothelial cells (EC) are characterized by glycolytic energy production and by a high capacity to increase this metabolic pathway if needed. However, additional energy-generating pathways may be important under certain conditions such as ischemia. The current project aims to clarify whether EC may undergo a metabolic switch to compensate for energy restriction under conditions of inhibited glycolysis.

Our study was performed in human umbilical vein EC (HUVEC). 2-deoxyglucose (2-DG, 20 mM, 5 min – 7h), a known inhibitor of glycolysis led to a rapid and longlasting decrease of intracellular ATP levels. A maximal reduction occurred after 1 h (60%) and was followed by a slow but incomplete recovery. In parallel, a rapid and sustained activation of the energy-sensing enzyme AMP-activated protein kinase (AMPK) was detected, which was monitored by phosphorylation of threonine 172, an activating phosphorylation site. Furthermore, AMPK-mediated inhibitory phosphorylation of acetyl-CoA carboxylase (ACC), a negative regulator of fatty acid oxidation, and enhanced fatty acid oxidation from exogenous or endogenous fatty acids were seen. However, changes in fatty acid oxidation were only marginally dependent on the AMPK-ACC axis as evidenced by siRNA-mediated downregulation of the respective enzymes. Importantly, 2-DG led to a transient activation of autophagy, a self-digestion pathway, which supplies nutrients and contributes to stress adaptations and longevity. Autophagy induction was partially mediated by AMPK as indicated by AMPK-mediated phosphorylation of ULK1, which is involved in the initiation of autophagy. In addition, inhibition of the mTOR pathway, known to repress autophagy, was seen. Autophagy was monitored by detecting conjugated LC3B, a marker protein of autophagosomes, in Western-Blots and immunofluorescence. Taken together, our results demonstrate that endothelial cells respond to inhibition of glycolysis with an increased fatty acid oxidation and activation of autophagy. The observed adaptive mechanisms may support survival under metabolic stress and help to prevent endothelial dysfunction, which is a hallmark of endothelial ageing and age-related vascular diseases.
Colorectal cancer (CRC), which accounts for over 9% of all cancer incidence, is a major cause of morbidity and mortality throughout the world. An increased body mass index (BMI) is an established risk factor for CRC. BMI was related to CRC risk for younger but not for older individuals. Therefore, diet can strongly influence the risk of CRC in younger persons. Changes in food habits might reduce up to 70% of this cancer burden, while diets high in red meat, refined foods, and unhealthy fats have been linked to an increased risk of the disease. Furthermore, higher dietary glycemic load and total carbohydrate intake were statistically significant associated with an increased risk of recurrence and mortality in patients with advanced CRC. The long-term objective of our study is to characterize the role of energy balance factors in colon cancer progression at the molecular level. cDNA arrays of the two CRC cell lines HT29 and RKO before and after nutrient depletion revealed an altered gene expression pattern with 6,662 and 5,861 annotated, differentially expressed genes for HT29 and RKO, respectively (p<0.005). Therefore, depletion of nutrients has significant impact to transcriptome in both cell lines. Energy-dependent activation of AMP-activated protein kinase (AMPK), which detect and react to fluctuations in the AMP:ATP ratio that take place during nutrient depletion, modulates longevity via post-translational modification CREB-regulated transcriptional co-activator-1 (CRTC-1). Since the resulting down-regulation of crtc-1 increases lifespan/longevity, the changes within the gene expression pattern after nutrient depletion could also be relevant with respect to lifespan extension and aging.
It is widely recognized that stress causes universal detrimental effects on health and longevity due to intensified molecular damage. Stress-induced increase of production of reactive oxygen species is a potent contributor to progressive loss of cellular homeostasis and accelerated ageing. Nevertheless the organism possesses an ability to withstand the oxidative lesions due to activity of antioxidant systems. The extent of repair potential presumably depends on gender and age. We found that a short term acute psychosocial stress caused a significant increase of catalase activity in whole saliva of young people that was accompanied by a pronounced decrease of concentration of oxidatively modified proteins. The hydrogen peroxide scavenger catalase is an important player in antioxidant protection. Both total protein content and concentration of thiobarbituric acid reactive substances did not change significantly. We identified significant gender differences in the prooxidant-antioxidant response of whole saliva of the participants. The increase of catalase activity as well as the decrease of concentration of oxidatively modified proteins was much greater in women compared to men. Our findings indicate that acute short-term psychosocial stress has a supposed beneficial effect in young people due to activation of antioxidant protection mechanisms in whole saliva. Furthermore, the boost of the antioxidant function is more manifested in women. Therefore it is important to explore whether a similar trend is observed at older ages. In addition, our findings suggest that antioxidant response in whole saliva is activated immediately after the onset of acute psychosocial stress that can have implications in illumination of mechanisms by which antioxidant function is activated.
The forkhead box protein O1 (FoxO1) is a member of the forkhead box family. FoxO1 is a transcription factor and involved in different cellular processes, like DNA damage repair, oxidative stress response or gluconeogenesis. It is dysregulated in various diseases. Knockout of FoxO1 in mouse leads to early embryo lethality (E10.5). Here we report on the regulation of FoxO1 in preimplantation rabbit embryos.

Expression and phosphorylation levels of FoxO1 were measured by RT-qPCR and Western Blot in blastocysts of different gastrulation stages at day 6 post coitum. In blastocysts FoxO1 was higher expressed in embryoblast than in trophoblast cells. The phosphorylation rate decreased significantly from gastrulation stage 1 to 2.

To test the influence of nutrient stimulation on FoxO1 phosphorylation, we cultured day 6 blastocysts in vitro with L-leucine, glucose and insulin. L-leucine supplementation led to a 1.7 fold increase in FoxO1 phosphorylation after 30 minutes. When rabbit blastocysts were cultured with various glucose concentrations (0mM, 10mM and 25mM) for 6 hours, phosphorylation and protein amount of FoxO1 were not altered. In vitro stimulation with 17nM insulin led to an enhanced FoxO1 phosphorylation after 30 minutes.

Experimental diabetes type 1 in female rabbits was used to analyse the regulation of FoxO1 in early diabetic pregnancy. Maternal diabetes had no effect on expression and phosphorylation of FoxO1 in day 6 blastocysts.

Our results demonstrate that FoxO1 is sensitive to L-leucine and insulin and may function as a regulatory protein in preimplantation embryos. So far, we could not show effects of a maternal diabetes type 1 on embryonic FoxO1 expression.

(Supported by EU FP 7 EpiHealth (N°278418) and Cost Action TD 1101: RGB-Net)
TSG (2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside) from the Chinese herb Polygonum multiflorum increases lifespan and stress resistance in Caenorhabditis elegans

Zhao L.², Büchter C.¹, Wätjen W.*¹

¹Martin-Luther-Universität Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, “Biofunctionality of Secondary Plant Compounds”, Weinbergweg 22, 06120 Halle (Saale), Germany,
²Heinrich-Heine-Universität, Institute of Toxicology, P.O. Box 101007, 40001 Düsseldorf, Germany;

Tetrahydroxystilbeneglucoside (TSG) was isolated from Polygonum multiflorum, a plant that is widely used as a traditional anti-aging agent in East Asian countries. It is known that the stilbene resveratrol increases the lifespan of Caenorhabditis elegans, however for the structural analogue TSG this is not known. We analysed the effects of TSG in C. elegans in comparison to resveratrol; the flavonoid quercetin was used as a reference compound.

TSG showed strong antioxidative effects in a cell-free assay (TEAC assay) and also in the nematode (DCF-assay). Treatment with TSG decreased the level of lipofuscin, a pigment consisting of highly oxidized and cross-linked proteins. This pigment is generally considered as a biomarker of ageing in diverse species. TSG prolongs the mean, median and maximum adult lifespan of C. elegans by 23.5, 29.4 and 7.2 %, respectively. These results are comparable to the effects of quercetin and resveratrol. TSG-mediated extension of lifespan was abolished in a daf-16 loss of function mutant strain, but not in a sir-2.1 loss of function mutant strain. Our results show that TSG has a longevity promoting effect in C. elegans, depending at least in parts on the FoxO homologue DAF-16. Modulation of this pathway by TSG may be a molecular mechanism responsible for the “anti-ageing”-effect of Polygonum multiflorum.

*e-mail: wim.waetjen@landw.uni-halle.de
(41) Effect of cell stretching on the gene expression in lung epithelial cells

Bettina Weber¹, Nancy Bader¹, Andreas Simm¹,², Holger Lehnich², Rolf-Edgar Silber¹ & Babett Bartling¹

¹ Department of Cardio-thoracic Surgery, University Hospital Halle (Saale), Germany
² Centre of Medical Basic Research, Medical Faculty, Halle (Saale), Germany

Background: Lung epithelial cells, and especially alveolar epithelial cells type I which express the cell adhesion molecule RAGE, are exposed to cyclic stretch in vivo due to breathing. Therefore, our study aimed at the identification of genes whose mRNA expressions are altered by cyclic stretch dependent or independent of RAGE.

Methods: We developed a multi-axial stretch device that allows the cyclic stretching of adherent cells on a flexible silicon membrane. We also generated human alveolar A549 cells stably over-expressing RAGE. A549 cells with and without RAGE overexpression (n = 3 each) and further human lung epithelial cell lines (H322, Calu-3, H358, Beas-2B; n = 1 each) were exposed to cyclic stretch (~20 % surface extension at 12 min⁻¹) for 24 h. The mRNA expression was studied by Affymetrix GeneChip analysis. Genes of interest were then checked in further experiments by qPCR (n ≤ 10 each).

Results: GeneChip analysis identified for all types of epithelial cells several genes with moderate alterations in their mRNA expression, such as urokinase plasminogen activator (PLAU), tissue plasminogen activator (PLAT) and protein kinase Cζ (PKCζ) which were up-regulated. The mRNA expression of other genes was altered in individual cell lines only, such as Lipocalin 2 (LCN2) which was specifically down-regulated in H322 cells. In contrast, the anterior gradient 2 homolog (AGR2) was down-regulated in A549 and Beas-2B only. Out of the up-regulated genes we finally selected PLAU, PLAT and their inhibitor PAI-1 as well as some other genes for more detailed analyses. In A549 cells we confirmed the increased mRNA expression of PLAU, PLAT and PAI-1. This effect was independent of RAGE. Other cell types specifically up-regulated PLAU (H322, Calu-3) or PAI-1 (Beas-2B). However, most genes of interest could not be verified in further experiments by qPCR.

Conclusion: Breathing-mediated cyclic stress moderately affects the gene expression in lung epithelial cells only.
(42) Modulation of stress resistance and lifespan in C. elegans by dietary polyphenols

Havermann S.¹, Wilke S.¹, Koch K.¹, Stolz S.¹, Humpf H.-U.², Wätjen W.¹

¹ Martin-Luther-Universität Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, “Biofunctionality of Secondary Plant Compounds”, Weinbergweg 22, 06120 Halle/Saale, Germany
² Universität Münster, Institute of Food Chemistry, Corrensstr. 45, 48149 Münster, Germany

Flavonoids are secondary plant compounds ubiquitously present in fruit and vegetables. These substances possess a remarkable spectrum of biochemical and pharmacological activities, e.g. antioxidative and anti-inflammatory activities and they are also discussed as compounds that prolong the lifespan of organisms. We used the model organism Caenorhabditis elegans to investigate the effects of dietary polyphenols (e.g. the flavonoid baicalein and the methylated derivates oroxylin A, negletein and baicaleintrimethylether) on intracellular signalling pathways, stress resistance and lifespan.

All flavonoids (except baicaleintrimethylether) possess a high radical scavenging activity in the cell-free TEAC assay; comparable or even better than the reference compound trolox. Baicalein was able to decrease intracellular reactive oxygen species in C. elegans (DCF assay). This flavonoid activates SKN-1, a key transcription factor that binds to the antioxidant responsive element in intestinal cells (SKN-1: homologue to Nrf2); DAF-16, another important transcription factor in the regulation of ageing was not affected. Baicalein increases the resistance of C. elegans against thermal stress (SYTOX-assay); this effect was mediated by SKN-1. Lifespan analysis revealed that baicalein extends the mean, median and maximum lifespan of the nematode by 45%, 57% and 24%, respectively. The prolongation of lifespan by baicalein is not caused by dietary restriction, since the pharyngeal pumping rate was not modulated by this flavonoid. RNAi-mediated knockdown of skn-1 abolished the lifespan extension induced by baicalein; therefore we suggest that the lifespan prolongation depends at least partially on SKN-1.

e-mail: susannah.havermann@landw.uni-halle.de
List of Speakers (A-F)

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Address</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldwin, Carolyn</td>
<td>Human Development &amp; Family Sciences Research in Human Development</td>
<td>Oregon State University, Corvallis, OR 97331</td>
<td>E-Mail: <a href="mailto:Carolyn.Aldwin@oregonstate.edu">Carolyn.Aldwin@oregonstate.edu</a> Tel.: +1 (0) 541 - 737 2024 Fax: +1 (0) 541 - 737 4001</td>
</tr>
<tr>
<td>Austad, Steven</td>
<td>Barshop Institute for Longevity &amp; Aging Studies, University of Texas Health Sciences Center</td>
<td>University of Texas Health Sciences Center, San Antonio, TX 78245, USA</td>
<td>E-Mail: <a href="mailto:austad@uthscsa.edu">austad@uthscsa.edu</a> Tel.: +1 (0) 210 - 562 6011 Fax: +1 (0) 210 - 562 9053</td>
</tr>
<tr>
<td>Bartling, Babett</td>
<td>University Hospital Halle (Saale) Department of Cardiothoracic Surgery</td>
<td>University Hospital Halle, Ernst-Grube-Str. 40, D - 06120 Halle</td>
<td>E-Mail: <a href="mailto:babett.bartling@uk-halle.de">babett.bartling@uk-halle.de</a> Tel.: +49 (0) 345 - 557 3314 Fax: +49 (0) 345 - 557 3317</td>
</tr>
<tr>
<td>Campisi, Judith</td>
<td>Buck Institute for Research on Aging</td>
<td>Buck Institute for Research on Aging, Novato, CA 94945, USA</td>
<td>E-Mail: <a href="mailto:jcampisi@buckinstitute.org">jcampisi@buckinstitute.org</a> Tel.: +1 (0) 415 - 209 2043</td>
</tr>
<tr>
<td>Fedorova, Maria</td>
<td>Institut für Bioanalytische Chemie der Fakultät für Chemie und Mineralogie Biotechnologisch-Biomedizinisches Zentrum (BBZ) Universität Leipzig</td>
<td>Universität Leipzig, Deutscher Platz 5, D - 04103 Leipzig</td>
<td>E-Mail: <a href="mailto:maria.fedorova@bbz.uni-leipzig.de">maria.fedorova@bbz.uni-leipzig.de</a> Tel.: +49 (0) 341 - 973 1336 Fax: +49 (0) 341 - 973 1339</td>
</tr>
<tr>
<td>Fuellen, Georg</td>
<td>Institute for Biostatistics and Informatics in Medicine and Ageing Research</td>
<td>Institute for Biostatistics and Informatics in Medicine and Ageing Research, Ernst-Heydemann-Str. 8, D - 18057 Rostock</td>
<td>E-Mail: <a href="mailto:fuellen@alum.mit.edu">fuellen@alum.mit.edu</a> Tel.: +49 (0) 381 - 494 7360 Fax: +49 (0) 381 - 494 7203</td>
</tr>
</tbody>
</table>
## List of Speaker (G-L)

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Address</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Griffiths, Helen</td>
<td>Associate Research Life and Health Sciences, Aston Triangle</td>
<td>GB - Birmingham B4 7ET UK</td>
<td>E-Mail: <a href="mailto:helen.griffiths@aston.ac.uk">helen.griffiths@aston.ac.uk</a> Tel.: +44 121 - 204 3950 Fax: +44 121 - 204 4175</td>
</tr>
<tr>
<td>Günther, Andreas</td>
<td>Justus Liebig University Giessen University of Giessen Lung Center Medical Clinic II, Klinikstrasse 36</td>
<td>D - 35392 Giessen</td>
<td>E-Mail: <a href="mailto:Andreas.Guenther@innere.med.uni-giessen.de">Andreas.Guenther@innere.med.uni-giessen.de</a> Tel.: +49 (0) 641 - 985 42502 Fax: +49 (0) 641 - 985 42508</td>
</tr>
<tr>
<td>Hedtke-Becker, Astrid</td>
<td>Hochschule Mannheim University of Applied Sciences Fakultät für Sozialwesen</td>
<td>Paul-Wittsack-Str. 10 D - 68163 Mannheim</td>
<td>E-Mail: <a href="mailto:a.hedtke-becker@hs-mannheim.de">a.hedtke-becker@hs-mannheim.de</a> Tel.: +49 (0) 621 - 292 6398 Fax: +49 (0) 621 - 292 6720</td>
</tr>
<tr>
<td>Höhn, Annika</td>
<td>Friedrich-Schiller-University Jena Institute of Nutrition Department of Nutritional Toxicology</td>
<td>Dornburgerstrasse 24 D - 07743 Jena</td>
<td>E-Mail: <a href="mailto:annika.hoehn@uni-jena.de">annika.hoehn@uni-jena.de</a> Tel.: +49 (0) 3641 - 949 695 Fax: +49 (0) 3641 - 949 70</td>
</tr>
<tr>
<td>Ito, Kazuhiro</td>
<td>Airway Disease, National Heart and Lung Institute, Imperial College London Dovehouse street SW3 6LY, London, GB</td>
<td></td>
<td>E-Mail: <a href="mailto:k.ito@imperial.ac.uk">k.ito@imperial.ac.uk</a> Tel.: +44 (0) 207 - 193 9210</td>
</tr>
<tr>
<td>Johnson, Thomas E.</td>
<td>Molecular and Behavioral Genetics Box 447 University of Colorado Boulder Boulder CO, 80309, USA</td>
<td></td>
<td>E-Mail: <a href="mailto:johnsont.@colorado.edu">johnsont.@colorado.edu</a> Tel.: +1 303 - 492 7362 Fax: +1 303 - 492 8063</td>
</tr>
<tr>
<td>Klöckner, Udo</td>
<td>Julius-Bernstein-Institute for Physiology Magdeburger Str. 6 D - 06097 Halle (Saale)</td>
<td></td>
<td>E-Mail: <a href="mailto:udo.kloeckner@medizin.uni-halle.de">udo.kloeckner@medizin.uni-halle.de</a> Tel.: +49 (0) 345 - 557 1392 Fax: +49 (0) 345 - 557 4019</td>
</tr>
<tr>
<td>Larsson, Lars</td>
<td>Department of Clinical Neurophysiology Uppsala University Hospital SE - 751 85 Uppsala</td>
<td></td>
<td>E-Mail: <a href="mailto:Lars.Larsson@neurofys.uu.se">Lars.Larsson@neurofys.uu.se</a> Tel: +46 (0)18 - 611 34 45 Fax : +46 (0)18 - 55 61 06</td>
</tr>
</tbody>
</table>
## List of Speaker (L-S)

<table>
<thead>
<tr>
<th>Speaker</th>
<th>Institution</th>
<th>Address</th>
<th>Contact Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ladwig, Karl-Heinz</td>
<td>Institute of Epidemiology Helmholtz Zentrum München</td>
<td>D - 85764 Neuherberg</td>
<td>E-Mail: <a href="mailto:ladwig@helmholtz-muenchen.de">ladwig@helmholtz-muenchen.de</a> Tel.: +49 (0) 89 - 3187 3623 Fax: +49 (0) 89 - 3187 3667</td>
</tr>
<tr>
<td>Lepperdinger, Günter</td>
<td>Institute for Biomedical Aging Research University of Innsbruck</td>
<td>Rennweg 10</td>
<td>E-Mail: <a href="mailto:Guenter.Lepperdinger@uibk.ac.at">Guenter.Lepperdinger@uibk.ac.at</a> Tel.: +43 - 512 - 507 508 40 Fax: +43 - 512 - 507 508 8</td>
</tr>
<tr>
<td>Näbauer, Michael</td>
<td>Medizinische Klinik I Klinikum Großhadern</td>
<td>D - 81377 München</td>
<td>E-Mail: <a href="mailto:michael.nabauer@med.uni-muenchen.de">michael.nabauer@med.uni-muenchen.de</a> Tel.: +49 (0) 89 - 7095 3162 Fax: +49 (0) 89 - 7095 8788</td>
</tr>
<tr>
<td>Pierce, Grant N.</td>
<td>St. Boniface Hospital Research</td>
<td>351 Tache Avenue</td>
<td>E-Mail: <a href="mailto:gpierce@sbrc.ca">gpierce@sbrc.ca</a> Tel.: +1 (204) - 235 - 3206 Fax: +1 (204) - 235 - 0793</td>
</tr>
<tr>
<td>Pitkälä, Kaisu</td>
<td>University of Helsinki, Department of General Practice and Primary Health Care</td>
<td>PO Box 20</td>
<td>E-Mail: <a href="mailto:kaisu.pitkala@helsinki.fi">kaisu.pitkala@helsinki.fi</a> Tel.: + 358 - 50 - 338 5546 Fax: + 358 - 9 - 191 2753 6</td>
</tr>
<tr>
<td>Sasson, Shlomo</td>
<td>The Institute for Drug Research Department of Pharmacology The Hebrew University Faculty of Medicine POBox 12272 Jerusalem 91120, Israel</td>
<td>E-Mail: <a href="mailto:shlomo.sasson@mail.huji.ac.il">shlomo.sasson@mail.huji.ac.il</a> Tel.: + 972 - 2 - 675 8798 Fax: + 972 - 2 - 675 8741</td>
<td></td>
</tr>
</tbody>
</table>
## List of Speaker (S-W)

<table>
<thead>
<tr>
<th>Name</th>
<th>Position/Institution</th>
<th>Address</th>
<th>Contact Information</th>
</tr>
</thead>
</table>
| Schulz, Ralf-Joachim | Lehrstuhl für Geriatrie der Universität zu Köln, Klinik für Geriatrie am St. Marien-Hospital | Kuniberts Kloster 11–13, D - 50668 Köln | E-Mail: ralf-joachim.schulz@dggeriatrie.de  
Tel.: +49 (0) 221 - 1629 2350  
Fax: +49 (0) 221 - 1629 2351 |
| Vierecke, Juliane | VAD-Team  
Deutsches Herzzentrum Berlin, Augustenburgerplatz 1 | D - 13353 Berlin | E-Mail: Vierecke@dhzb.de  
Tel.: +49 (0) 30 - 4593 2160  
Fax: +49 (0) 30 - 4593 2209 |
| Wahl, Hans-Werner | Heidelberg University, Institute of Psychology Department of Psychological Aging Research | Bergheimer Strasse 20, D - 69115 Heidelberg | E-Mail: h.w.wahl@psychologie.uni-heidelberg.de  
Tel.: +49 (0) 6221 - 548 111  
Fax: +49 (0) 6221 - 548 112 |
| Werdan, Karl | University Hospital Halle (Saale), Department of Internal Medicine III  
Ernst-Grube-Str. 40 | D - 06120 Halle (Saale) | E-Mail: karl.werdan@medizin.uni-halle.de  
Tel.: +49 (0) 345 - 557 2601  
Fax: +49 (0) 345 - 557 2072 |
| Wurm, Susanne | DZA Deutsches Zentrum für Altersfragen, German Centre of Gerontology  
Manfred-von-Richtofen Str.2 | D - 12101 Berlin | E-Mail: susanne.wurm@dza.de  
Tel.: +49 (0) 30 - 260 740 78  
Fax: +49 (0) 30 - 785 435 0 |

106
Organizers

**Silber, Rolf-Edgar**  
Universitätsklinikum Halle (Saale)  
Director of the  
Department of Cardiothoracic Surgery  
Ernst-Grube-Str. 40  
D - 06120 Halle (Saale)

E-Mail: edgar.silber@medizin.uni-halle.de  
Tel.: +49 (0) 345 - 557 2720  
Fax: +49 (0) 345 - 557 2782

**Simm, Andreas**  
Universitätsklinikum Halle (Saale)  
Department of Cardiothoracic Surgery  
Ernst-Grube-Str. 40  
D - 06120 Halle (Saale)

E-Mail: andreas.simm@medizin.uni-halle.de  
Tel.: +49 (0) 345 - 557 2647  
Fax: +49 (0) 345 - 557 7070

**Müller-Werdan, Ursula**  
Universitätsklinikum Halle (Saale)  
Department of Internal Medicine III  
Ernst-Grube-Str. 40  
D - 06120 Halle (Saale)

E-Mail: ursula.mueller-werdan@medizin.uni-halle.de  
Tel.: +49 (0) 345 - 557 2816  
Fax: +49 (0) 345 - 557 2072
Involved Societies

**German National Academy of Sciences Leopoldina**  
Jägerberg 1  
D - 06108 Halle (Saale)  
E-Mail: leopoldina@leopoldina-halle.de  
Tel.: +49 (0) 345 - 47239 600  
Fax: +49 (0) 345 - 47239 919

**DGGG**  
German Society of Gerontology and Geriatrics  
Geschäftsstelle  
Seumestr. 8  
D - 10245 Berlin  
E-Mail: gs@dggg-online.de  
Tel.: +49 (0) 30 - 52137 271  
Fax: +49 (0) 30 - 52137 372

**DGTHG**  
German Society of Thoracic and Cardiovascular Surgery  
Langenbeck-Virchow-Haus  
Luisenstraße 58/59  
D - 10117 Berlin  
E-Mail: sekretariat@dgthg.de  
Tel.: +49 (0) 30 - 28004 370  
Fax: +49 (0) 30 - 28004 379
Involved Societies

**European Section of the International Academy of Cardiovascular Science**
IACS
c/o Institute of Cardiovascular Sciences
St. Boniface General Hospital Research Centre
351 Tache Avenue
Winnipeg, Manitoba
R2H 2A 6 Canada

**IZAH**
Interdisciplinary Centre on Ageing Halle
Ernst-Grube-Str. 40
06120 Halle

E-Mail: andreas.simm@medizin.uni-halle.de
Tel.: +49 (0) 345 - 557 2647
Fax: +49 (0) 345 - 557 7070

**Friends and Sponsors of the Martin-Luther-University Halle-Wittenberg e.V.**
Universitätsplatz 10
06108 Halle

E-Mail: ramona.mitsching@vff.uni-halle.de
Tel.: +49 (0) 345 - 552 2912
Fax: +49 (0) 345 - 552 7076
Author Index

Aldwin, C. M. 36, 103
Al-Robaiy, S. 61
Aurich, A.-C. 79
Austad, S. 12, 103
Bache, M. 68
Bader, N. 101
Bakan, B. 91
Baraibar, M. A. 62, 66
Bartel, F. 63
Bartling, B. 30, 61, 103
Bergheim, I. 67
Bethmann, D. 96
Böning, A. 86
Bouche, M. 66
Büchter, C. 64, 100
Bürke, M. 79
Bulteau, A.-L. 62
Butler-Browne, G. 62
Campisi, J. 10, 103
Dalgalarondo, M. 91
Ebelt, H. 83
Englert, C. 72
Fasshauer, M. 90
Fedorova, M. 26, 103
Fischer, B. 73, 99
Frary, C. E. 65
Friguet, B. 62, 66
Fuellen, G. 18, 103
Gärttner, S. 5, 67
Gekle, M. 70, 95
Gläser, C. 90
Glomb, M. A. 72, 74
Gnanapragassam, V.S., 68
Gogol, M. 69
Griffiths, H. 20, 104
Groß, S. 63
Großmann, C. 70
Großmann, M. 75
Grune, T. 73, 77, 80, 87
<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Günther, A.</td>
<td>34, 04</td>
</tr>
<tr>
<td>Gürke, J.</td>
<td>71, 99</td>
</tr>
<tr>
<td>Hagemann, M.</td>
<td>90</td>
</tr>
<tr>
<td>Hanisch, F.</td>
<td>75</td>
</tr>
<tr>
<td>Hartmann, H.</td>
<td>69</td>
</tr>
<tr>
<td>Hartmann, N.</td>
<td>72</td>
</tr>
<tr>
<td>Haucke, E.</td>
<td>5, 71, 73, 99</td>
</tr>
<tr>
<td>Havermann, S.</td>
<td>64, 102</td>
</tr>
<tr>
<td>Hedtke-Becker, A.</td>
<td>58, 104</td>
</tr>
<tr>
<td>Heinemann, S. H.</td>
<td>94</td>
</tr>
<tr>
<td>Heller, R.</td>
<td>80, 96</td>
</tr>
<tr>
<td>Henning, Chr.</td>
<td>5, 74</td>
</tr>
<tr>
<td>Henning, E.</td>
<td>82</td>
</tr>
<tr>
<td>Hirche, F.</td>
<td>71</td>
</tr>
<tr>
<td>Hirose, M.</td>
<td>93</td>
</tr>
<tr>
<td>Hofmann, B.</td>
<td>76</td>
</tr>
<tr>
<td>Hoffmann, K.</td>
<td>90</td>
</tr>
<tr>
<td>Höhn, A.</td>
<td>14, 87, 104</td>
</tr>
<tr>
<td>Hollemann, Th.</td>
<td>89</td>
</tr>
<tr>
<td>Holzhausen, H.-J.</td>
<td>75</td>
</tr>
<tr>
<td>Honnen, S.</td>
<td>64</td>
</tr>
<tr>
<td>Höpfner, F.</td>
<td>83</td>
</tr>
<tr>
<td>Horstkorte, R.</td>
<td>68, 75</td>
</tr>
<tr>
<td>Huber, O.</td>
<td>67</td>
</tr>
<tr>
<td>Humpf, H.-U.</td>
<td>102</td>
</tr>
<tr>
<td>Hünerberg, M.</td>
<td>95</td>
</tr>
<tr>
<td>Hyzewicz, J.</td>
<td>62</td>
</tr>
<tr>
<td>Ibrahim, S.</td>
<td>93</td>
</tr>
<tr>
<td>Immel, U.-D.</td>
<td>63</td>
</tr>
<tr>
<td>Issa, H.</td>
<td>86</td>
</tr>
<tr>
<td>Ito, K.</td>
<td>32, 104</td>
</tr>
<tr>
<td>Jacobs, K.</td>
<td>76, 88</td>
</tr>
<tr>
<td>Johnson, Th. E.</td>
<td>54, 104</td>
</tr>
<tr>
<td>Johnson, Y. J.</td>
<td>82</td>
</tr>
<tr>
<td>Joshi, P. R.</td>
<td>74</td>
</tr>
<tr>
<td>Jung, T.</td>
<td>77</td>
</tr>
<tr>
<td>Kadamov, Y.</td>
<td>98</td>
</tr>
<tr>
<td>Kassem, M.</td>
<td>65</td>
</tr>
<tr>
<td>Klepsch, S.</td>
<td>78</td>
</tr>
<tr>
<td>Klintschar, M.</td>
<td>63</td>
</tr>
<tr>
<td>Klöckner, U.</td>
<td>44, 92, 104</td>
</tr>
<tr>
<td>Koch, K.</td>
<td>102</td>
</tr>
</tbody>
</table>
Koenig, J. 87
Kottek, T. 93
Kunz, M. 93
Ladwig, K.-H. 42, 105
Larsson, L. 22, 66, 104
Le Boulch, L. 65
Lehnich, H. 101
Leipold, E. 94
Lepperdinger, G. 52, 78, 105
Li, L. 79, 86
Liehr, K. 74
Lindenmüller, S. 96
Lochmann, P. 84
Loppnow, H. 88
Marion, D. 91
Meçe, O. 5, 80
Meyer, G. 81
Miehle, K. 90
Mildenberger, S. 95
Mitter, D. 90
Mohamed, S. A. 82, 84
Müller-Werdan, U. 83, 85
Näbauer, M. 48, 105
Navarrete Santos, Alexander 73, 76, 84, 88, 91
Navarrete Santos, Anne 71, 73, 99
Neef, R. 85
Nehlin, J. 65
Niemann, B. 79, 83, 86
Nuding, S. 83
Ott, Chr. 87
Petruschke, H. 88
Pfirrmann, Th. 89
Pierce, G. 28
Pitkälä, K. 16, 105
Porrmann, J. 90
Pötzsch, S. 5, 88, 91
Prip-Buus, C. 62
Rabe, S. 95
Reitinger, S. 78
Rogowska-Wrzesinska, A. 62
Rohrbach, S. 79, 86
Rückschloss, U. 92
<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruhs, S.</td>
<td>70</td>
</tr>
<tr>
<td>Santos, S.</td>
<td>66</td>
</tr>
<tr>
<td>Sasson, S.</td>
<td>24, 105</td>
</tr>
<tr>
<td>Schauer, M.</td>
<td>93</td>
</tr>
<tr>
<td>Schindler, M.</td>
<td>71, 99</td>
</tr>
<tr>
<td>Schink, M.</td>
<td>5, 94</td>
</tr>
<tr>
<td>Schirmeyer, J.</td>
<td>94</td>
</tr>
<tr>
<td>Schönherr, R.</td>
<td>94</td>
</tr>
<tr>
<td>Schreier, B.</td>
<td>95</td>
</tr>
<tr>
<td>Schulz, R.-J.</td>
<td>56, 106</td>
</tr>
<tr>
<td>Seliger, Barbara</td>
<td>97</td>
</tr>
<tr>
<td>Seliger, B.</td>
<td>72, 96</td>
</tr>
<tr>
<td>Sellmann, C.</td>
<td>67</td>
</tr>
<tr>
<td>Siekmann, H.</td>
<td>85</td>
</tr>
<tr>
<td>Sievers, H. H.</td>
<td>82, 84</td>
</tr>
<tr>
<td>Silber, R.-E.</td>
<td>61, 76, 84, 86, 88, 91, 101</td>
</tr>
<tr>
<td>Simm, A.</td>
<td>61, 69, 72, 76, 84, 88, 91, 101</td>
</tr>
<tr>
<td>Somoza, V.</td>
<td>91</td>
</tr>
<tr>
<td>Spengler, K.</td>
<td>96</td>
</tr>
<tr>
<td>Spruss, A.</td>
<td>67</td>
</tr>
<tr>
<td>Stehle, F.</td>
<td>97</td>
</tr>
<tr>
<td>Stephan, A.</td>
<td>81</td>
</tr>
<tr>
<td>Stock, F.</td>
<td>90</td>
</tr>
<tr>
<td>Stöckl, G.</td>
<td>83</td>
</tr>
<tr>
<td>Stoltenburg, G.</td>
<td>75</td>
</tr>
<tr>
<td>Stolz, S.</td>
<td>102</td>
</tr>
<tr>
<td>Stumpf, D.</td>
<td>79</td>
</tr>
<tr>
<td>Tarasenko, L.</td>
<td>98</td>
</tr>
<tr>
<td>Tsuber, V.</td>
<td>98</td>
</tr>
<tr>
<td>Uhde, K.</td>
<td>99</td>
</tr>
<tr>
<td>Vierecke, J.</td>
<td>50, 106</td>
</tr>
<tr>
<td>Villmow, M.</td>
<td>92</td>
</tr>
<tr>
<td>Vordermark, D.</td>
<td>68</td>
</tr>
<tr>
<td>Wahl, H.-W.</td>
<td>40, 106</td>
</tr>
<tr>
<td>Wätjen, W.</td>
<td>64, 99, 100, 102</td>
</tr>
<tr>
<td>Weber, B.</td>
<td>101</td>
</tr>
<tr>
<td>Weber, D.</td>
<td>87</td>
</tr>
<tr>
<td>Weidemann, W.</td>
<td>75</td>
</tr>
<tr>
<td>Weigel, D.</td>
<td>96</td>
</tr>
<tr>
<td>Weis, J.</td>
<td>75</td>
</tr>
<tr>
<td>Wendler, S.</td>
<td>72</td>
</tr>
<tr>
<td>Werdan, K.</td>
<td>46, 83, 106</td>
</tr>
<tr>
<td>Name</td>
<td>Page</td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
</tr>
<tr>
<td>Wilke, S.</td>
<td>102</td>
</tr>
<tr>
<td>Wolf, U.</td>
<td>85</td>
</tr>
<tr>
<td>Wurm, S.</td>
<td>38, 106</td>
</tr>
<tr>
<td>Zhao, L.</td>
<td>100</td>
</tr>
<tr>
<td>Zierz, S.</td>
<td>75</td>
</tr>
</tbody>
</table>
Thank’s to our Sponsors
(in alphabetical order)

<table>
<thead>
<tr>
<th>Bayer HealthCare</th>
<th>Edwards Lifesciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETHICON a Johnson &amp; Johnson company</td>
<td>FUMEDICA</td>
</tr>
<tr>
<td>MAQUET GETINGE GROUP</td>
<td></td>
</tr>
<tr>
<td>Medtronic</td>
<td>Novartis Pharmaceuticals</td>
</tr>
<tr>
<td><a href="http://www.servier.de">www.servier.de</a></td>
<td>SORIN Group</td>
</tr>
<tr>
<td>THORATEC Corporation</td>
<td></td>
</tr>
</tbody>
</table>

Bayer 1.500 €/ Edwards Lifesciences 6.000 €/ ETHICON 1.000 €/ FUMEDICA 1.000 €/ JR 2.500 €/ MAQUET 3.000 €/ Medtronic 10.000 €/ NOVARTIS 4.000 €/ SERVIER 2.000 €/ SORIN GROUP 2.000 €/ THORATEC 2.000 €
Map of Halle (Saale)