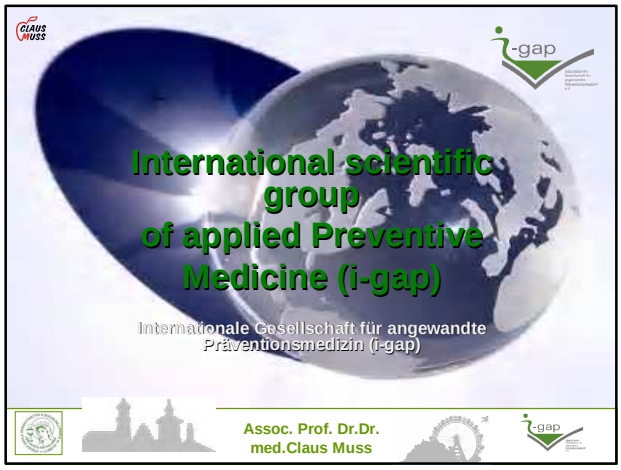
International scientific group of applied Preventive Medicine (i-gap)
Internationale Gesellschaft für angewandte Präventionsmedizin (i-gap)
Assoc. Prof. Dr.Dr. med.Claus Muss


Welcome
Willkommen
International Symposium
Nitrosative stress
Internationales Symposium
Nitrosativer Stress
Augsburg-Bratislava
Internationale Gesellschaft für angewandte Präventionsmedizin
Assoc.Prof.DDR.C.Muss
Vienna


Assoc. Prof. Dr. Dr. med.Claus Muss
Nutritional medicine & Integrative medicine
Preventive medicine & Environmental medicine
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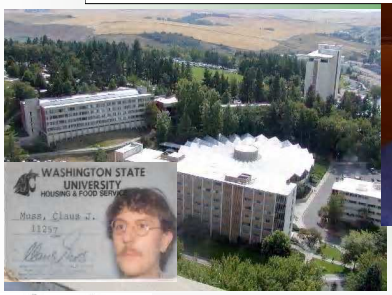
Augsburg Bratislava
Vienna
Assoc. Prof. Dr.Dr. med.Claus Muss

Birthplace of Science investigating Nitrosative Stress



Prof. Martin Pall

Augsburg-Bratislava

Internationale Gesellschaft für angewandte Präventionsmedizin
International scientific group for apllied preventive Medicine
Assoc.Prof.DDR.C.Muss

Vienna

Topics I Science

- On the search for further transmitters of the NO/ONOO cycle into different body compartments
- Does the NO/ONOO cycle effect homorne activity?
- Is the a realtionship beween the NO/ONOO-cycle and homone production related to metablolism?
- Do we have eveidence to believe that the metabolic syndrome is related to the effects of NO/ONOO cycle?

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Topic II
Practical clinical protocols in the approach of treating patients with NO/ONOO problems

- Medication in favor of prevention
- Medication in favor of treatment
- How to monitor patients with nitrosative stress

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Vienna

Impact of Peroxynitite on redox status

- Reactive nitrogen oxide species (RNS), which include the free radical nitric oxide (•NO), peroxynitrite (ONOO–), and nitrogen dioxide (NO2), play significant roles as redox active molecules
- **Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991;43:109–142.**

SOD & N0

- Superoxide reacts with NO to generate peroxynitrite and other nitrogen species that are able to alter the structure and function of several other mitochondrial proteins, notably complex I.
- **Liaudet L, Soriano FG, Szabo C. Biology of nitric oxide signaling. *Crit Care Med*. 2000;28:N37–N52 .**

Nitrosation targets transcription factors

- Over 200 different proteins have been found to be targeted by *S*-nitrosation, including metabolic enzymes, phosphatases, transcription factors, and others
- **Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein Snitrosylation: purview and parameters. Nat Rev Mol Cell Biol. 2005;6:150–166 .**

NO blocks mitochondrial respiration

- Nitric oxide (NO), reactive oxygen species and other inflammatory mediators are produced in excess and can directly inhibit mitochondrial respiration.
- NO competes with oxygen in binding to cytochrome oxidase (complex IV), thereby decreasing the activity of the enzyme. This will block the electron transport chain and lead to overproduction of superoxide.

- **Liaudet L, Soriano FG, Szabo C. Biology of nitric oxide signaling. *Crit Care Med.* 2000;28:N37–N52 .**

N0 contributes to protein misfolding in mitochondria

- Recent studies have suggested that nitrosative stress due to generation of excessive nitric oxide (NO) can mediate excitotoxicity by triggering protein misfolding and aggregation.
- S-Nitrosylation, or covalent reaction of NO with specific protein thiol groups, represents a convergent signal pathway contributing to NO-induced protein misfolding and aggregation, compromised dynamics of mitochondrial fission-fusion process, thus leading to neurotoxicity.

- **Gu Z, Nakamura T, Lipton SA: Redox Reactions Induced by Nitrosative Stress Mediate Protein Misfolding and Mitochondrial Dysfunction in Neurodegenerative Diseases.** Mol Neurobiol. 2010 Mar 25.

Acta Neuropathol (1991) 83: 61 – 65
Acta Neuropathologica
© Springer-Verlag 1991

Mitochondrial abnormalities in the postviral fatigue syndrome*

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Received February 1, 1991/Revised April 24, 1991/Revised, accepted August 5, 1991

Summary. We have examined the muscle biopsies of 50 patients who had postviral fatigue syndrome (PFS) for from 1 to 17 years. We found mild to severe atrophy of type II fibres in 39 biopsies, with a mild to moderate excess of lipid. On ultrastructural examination, 35 of these specimens showed branching and fusion of mitochondrial cristae. Mitochondrial degeneration was obvious in 40 of the biopsies with swelling, vacuolation, myelin figures and secondary lysosomes. These abnormalities were in obvious contrast to control biopsies, laboratory have revealed defects in sustained concentration and a curious sensitivity to visually disturbing patterns (in preparation).

The lack of specific diagnostic criteria has made it very difficult to study the disease and, until recently, it has not been the subject of scientific investigation. Attempts have now been made to set strict guidelines for diagnosis, with a working case definition [18]. Unfortunately, the term "chronic fatigue syndrome" has been selected and this will certainly lead to severe problems

CFS & Mitochondria

- A remarkable correlation is observed between the degree of mitochondrial dysfunction and the severity of illness.
- The individual factors indicate which remedial actions, in the form of dietary supplements, drugs and detoxification, are most likely to be of benefit, and what further tests should be carried out.
- **Myhill S, Booth NE, McLaren-Howard J.: Chronic fatigue syndrome and mitochondrial dysfunction. Int J Clin Exp Med. 2009;2(1):1-16.**

Downregulation of Glutathione system

- In the presence of oxygen, endogenously produced •NO can rapidly lead to *S*-nitrosation of protein thiols (SNO) and glutathione (GSNO), presumably via the formation of other reactive nitrogen oxide species, such as N_2O_3, NO_2, and thiyl radicals
- **Schrammel A, Gorren AC, Schmidt K, Pfeiffer S, Mayer B. S-nitrosation of glutathione by nitric oxide, peroxynitrite, and (*)NO/O(2)(*-). Free Radic Biol Med. 2003;34:1078–1088 .**

Topics I Science

- On the search for further transmitters of the NO/ONOO cycle into different body compartments
- Does the NO/ONOO cycle effect homorne activity?
- Is the a realtionship beween the NO/ONOO-cycle and homone production related to metablolism?
- Do we have eveidence to believe that the metabolic syndrome is related to the effects of NO/ONOO cycle?

Tyrosine Phosphorylation

- Reversible protein tyrosine phosphorylation and dephosphorylation are important posttranslational protein modifications involved in a variety of cellular signal transduction cascades that control metabolism, motility, cell growth, proliferation, differentiation, and survival
- **Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T. Protein tyrosine phosphatases in the human genome. Cell. 2004;117:699–711**

Explaining the multi-symtomes of nitrosative stress

- Is there a hormone responsible for the multiple effects of nitrosative stress.
- Explaining multiple syndromes of nitrosative stress by hormone effect

Tissue producing Leptin

- Leptin is produced predominantly by adipocytes, although low levels have been detected in the hypothalamus, pituitary, stomach , skeletal muscle , mammary epithelia , chondrocytes and a variety of other tissues
- **Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. Int J Obes Relat Metab Disord. 2002;26:1407–1433**

- The leptin receptors are localized in the brain, pancreas, liver and heart

- **Sader S, Nian M, Liu P. (2003) Leptin: a novel link between obesity, diabetes, cardiovascular risk, and ventricular hypertrophy. Circulation. 108: 644-6.**

- Leptin modulates mitochondrial cell respiration in acute stress situations.

- Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrao AB, Gold P, Chrousos GP. Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm, in cortisol and leptin secretion. *J Clin Endocrinol Metab.* 1998;**83**:280–283.

Modulators of Mitochondrial activity

- Among others, thyroid and sex hormones, insulin, glucocorticoids and leptin positively modulate mitochondrial energy production, protein synthesis and biogenesis.

- **Weitzel JM, Iwen KA, Seitz HJ. Regulation of mitochondrial biogenesis by thyroid hormone. *Exp Physiol.* 2003;88:121–128 .**
- **Stirone C, Duckles SP, Krause DN, Procaccio V. Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. Mol Pharmacol. 2005;68:959–965**
- **Orci L, Cook WS, Ravazzola M, Wang MY, Park BH, Montesano R, Unger RH. Rapid transformation of white adipocytes into fat-oxidizing machines. Proc Natl Acad Sci USA. 2004;101:2058–2063**

Leptin increases N0-Production

- The intravenous injection of leptin increased nitrite and nitrate concentrations in blood serum of normotensive Wistar rats but not in obese Zucker rats, which have a mutation in their leptin receptor gene.

- **Frühbeck G.: Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes* 48: 903–908, 1999**

Leptin increases endothelial production of 0N00

- In obesity, leptin increases eNOS expression and decreases intracellular L-arginine, resulting in eNOS an uncoupling and depletion of endothelial NO and an increase of cytotoxic ONOO–.
- Hyperleptinemia triggers an endothelial NO/ONOO– imbalance characteristic of dysfunctional endothelium observed in other vascular disorders, i.e., atherosclerosis and diabetes.
- **Mykhaylo Korda, Ruslan Kubant, Stephen Patton, and Tadeusz Malinski .: Leptin-induced endothelial dysfunction in obesity. *Am J Physiol Heart Circ Physiol* 295: 1514-1521, 2008**

Leptin induces oxidative stress in human endothelial cells

ANNE BOULOUMIÉ,[1] TAKESHI MARUMO, MAX LAFONTAN,* AND RUDI BUSSE
Institut für Kardiovaskuläre Physiologie, Klinikum der J. W. Goethe-Universität, Frankfurt/Main, Germany; and *INSERM U317, Institut Louis Bugnard, CHU Rangueil, Toulouse, France

ABSTRACT Human umbilical vein endothelial cells (HUVEC) express functional receptors to leptin, the product of the *ob* gene. As human obesity is associated with atherosclerosis and hyperleptinemia, we investigated whether leptin, in addition to its angiogenic properties, exerts atherogenic effects through the generation of oxidative stress in endothelial cells. In HUVEC leptin increased the accumulation of reactive oxygen species (ROS), as assessed by the oxidation of 2′,7′- dichlorodihydrofluorescein, in a time- and concentration-dependent manner. In addition, leptin activated the NH_2-terminal c-Jun kinase/stress-activated protein kinase pathway as demonstrated by enhanced JNK activity and AP-1 DNA binding. Both effects were sensitive to antioxidant treatment with N-acetylcysteine. NF-κB, an-

cently, new peripheral roles for leptin have been identified, i.e., regulation of hematopoietic processes (5, 6) and proinflammatory immune responses (7), as well as stimulation of endothelial cell growth and angiogenesis (8, 9). Moreover, the leptin receptor with the longest cytoplasmic domain (Ob-Rb), which is responsible for the leptin-mediated activation of the Janus kinases (JAK)/STAT pathway (10), has been found to be expressed in various peripheral cells such as endothelial cells (8, 9) and cells from the immune system (5). The plasma concentrations of leptin are markedly increased in human obesity and positively correlated to body fat mass (11). As human obesity is associated with hyperleptinemia and atherosclerosis, we hypothesized that leptin, in addition to its angiogenic properties,

Leptin & Antioxidants

thelial cells. In HUVEC leptin increased the accumulation of reactive oxygen species (ROS), as assessed by the oxidation of 2′,7′- dichlorodihydrofluorescein, in a time- and concentration-dependent manner. In addition, leptin activated the NH_2-terminal c-Jun kinase/stress-activated protein kinase pathway as demonstrated by enhanced JNK activity and AP-1 DNA binding. Both effects were sensitive to antioxidant treatment with N-acetylcysteine. NF-κB, another redox-sensitive transcription factor, was also activated by leptin stimulation in an oxidant-dependent manner. Finally, activation of both AP-1 and

Bouloumié, A., Marumo, T., Lafontan, M., Busse, R. Leptin induces oxidative stress in human endothelial cells. *FASEB J.* 13, 1231–1238 (1999)

Leptin levels predictor of survivial in citrical illness

- Low tri-iodothyronine (T3) syndrome, hypogonadism, insulin resistance, adrenal insufficiency and decreased circulating leptin levels were observed in … prolonged sepsis and critical illness
- **Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: acute and prolonged critical illness as different neuroendocrine paradigms. *J Clin Endocrinol Metab.* 1998;83:1827–1834.**

Inducers of Leptin

- Like majority of neurohormones leptin levels exhibit important circadian rythms (with peak during night). Several release from adipocytes.
- Agonists increasing leptin: These include TNF-alpha and other pro-inflammatory cytokines, **insulin, glucose,** angiotensin II **estrogens**.
- **Takamura T, Honda M, Sakai Y, Ando H, Shimizu A, Ota T, Sakurai M, Misu H, Kurita S, Matsuzawa-Nagata N, Uchikata M, Nakamura S, Matoba R, Tanino M, Matsubara K, Kaneko S. Gene expression profiles in peripheral blood mononuclear cells reflect the pathophysiology of type 2 diabetes. *Biochem Biophys Res Commun.* 2007;361:379–384.**

Expression of Leptin recepors

- Such ubiquitous expression of the receptors in humans and widespread binding of leptin in various organs, indicates it's role in a constellation of vital processes including growth, metabolic control, immune regulation, insulin sensitivity regulation, reproduction.
- **Storlien L, Oakes ND, Kelley DE. Metabolic flexibility. *Proc Nutr Soc.* 2004;63:363–368**

Leptin receptor on immune cells

- Although leptin is not a classical cytokine several immune cells (including polymorphonuclear leukocytes, monocytes, macrophages and lymphocytes) bear leptin receptors and their activity can be modulated by leptin
- **Zhao Y, Sun R, You L, Gao C, Tian Z. Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. Biochem Biophys Res Commun 2003; 300: 247-252**

Adipocytokine	Immune system effects	Vascular effects
Leptin	Pro-inflammatory Increase in T cell activation, and cytokine release proliferation Promotes Th1 response Increases NK cell activation Increases macrophage activation and cytokine release (TNF-α/IL-6 etc) Activates neutrophils and increases their chemotaxis and oxidative burst. ?Prevents inflammatory damage in conditions of overt immune system stimulation?	Induces endothelial dysfunction Increases blood pressure Atherosclerosis Increases ICAM, VCAM Plasma levels related to hard clinical endpoints but acutely releases NO from endothelium

Leptin in T cells

- Activated T cells themselves have been shown to express and secrete leptin, which sustained their proliferation in an autocrine loop.

- **Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S, Matarese G. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest. 2003;111:241–250**

Leptin impact on TH1

- Leptin induces cytokine producing capacity switch towards Th1 producing cells, particularly by increasing interferon gamma and TNF-alpha and IL-2 producing capacity.

Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 1998; 394: 897-901.

Matarese G.Leptin and the immune system: how nutritional status influences the immune response. Eur Cytokine Netw 2000; 11:7-14

Leptin and phagocytic activity

- Impaired phagocytic functions resulting in reduced bacterial elimination have been described for macrophages from leptin-deficient mice during infections with *Escherichia coli*, *Candida albicans and Klebsiella pneumoniae*

- **Mancuso P, Gottschalk A, Phare SM, Peters-Golden M, Lukacs NW, Huffnagle GB. Leptin-deficient mice exhibit impaired host defense in Gram-negative pneumonia. J Immunol. 2002;168:4018–4024**

MAPK in Leptin receptor

- Stimulation of leptin receptor triggers activation of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) . This is typical for IL 6 activity
- Martin-Romero C, Sanchez-Margalet V. Human leptin activates PI3K and MAPK pathways in human peripheral blood mononuclear cells: possible role of Sam68. Cell Immunol. 2001;212:83–91

Proinflammatory stimmulation of Leptin

- LPS, as well as proinflammatory mediators such as tumor necrosis factor (TNF)-α and IL-1, increase the expression of leptin mRNA in adipose tissue
- **Grunfeld C, Zhao C, Fuller J, Pollack A, Moser A, Friedman J, Feingold KR. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. J Clin Invest. 1996;97:2152–2157**

Antiinflammatory effects of Leptin

- Anti-inflammatory effects of leptin were further demonstrated by reduced TNF-α and IL-6 responses in endotoxin treated primates
- Xiao E, Xia-Zhang L, Vulliemoz NR, Ferin M, Wardlaw SL. Leptin modulates inflammatory cytokine and neuroendocrine responses to endotoxin in the primate. Endocrinology. 2003

Leptin stimulates Th1

- Leptin was reported to stimulate the proliferation of T cells *in vitro*, to promote T helper (Th)1 responses
- Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature. 1998;394:897–901

Leptin stimmulates NFκß signaling

In the present study, we report that stimulation of cultured human endothelial cells with leptin leads to an enhanced intracellular accumulation of reactive oxygen species (ROS). This effect was associated with activation of the JNK/SAPK-dependent pathway as well as the redox-sensitive transcription factor NF-κB. The oxidant-dependent increase in MCP-1 expression observed after leptin stimulation further supports the hypothesis that ROS play a major role in the leptin-activated intracellular signaling pathway in endothelial cells.

Bouloumié, A., Marumo, T., Lafontan, M., Busse, R. Leptin induces oxidative stress in human endothelial cells. *FASEB J.* 13, 1231–1238 (1999)

Th1/Th2 Modulation by Leptin

- In models of severe and chronic inflammation leptin leads to decrease Th1 type cytokines and increase of Th2 cytokines and decrease in T cell proliferation.

Matarese G. Leptin and the immune system: how nutritional status influences the immune response. Eur Cytokine Netw 2000; 11: 7-14.

N0 contributes to Mastcell degeneration

- Nitric oxide may also regulate mast cell function. Hence, activation of nitric oxide-producing cells in the tissue microenvironment may be important in the control of mast cell-dependent allergic reactions.
- **Eastmond NC, Banks EM, Coleman JW. Nitric oxide inhibits IgE-mediated degranulation of mast cells and is the principal intermediate in IFN-gamma-induced suppression of exocytosis. J Immunol 1997; 159: 1444-1450**

Leptin and hypertension

- **Department of Cardiology, Elena Venizelou Hospital, Athens, Greece.:**
- Free leptin surrogates are associated with masked hypertension in nonobese normoglycemic subjects. Free leptin is almost equally increased in masked and sustained hypertension, suggesting a similar leptin-related vascular impairment.
- **Thomopoulos C et al.: Free leptin is associated with masked hypertension in nonobese subjects: a cross-sectional study. Hypertension Jun;53(6):965-72 (2009).**

Leptin and cartilage demage

- Leptin indeed exhibits, in concert with other pro-inflammatory cytokines, a detrimental effect on articular cartilage by promoting nitric oxide synthesis in chondrocytes.
- **Otero M.: FEBS Lett Jan;579(2):295-301 (2005)**

Thymus & Leptin

- Leptin deficiency in mice results in chronic thymic atrophy, suppressed cell-mediated immunity, and decreased numbers of total lymphocytes, suggesting a **key role for the metabolic hormone leptin in regulating thymopoiesis** and overall immune homeostasis.
- Furthermore, the studies have revealed that leptin treatment increases thymic expression of IL-7, an important soluble thymocyte growth factor produced by medullary thymic epithelial cells.
- **Gruver A, Ventevogel M, Sempowski G Leptin receptor is expressed in thymus medulla and leptin protects against thymic remodeling during endotoxemia-induced thymus involution. J Endocrinol (2009).**

National Cancer Institute Bethesda, USA.

- Leptin induces the production of chemokines which may activate macrophages and promote macrophage cell chemotaxis.
- These data provide a rational basis for leptin-induced cross-talk between preneoplastic epithelial cells and immune cells that may influence the promotional phase of carcinogenesis.
- **Fenton JI, Hursting SD, Perkins SN, Hord NG:Leptin induces an Apc genotype-associated colon epithelial cell chemokine production pattern associated with macrophage chemotaxis and activation. Carcinogenesis Feb;28(2):455-64 (2007).**

Breast Cancer risk & Leptin

- Leptin is overexpressed in human breast tumors and is produced by breast cancer cells in response to obesity-related stimuli. The leptin promoter polymorphism Lep-2548G/A can be associated with increased leptin secretion by adipocytes and elevated cancer risk.

Terrasi M, Fiorio E, Mercanti A, Koda M, Moncada CA, Sulkowski S, Merali S, Russo A, Surmacz. Int J Cancer Sep;125(5):1038-44 (2009).

Leptin reduces estrogen receptors

- Increased expression of leptin in visceral adipose depot together with increased expressions of proinflammatory cytokines may contribute to reduced expressions of estrogen receptors in subcutaneous fat may.
- **Molecular and cellular endocrinology. 2010 Jan 15; 314(1):150-6 Expression of adipokines and estrogen receptors in adipose tissue and placenta of patients with gestational diabetes mellitus.**

Brain weight under Leptin control

- Reduced brain weight, myelinization and synaptogenesis, the indicators of impaired CNS development and predictors of neurological ailments, have been reported in congenital leptin-deficient humans and rodents

Ahima RS, Bjorbaek C, Osei S, Flier JS. Regulation of neuronal and glial proteins by leptin: implications for brain development. Endocrinology. 1999;140:2755–62

Learning and Leptin

- Diabetes mellitus was associated with cognitive deficits, including psychomotor efficiency, attention, learning and memory, intelligence and executive function.
- **Ahima RS, Bjorbaek C, Osei S, Flier JS. Regulation of neuronal and glial proteins by leptin: implications for brain development. Endocrinology. 1999;140:2755–62**

Leptin & neuropathic pain

- Accumulating evidence suggests the existence of a molecular substrate for neuropathic pain produced by neurons, glia, and immune cells.
- A report from the Department of Pharmacology, Wakayama Medical University, Japan shows that adipocytes (Leptin)associated with primary afferent neurons may be involved in the development of neuropathic pain through adipokine secretion.
- **Maeda T, Kiguchi N, Kobayashi Y, Ikuta T, Ozaki M, Kishioka S: Leptin derived from adipocytes in injured peripheral nerves facilitates development of neuropathic pain via macrophage stimulation. Proc Natl Acad Sci U S A 2009**

Leptin and the skin: a new frontier.

Department of Dermatology, University of Lübeck, Germany

- Leptin, is a major player in the biology and pathology of mammalian skin and its appendages.
- Specifically, the potent metabolic effects of leptin and its mimetics may be utilized to improve, preserve and restore skin regeneration and hair cycle progression, and may halt or even partially reverse some aspects of skin ageing.
- **Poeggeler B, Schulz C, Pappolla MA, Bodó E, Tiede S, Lehnert H, Paus R Exp Dermatol 2009**

Leptin and leptin receptor expression in asthma.

- **The adipokine leptin is a potential new mediator for bronchial epithelial homeostasis. In vitro bronchial epithelial cells express leptin/leptin receptor.**
- **TGF-beta decreases and fluticasone propionate increases leptin receptor expression, and leptin decreases the spontaneous release of TGF-beta and increased cell proliferation.**
- **Severe asthma is associated with a reduced expression of leptin and its receptor.**
- Istituto di Biomedicina e Immunologia Molecolare Palermo, Italy: Bruno A, et al.: J Allergy Clin Immunol Aug;124(2):230-237 (2009).

Alzheimer and Leptin

- Leptin was shown to modulate amyloid plaque formation in vivo and in vitro
- **Fewlass DC, Noboa K, Pi-Sunyer FX, Johnston JM, Yan SD, Tezapsidis N. Obesity-related leptin regulates Alzheimer's Abeta. Faseb J. 2004;18:1870–8.**

Increase of leptin in the brain activates spatial learning and memory

- The presented studies suggest that leptin signaling in the brain may have important implications for cognitive function.
- Therefore behavioral performance related to spatial learning and memory was improved by leptin in vivo applications.
- **Oomura Y, Aou S, Fukunaga K .:Prandial increase of leptin in the brain activates spatial learning and memory.**

  Pathophysiology (2009)

Leptin in intestinal inflammation: good and bad gut feelings.

- Leptin has a the potent effect on T cell mediated intestinal autoimmunity and may have a role in the development of such diseases.
- Matarese G, Lechler RI : Gut Jul;53(7):921-2 (2004)

Colonic leptin: source of a novel proinflammatory cytokine involved in IBD.

- Leptin induced epithelial wall damage and neutrophil infiltration that represent characteristic histological findings in acute intestinal inflammation.
- These observations provide evidence for an intraluminal biological signaling of leptin and a new pathophysiological role for intraluminal leptin during states of intestinal inflammation such as inflammatory bowel disease.

- **Sitaraman S.: FASEB J 2004 Apr;18(6):696-8. Colonic leptin: source of a novel proinflammatory cytokine involved in IBD.**

Mast cell cary receptors for leptin

- **Department of Pathology, Ghent University Hospital, Ghent University, Belgium.**
- **Recent findings demonstrate the expression of leptin and leptin receptors on mast cells, suggesting paracrine and/or autocrine immunomodulatory effects of leptin on mast cells.**
- **Taildeman J, Pérez-Novo CA, Rottiers I, Ferdinande L, Waeytens A, De Colvenaer V, Bachert C, Demetter P, Waelput W, Braet K, Cuvelier CA: Human mast cells express leptin and leptin receptors. Histochem Cell Biol Jun;131(6):703-11 (2009).**

Mast cells induce leptin

- The expression of leptin and leptin receptors on mast cells, suggesting paracrine and/or autocrine immunomodulatory effects have been shown late

Taildeman J, Pérez-Novo CA, Rottiers I, Ferdinande L, Waeytens A, De Colvenaer V, Bachert C, Demetter P, Waelput W, Braet K, Cuvelier CA Human mast cells express leptin and leptin receptors. Histochem Cell Biol 2009 Jun;131(6):703-11

Histamin intolerance


Ursachen entzündlicher Darmerkrankungen

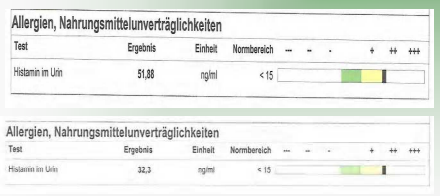
Test	Ergebnis	Einheit	Normbereich	---	--	-	+	++	+++
Nahrungsmittelallergien: EPX	599,8	ng/ml	< 350						

Nitrosativer Stress

Stufe 3a: Nitrosativer Stress

Test	Ergebnis	Einheit	Normbereich	---	--	-	+	++	+++
Histamin im Stuhl	1780	ng/ml	< 600						

Histaminintolerance after 6 weaks of diet

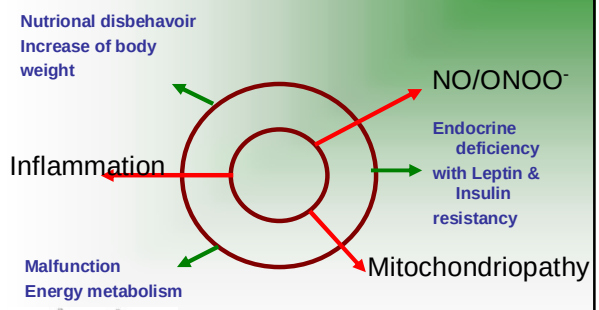

Allergien, Nahrungsmittelunverträglichkeiten

Test	Ergebnis	Einheit	Normbereich	---	--	-	+	++	+++
Histamin im Urin	51,88	ng/ml	< 15						

Allergien, Nahrungsmittelunverträglichkeiten

Test	Ergebnis	Einheit	Normbereich	---	--	-	+	++	+++
Histamin im Urin	32,0	ng/ml	< 15						

Cycle of chronic disease



A new approach to treatmeatment

- Nutrition: low carb diet, low inflammatory response diet, low histamine diet
- control of thyreoid gland activity
- **Vitamin D**
- **Glutathione**
- **Q 10 and L-Acetyl Carnithine**
- **Methyl B12**

High Glucose levels contribute to SOD production in mitochondria

- High levels of glucose result in increased mitochondrial superoxide generation and ROS
- **Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, Brownlee M. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest.* 2003;112:1049–1057.**

Glucose is an inflammatory mediator

- **Glucose could, therefore, be viewed as an inflammatory mediator**

Dandona P, Aljada A, Dhindsa S, Garg R. Insulin as an anti-inflammatory and antiatherosclerotic hormone. *Clin Cornerstone.* 2003:S13–S20

Overflow of glucose contributes to nitrosative stress in CNS

- In obesity, the brain becomes insulin resistant and can have too much glucose, which is associated with accelerated brain aging and may involve NO-induced oxidative damage to neuronal mitochondria

- **Mastrocola R, Restivo F, Vercellinatto I, Danni O, Brignardello E, Aragno M, Boccuzzi G. Oxidative and nitrosative stress in brain mitochondria of diabetic rats. *J Endocrinol.* 2005;187:37–44.**

Hyperglycaemia activates inflammatory system

- Hyperglycaemia can activate the inflammatory system via advanced glycation end-products (AGE), which are known to increase NFκB activity

Yeh CH, Sturgis L, Haidacher J, Zhang XN, Sherwood SJ, Bjercke RJ, Juhasz O, Crow MT, Tilton RG, Denner L. Requirement for p38 and p44/p42 mitogen-activated protein kinases in RAGE-mediated nuclear factor-kappaB transcriptional activation and cytokine secretion.

***Diabetes.* 2001;50:1495–1504**

High carb food fuels metabolic syndrome

- **Hence, excessive levels of high glycaemic index carbohydrates could not only result in a large amount of saturated fat being created** (as glycogen stores become saturated), but if the pancreas was unable to cope, hyperglycaemia. Certainly, there is good evidence that high carbohydrate diets are more likely to result in the metabolic syndrome, which is supportable by basic biochemistry.

- **Accurso A, Bernstein RK, Dahlqvist A, Draznin B, Feinman RD, Fine EJ, Gleed A, Jacobs DB, Larson G, Lustig RH, Manninen AH, McFarlane SI, Morrison K, Nielsen JV, Ravnskov U, Roth KS, Silvestre R, Sowers JR, Sundberg R, Volek JS, Westman EC, Wood RJ, Wortman J, Vernon MC. Dietary carbohydrate restriction in type 2 diabetes mellitus and metabolic syndrome: time for a critical appraisal. *Nutr Metab* 2008;5:9.**

Impact of brain function on insulin resistance

- The brain is almost totally dependent on glucose: although it constitutes only 2% of the body mass, its metabolism accounts for 50% of total body glucose utilization. Although the brain does not require insulin to take up glucose, insulin receptors are found in many areas of the brain and are vital for normal function.

Chronic fatigue syndrome is associated with metabolic syndrome: results from a case-control study in Georgia

- We hypothesized that persons with chronic fatigue syndrome (CFS) would have a higher prevalence of metabolic syndrome compared with well controls, and that unwell persons with insufficient symptoms or fatigue for CFS (termed ISF) would have a prevalence of metabolic syndrome intermediate between those with CFS and the controls. The analysis was based on a population-based case-control study conducted in metropolitan, urban, and rural areas of Georgia, United States, between September 2004 and July 2005.
- Persons with CFS were 2-fold as likely to have metabolic syndrome (odds ratio = 2.12, confidence interval = 1.06, 4.23) compared with the controls. There was a significant graded relationship between the number of metabolic syndrome factors and CFS; each additional factor was associated with a 37% increase in likelihood of having CFS.
- **Elizabeth M Maloney; Roumiana S Boneva; Jin-Mann S Lin; William C Reeve: Metabolism: clinical and experimental (2010)**

CHRONIC FATIGUE SYNDROME AND METABOLIC SYNDROME X IN CHERNOBYL ACCIDENT SURVIVORS

- *Frequency, the trends of development, and outcomes of Chronic Fatigue Syndrome (CFS) and Metabolic Syndrome X (MSX) in clean-up workers of the consequences of the Chernobyl accident (liquidators) were studied.*
- *During 1990–2001 there were examined randomly selected 367 liquidators of 1986–1987.*
- *It was established that CFS frequency significantly (p<0,001) decreased (from 65,5% in 1990–1995 to 10,5% in 1996–2001) and MSX frequency significantly (p<0,001) increased (from 15 to 48,2%).*
- *CFS and MSX are considered to be the stages of another neuropsychiatric and physical pathology development, and CFS can transform towards MSX. Mitochondrial genome disorders together with changes of transmembrane ionic transport could be the basis of CFS and MSX.*
- **Kovalenko A. N., Loganovsky K. N. Ukrainian Medical Journal №6(26) XI - XII 2001**

Melatonin is a scavenger of peroxynitrite

It was demonstrated that the pineal neurohormone melatonin inhibits peroxynitrite-mediated oxidant processes.

Melatonin caused a dose-dependent inhibition of the oxidation of dihydrorhodamine 123 to rhodamine *in vitro*.

Moreover, in cultured J774 macrophages, melatonin inhibited the development of DNA single strand breakage in response to peroxynitrite and reduced the suppression of mitochondrial respiration.

Thus, melatonin appears to be a scavenger of peroxynitrite. This action may contribute to the antioxidant and antiinflammatory effects of melatonin in various pathophysiological conditions

- **Eli Gilad, Salvatore Cuzzocrea, Basilia Zingarelli, Andrew L. Salzman and Csaba Szabó Life Sciences (60),10, 1997, 169-174**

Melatonin receptors in adipocytes

- Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Brazil.:
- **Melatonin receptors were recently described in adipocytes, where leptin is synthesized.** Here, we investigated the influence of melatonin and its interaction with insulin and dexamethasone on leptin expression.
- It was concluded that **melatonin interacts with insulin and upregulates insulin-stimulated leptin expression**

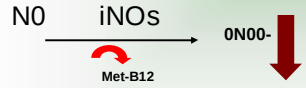
- **Alonso-Vale MI et al.: Melatonin enhances leptin expression by rat adipocytes in the presence of insulin. Am J Physiol Endocrinol Metab 2005 Apr;288(4):E805-12**

Melatonin & Leptin

- **Leptin and melatonin play an important role in the regulation of body mass and energy balance: Both hormones show a circadian rhythm, with increasing values at night.**
- Lean subjects:
- → Melatonin (at night) ▲ → Leptin▲-> **satiety at night**

Scavengers of N0/0N00^{-} Cycle

- Among a few chemical substances Methyl-Cobalamin scavenges N0 production via reducing the activity of the iN0s:



Pall ML. Cobalamin used in chronic fatigue syndrome therapy is a nitric oxide scavenger. Journal of Chronic Fatigue Syndrome, 2001;8(2):39-44

Reduced bioavailbility of Vitamin B12

- As we grow older, absorption of all forms of B12 diminishes.
- Weak stomach acid and other gastrointestinal conditions, medications, acid blockers, and reduced production of a protein called "intrinsic factor" all impair B12 absorption.
- Use of laxatives depletes storage of B12.

Ann Pharmacother 1999 May;33:641-3

Effects of nitrosative stress

- *Vitamin B12 inhibits the synthesis of iNO. Thus chronic nitrosative stress triggers the deficiency of vitamin B12.*
- *B12 is also the most powerful scavenger of nitric oxide and will therefore reduce the symptoms of CFS and inflammtion regardless of the cause*

Pall ML. Cobalamin used in chronic fatigue syndrome therapy is a nitric oxide scavenger. Journal of Chronic Fatigue Syndrome, 2001;8(2):39-44

Scavenging N0

- Methyl-Vitamin B12 is one of two active coenzymes used by B-12 dependent enzymes in the body, and is specifically used by 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), also known as methionine synthase.
- When Vitamin B12 succeeds at entering the bloodstream it then must be **converted to an active coenzyme before it is beneficial to our health**.

double-blind crossover trial with Met B12 CFS

- Twenty eight subjects suffering from non-specific fatigue were evaluated in a double-blind crossover trial of 5 mg of hydroxocobalamin twice weekly for 2 weeks, followed by a 2-week rest period, and then a similar treatment with a matching placebo.
- The placebo group in the first 2 weeks had a favourable response to the hydroxocobalamin during the second 2 week period with respect to enhanced general well being. Subjects who received hydroxocobalamin in the first 2-week period showed no difference between responses to the active and placebo treatments, which suggests that the effect of vitamin B12 lasted for over 4 weeks. **It is noted there was no direct correlation between serum vitamin B12 concentrations and improvement.** Whatever the mechanism, the improvement after hydroxocobalamin may be sustained for 4 weeks after stopping the medication
- **A Pilot Study of Vitamin B12 in the Treatment of Tiredness," Ellis, F.R., and Nasser, S., British Journal of Nutrition, 1973;30:277-283**

B12 Deficiency

- The confusion is that people with low, low-normal, or even normal levels of the inactive form of B12 circulating in their blood can be deficienct in the required active forms of B12.

- **Tucker KL, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PW, Selhub J Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. Am J Clin Nutr. 2000 Feb;71(2):514-22.**

Vit B12 Deficiency in Aging

- Vitamin B12 deficiency is estimated to affect 10 to 15% of people over the age of 40, and approximately 40% of all adults may have B12 levels at or below the "low normal" range.

Baik HW and Russell RM. Vitamin B12 deficiency in the Elderly. Annu Rev Nutr 1999; 19:357-77

Long term bioavailability of CN B12

- The amount of B12 (simple cobalamin) excreted in the urine after a dose of methylcobalamin is about one-third that of a similar dose of cyanocobalamin.

- **Okuda K, Yashima K, Kitazaki T, Takara I. Intestinal absorption and concurrent chemical changes of methylcobalamin. J Lab Clin Med 1973;81:557-567**

Methyl-B12

- Once absorbed Methyl- B12 may be retained in the body better than cyanocobalamin
- Methyl B12 has a high bioavailablity

Bioavailability of Vit B12

- It takes at least three steps to convert the manmade CN-B12 in most vitamin supplements (called cyanocobalamin) into methylcobalamin, the only form of B12 your body can use to neutralize homocysteine.

- Pezacka E, Green R, Jacobsen DW. Glutathionylcobalamin as an intermediate in the formation of cobalamin coenzymes. Biochem Biophys Res Comm 1990;2:443-450

Long term metabolism of CN B12

- It takes 1-2 months for cyanocobalamin to be converted to active B12, assuming an adequate supply of necessary cofactors is available .

- Heinrich HC, Gabbe EE. Metabolism of the vitamin B12-coenzyme in rats and man. Ann NY Acad Sci 1964;112:871-903

Sublingual application

- Research has also shown that **both the oral and the sublingual forms of B12 are identically effective** at doses of at least 500 mcg.

- Sharabi A, Cohen E, Sulkes J, Garty M. Br J Clin Pharmacol. 2003 Dec;56(6):635-8.

Vitamin B12 levels

- Blood levels > 600 pg/ml are considered optimal for preventive use, Patients without any clinical impairment should be leveled at 400 pg/ml.
- N.J. Mann et al., Eur J Clin Nutr 1999 Nov;53(11):895-9

Chromium III

- **University of Oxford Department of Psychiatry:**
- (Cr3+) is required for optimal insulin activity and normal carbohydrate and lipid metabolism.
- Cr3+ may have antidepressant properties, possibly by enhancement of monoamine function through its ability to increase amino acid transport to the brain.
- **Franklin M, Odontiadis J:Effects of treatment with chromium picolinate on peripheral amino acid availability and brain monoamine function in the rat. Pharmacopsychiatry 2003 Sep;36(5):176-80**

Vitamin D and Leptin

- **There was a significant increase in leptin levels after therapy and the leptin levels were positively correlated with 25(OH)D levels (r:0.45, p<0.05).**
- **Tarcin O, Yavuz DG, Ozben B, Telli A, Velioglu Ogunc A, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O, Akalin S Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 2009**

Curcumin eliminates leptin's effects

- **The** antioxidant **Curcumin interrupts leptin signaling by reducing phosphorylation levels of leptin receptor (Ob-R) and its downstream intermediators. In addition, curcumin suppresses gene expression of Ob-R in HSCs, which requires the activation**
- Curcumin abrogates the stimulatory effect of leptin activation by interrupting leptin signaling and attenuating leptin-induced oxidative stress.

- **Tang Y, Zheng S, Chen A: Endocrinology;150(7):3011-20 (2009)**

Take home message

- Leptin is a matching biomarker mediating nitrosative stress
- Leptin induces metabolic changes in patients with CFS
- Leptin induces autoimmunity and immundeficiency

Outlook

- We have entered an exciting era in clinical research of nutrigenomics that is expected to give answers to many important questions.
- Although we are now at a very early stage, it seems likely that these efforts will eventually provide tangible benefits to obese persons who are striving unsuccessfully to control excessive body weight.

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Augsburg-Bratislava

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Thank you for your final attention

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