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ABSTRACTS

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Central nervous system control of inflammation-induced muscle catabolism

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Background and aims: Skeletal muscle catabolism is a co-morbidity of many chronic diseases and is the result of systemic inflammation. While direct inflammatory cytokine action on muscle promotes atrophy, non-muscle sites of action for inflammatory mediators are less well described. We sought to demonstrate that inflammatory signaling limited to the central nervous system induces muscle catabolism.

Methods: Interleukin-1 beta (IL-1β) was injected centrally at doses that estimate pathophysiological concentrations found during illness. Control injections of the same dose were given peripherally. Both acute and chronic studies were performed in animals with pharmacological and surgical blockade of glucocorticoid signaling. Molecular and pathological analysis of muscle was performed.

Results: We have demonstrated that central nervous system-delimited IL-1β signaling alone potently evokes a catabolic program in muscle, rapidly inducing atrophy. This effect is dependent on hypothalamic–pituitary–adrenal (HPA) axis activation, as CNS IL-1β-induced atrophy is abrogated by adrenalectomy or pharmacological blockade of glucocorticoid signaling. Microarray analysis also demonstrated that a glucocorticoid-responsive gene expression pattern is present in the muscle of multiple models of inflammatory muscle atrophy. Adrenalectomy also blocks the atrophy program in response to systemic inflammation, demonstrating that glucocorticoids are requisite for this process. When circulating levels of corticosterone are clamped at a level equivalent to those produced under inflammatory conditions, profound muscle wasting occurs.

Conclusions: Together, these data suggest that a significant component of inflammation-induced muscle catabolism occurs indirectly via a relay in the central nervous system.

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Gαi2 signaling promotes skeletal muscle hypertrophy, myoblast differentiation and regeneration via PKC- and HDAC- dependent pathways

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Skeletal muscle atrophy results in increased loss of function and mortality. The signaling pathways downstream of G protein-coupled receptors (GPCRs) that are able to block atrophy have not been well studied. In this study, we demonstrate that activation of the heterotrimeric guanine nucleotide—binding protein (G protein) Gαi2 induces skeletal muscle hypertrophy. Gαi2 is required for hypertrophy induced by lysophosphatidic acid, which activates a Gαi-linked GPCR. A constitutively active mutant of Gαi2 results in myotube growth, characterized by increased protein synthesis and enhanced fusion. Gαi2 activates p70S6 kinase and inhibits GSK3β, thereby activating the pro-differentiation NFAT transcription factor. Gαi2 activity is dependent on PKC signaling, since PKC inhibitors block the effects induced by Gαi2, whereas activated PKCα induces hypertrophy. Gαi2 can also inhibit atrophy caused by the cachectic cytokine TNFα, and thereby blocks the upregulation of the atrophy-inducing E3 ubiquitin ligase *MuRF1* via inhibition of the HDAC/*Myogenin* pathway. We also found that Gαi2 activation enhances muscle regeneration and causes a switch to oxidative fibers; the fiber-type switch is coincident and perhaps caused by an upregulation of PGC-1β. This study thus identifies a previously undiscovered skeletal muscle hypertrophy and differentiation pathway, and links Gαi2 to the recently identified HDAC/myogenin/MuRF1 atrophy pathway, indicating that receptors that act through Gαi2 represent potential targets for preventing skeletal muscle wasting.

1–30

Down regulation of TWIST-1 and its target, the miR 199/214 cluster, in human myocardium of patients with dilated cardiomyopathy results in increased proteasome activity

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Background: TWIST-1 is a transcription factor that has been described to regulate the microRNA (miR) 199/214 cluster during development. Genetic disruption of TWIST-1 resulted in a cachectic phenotype and early death of the knockout mice. This might be connected to the activity of the ubiquitin-proteasome system (UPS), as miR 199a has been suggested to regulate the ubiquitin E2 ligases Ube2i and Ube2g1. A loss of cardiomyocyte diameter and left ventricular mass is regularly seen in dilated cardiomyopathy.

Methods: Cardiac tissue from explanted heart of 40 patients with dilated cardiomyopathy and 20 rejected donor hearts were analysed for protein expression of TWIST-1and its inhibitor Id-1, MuRF-1 and MAFbx, the expression of miR 199a, 199b and 214, as well as the activity of the UPS by using specific flurogenic substrates in an enzyme kinetics assay.

Results: TWIST-1 was downregulated by 43% compared to donors (p=0.003), while Id1 expression was unchanged. This resulted in a reduced expression of miR199a (−35.2%, p=0.107), miR 199b (−33%, p=0.042) and miR 214 (−41%, p=0.015) compared to donor hearts. An increased peptidylglutamyl-peptide hydrolysing activity (p<0.0001) was observed in the UPS, while the chymotrypsin-like and trypsin-like activities were unchanged (both p>0.2). The protein level of the rate-limiting ubiquitin E3-ligases MuRF-1 and MAFbx were up-regulated compared to donors (p=0.006 and 0.038, respectively).

Conclusion: In summary, the TWIST-1/miR199/214 axis is downregulated in dilated cardiomyopathy, which is likely to play a role in the increased activity of the UPS. This may contribute to the loss of cardiac mass during dilatation of the heart.

1–31

Does the exposition to GSM electromagnetic waves induce a no thermal effect on a fast-twitch skeletal muscle, the "edl" in rats?

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One of the important challenges developed in the last decades and could affect health is the population exposure to electromagnetic waves (EMW), particularly the 'Global System of Mobile Communication signals', GSM signals. These GSM signals are emitted from many sources as like cell phones and base stations antenna. Our study investigates the effects of EMW radiation emitted from GSM mobile phones antenna (frequency at 900 MHz, Eeff=25 V/m) on extensor digitorum longus (edl) muscle myofibrillar proteins. Results show that animals body weight was not affected by 6 weeks of continuous 24-h exposure, but edl absolute muscle mass was increased by 10.5% (control: 0.1744±0.00252, exposed: 0.1927±0.00291, n=12, p<0.05). Since myofibrillar proteins content is directly proportional to muscle mass, protein dosage using the Bradford technique was performed and protein concentration was calculated in mg/g of muscle. An increase by 59% in edl myofibrillar protein content after the period of exposure was observed (control 20.11±1.59, exposed 31.97±2.81, n=12, p<0.05). The analysis of MHC isoforms by SDS-PAGE as already described by Talmadge et al. shows an increasing by 431.54% in MHC I, 35.08% in MHC IIa a decreasing by 16.34% in MHC IIb without a significant modification in the percentage of expression of MHC IIx/d (n=12 and p< 0.05). Thus, this study shows that 6 weeks of continuous whole-body exposure to EMW can induce a no thermal effect in myofibrillar proteins of fast-twitch mammalian skeletal muscles.

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Role of lipases in cancer-associated cachexia

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Cancer-associated cachexia is a multi-organ syndrome associated mainly with gastrointestinal and lung cancer. It is characterized by progressive loss of body weight, reduced muscle strength, and loss of adipose tissue as well as skeletal muscle mass. We evaluated the role of lipases, adipose triglyceride lipase (ATGL), and hormone sensitive lipase (HSL) in the initiation and progression of cancer-associated cachexia using two tumor models, Lewis ung carcinoma and B16 mlanoma in wildtype (wt), ATGL knockout, and HSLknockout mice. Cachexia was evaluated based on various systemic parameters, body weight, and specific organ weights as well as body composition using NMR and MRI techniques. While wt mice lost ~90% white adipose tissue (WAT) and ~40% skeletal muscle (m. gastocnemius) weight due to tumor progression, ATGL knockout mice retained the tissue depots in spite of similar tumor development. HSL knockout mice showed an

attenuated cachectic phenotype in comparison to wildtype. This resulted in significant body weight decrease in tumor-bearing wt and HSL knockout mice but ATGL knockout mice remained protected from cachexia. Although serum lipolytic factors, IL-6, ZAG, and TNF-alpha levels were similarly enhanced in tumor mice of all genotypes, WAT from ATGL tumor-bearing mice showed reduced lipolysis compared to tumor-bearing wt mice. Increased proteosomal and caspase 3/7 activities were observed in tumor-bearing wt mice which are attenuated in tumor-bearing lipase knockout mice. Interestingly, total triglyceride hydrolase activity and ATGL activity in particular in WAT of cancer patients showed a significant negative correlation with patient body mass index. Enzyme activities in non-cancer patients failed to show any such correlation. The results propose that both ATGL and HSL plays instrumental role in the progression of cancer-associated cachexia and hence they might represent attractive targets for drugs development.

1–33

A in vitro model for skeletal muscle force and atrophy, myotransducer

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Background and aims: Skeletal muscle atrophy is characterized by a decrease in muscle mass and force. In the drug discovery for muscle atrophy, muscle hypertrophy or increased muscle mass can be easily monitored using cellular or animal model rather than force measurement. However, since previous researches have implicated that muscle force measurement is important for monitoring the effect of treatment in muscle atrophy, we tried to establish in vitro muscle force measurement system, myotransducer.

Methods: Electro pulse stimulation (EPS) of C2C12 cells was used for in vitro muscle force model. C2C12 cells were seeded on collagen membranes and differentiated to myotube (myosheet) then EPS (0.5 V, 0.5 Hz) was applied until maximal tetanus force (max-force) of myosheet reached to stable state. IGF-1 and dexamethasone (DEX) were added in order to induce for hypertrophy or atrophy and time course of max-force was monitored.

Results: After 10-days EPS treatment, IGF-1 myosheets showed hypertrophic feature and its max-force was significantly higher than control. DEX induced myotube disruption and its max-force was diminished. This was accompanied by increased mRNA expression of atrogin-1, MuRF-1 and Cbl-b. When EPS loading was decreased (0.5~0.05 V), max force was also reduced in a voltage intensity dependent manner, but IGF-1 treatment had still higher max force than control. Moreover, when EPS reloaded at higher level, max-force of IGF-1 myosheets was rapidly recovered up to initial level before voltage lowering started. At the end point of EPS, atrogin-1 and MuRF-1 mRNA expressions were suppressed by IGF-1 treatment. These results are similar to IGF-1 treatment on *in vivo* muscles atrophy.

Conclusions: Our in vitro system, myotransducer, could detect difference of muscle force by voltage loading (hypertrophy) and unloading (atrophy), and drug treatments (IGF-1 and glucocorticoid). These results suggest that myotransducer may be useful to estimate muscle force in vivo.

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Ghrelin secretion and anxiety in a rat model of anorexia–cachexia induced by methotrexate chemotherapy

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Background and aims: Peripheral acylated ghrelin has orexigenic and anxiolytic effects, but its expression in the brain and putative roles are uncertain. Here, we studied if peripheral and central ghrelin expressions are altered in chemotherapy-associated anorexia–cachexia.

Methods: Preproghrelin and GOAT mRNA were assayed in the stomach, hypothalamus and amygdala and plasma acyl- and des-acyl-ghrelin were measured in Sprague–Dawley rats treated with methotrexate (MTX, 2.5 mg/kg, SC for 3 days). Forced-swim (FST) and elevated plus maze (EPM) tests and tissues sampling were performed at day 5 after first MTX injection, corresponding to the maximal decrease in food and water intakes and a loss of 25% of body weight. Control rats received PBS; the EPM test was done in a separate series of rats.

Results: Preproghrelin mRNA expression levels were lower in the stomach but increased in the hypothalamus of MTX rats. GOAT mRNA was decreased in the stomach and amygdala but not in the hypothalamus of MTX rats. Plasma levels of acyl- and des-acyl ghrelin did not differ significantly from controls but acyl-/des-acyl ghrelin ratios was lower in MTX rats. No significant differences in FST were observed between MTX and control rats, but fewer entries in open and central zone of the EPM were found in MTX rats.

Conclusions: These data show that the hypothalamic preproghrelin gene expression is increased during MTX-induced anorexia. Considering activation of hypothalamic neurosecretory vasopressin neurons in MTX-treated rats (Hamze-Sinno M. et al.; Physiol Behav, 2010), and immunohistochemical detection of ghrelin in magnocellular neurons (own unpublished results), these data suggest that both acyl- and des-acyl-ghrelin can be secreted from the hypothalamus into the systemic circulation in order to compensate loss of gastric ghrelin and/or to counteract dehydration. However, the lower rates of acylation of systemic ghrelin in MTX rats may contribute to anorexia–cachexia and increased anxiety.

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Endothelial function and circulating angiogenic cells in Fabry disease

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Background: Fabry disease is an X-chromosomal recessive deficiency of the lysosomal hydrolase alpha-galactosidase A (alpha-Gal A), which catalyzes the hydrolytic cleavage of the terminal galactose from globotriaosylceramide (GL-3). This results in an accumulation of GL-3 in a variety of cells such as endothelial cells, smooth muscle cells, and cardiomyocytes leading to functional impairment. A majority of patients develop end-stage kidney disease depending on dialysis treatment. This is associated with the malnutrition-inflammation atherosclerosis syndrome, progressive cachexia, and endothelial dysfunction. Enzyme replacement therapy can prevent or attenuate onset of cardiovascular complications.

Methods: We investigated the impact of Fabry disease on the biology of circulating angiogenic cells (CACs) and endothelial function. Twenty-six patients with untreated Fabry disease, 16 patients after 12 months of enzyme replacement therapy (ERT), and 26 healthy controls were investigated. Endothelial function was assessed by the EndoPat 200 device. CAC numbers were assessed by flow-cytometry, CAC function by a modified Boyden chamber assay.

Results: Fabry patients showed an impaired endothelial function, which normalized after enzyme replacement therapy. Fabry patients displayed increased numbers, but impaired function of CACs. Immunofluorescence and electron microscopy identified an excessive accumulation of GL-3 in Fabry diseased CACs. Enzyme replacement therapy attenuated CAC dysfunction in Fabry patients via a reduction in GL-3 accumulation in vitro and in vivo. SiRNA-mediated knockdown of alpha-Gal A in healthy CACs impaired their migratory capacity underlining a key function of this enzyme in CAC function.

Conclusions: Fabry patients show a dysfunction of circulating CAC and an impairment of endothelial function. Enzyme replacement therapy improves CAC and endothelial function and thus may attenuate development of cardiovascular/kidney disease and finally cachexia development in the long-term in this patient population.

2–20

The miRNA-212/132 family regulates cardiomyocyte size and autophagy

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Purpose: Growth control of cardiomyocytes is of major importance for diseases such as cardiac hypertrophy and cardiac wasting. MiRNAs are novel master regulators of gene expression, but their role in such processes is not entirely clear and never has been investigated systematically.

Methods and results: Here, we performed a functional miRNA library screen in cardiomyocytes and show the microRNA (miRNA)-212/132 family to regulate hypertrophy and autophagy. Hypertrophic stimuli lead to the upregulation of miR-212 and miR-132 expression in cardiomyocytes, which are both necessary and sufficient to drive the hypertrophic growth of cardiomyocytes. MiR-212/132 null mice are protected from pressure-overload-induced heart failure, whereas cardiomyocyte-specific overexpression of the miR-212/132 family leads to impaired autophagic activity, pathological cardiac hypertrophy, heart failure, and finally increased lethality in mice. Mechanistically, both miR-212 and miR-132 directly target the anti-hypertrophic and pro-autophagic FoxO3 transcription factor and overexpression of these miRNAs leads to hyperactivation of pro-hypertrophic calcineurin/NFAT signalling and impaired autophagic response upon starvation. Pharmacologic inhibition of miR-132 by antagomir injection rescues cardiac hypertrophy and heart failure in mice, offering a possible therapeutic approach for cardiac failure.

Conclusions: The miRNA-212/132 family plays a dominant role in the development of cardiac hypertrophy and heart failure and serves as a novel therapeutic relevant target.

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Angiotensin II upregulates Pp2cα and inhibits AMPK signaling and energy balance leading to skeletal muscle wasting

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Background and aims: Several diseases, including congestive heart failure and chronic kidney disease are characterized by elevated angiotensin II (Ang II) and muscle wasting. Ang II causes muscle

wasting by reducing appetite and enhancing catabolism, but little is known about the effects of Ang II on muscle metabolism and energy stores. Muscle-specific overexpression of insulin-like growth factor 1 (IGF-1) prevents Ang II wasting despite the ability of Ang II to impair upstream IGF-1 signaling. In mice overexpressing IGF-1 in skeletal muscle (MLC-IGF-1), the serine/threonine kinase 5′-adenosine monophosphate-activated protein kinase (AMPK), which functions as a sensor of cellular energy status, is activated by 62%. We hypothesized that Ang II induces muscle wasting by inhibiting AMPK signaling and altering energy balance, and that AMPK activation mediates the protective effects of IGF-1 against Ang II wasting.

Methods: We infused 1,000 ng/k/min Ang II or sham infused FVB mice or MLC-IGF-1 mice for 7 days ± the pharmacological AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) and measured ATP, muscle weight; AMPK, AKT expression and phosphorylation; E3 ubiquitin ligase expression.

Results: Ang II infusion in mice reduced gastrocnemius muscle weight by 26% and depleted ATP by 74%. Furthermore, Ang II upregulated the protein phosphatase PP2Cα by 2.6-fold and reduced AMPK phosphorylation and signaling in muscle. Importantly, muscle-specific overexpression of IGF-1 and AICAR each blocked Ang II-induced PP2Cα upregulation, restored AMPK activity to levels of controls, and reversed Ang II-mediated ATP depletion and muscle wasting. Moreover, AICAR activated Akt and inhibited Ang II-induced increases in E3 ubiquitin ligase expression.

Conclusions: These results demonstrate critical roles for energy depletion and AMPK inhibition in Ang II-induced muscle wasting, and demonstrate a role for AMPK activation in IGF-1 mediated rescue from Ang II wasting. These findings suggest a therapeutic potential for AMPK activators in diseases characterized by muscle wasting.

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Signaling in the heart of rats with cancer cachexia compared myocardial infarction

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Background: Cachexia is a common co-morbidity in cancer and chronic heart failure (CHF) and increases the mortality of patients. It is well established that CHF and chemotherapeutics in cancer lead to an impaired heart function due to ventricular remodeling, but it is not known if the tumor itself affects the heart. Here, we compared the effects of cancer cachexia-induced to myocardial infarction-induced cardiac alterations in signaling.

Study design and methods: We used the AH-130 hepatoma model (13 days) as a model for cancer cachexia and the LAD model (56 and 210 days) to induce cardiac cachexia. In all studies, we assessed the body weight, body composition, cardiac function and quality of life. At the end of the studies, we sacrificed the animals and measured the weight of the organs. Finally, we looked for apoptosis, necrosis and fibrosis in the heart using fluorogenic and Luminex assays. Furthermore, we analysed atrophic proteins and anabolic signaling pathways by Western Blot.

Results: Myocardial infarction of rats caused an impaired heart function without the induction of true cachexia. We found neither apoptosis nor an increase in catabolic signaling, but a rise in the anabolic signaling in the heart. The rat cancer model caused severe cachexia with a strongly impaired cardiac function. There was a 58% loss of LV mass due to an increased catabolism (UPS-System), more apoptosis (increased activity of caspase-3 and -6), necrosis and a downregulation of the anabolic signaling (Akt, 4EBP1, p70S6K).

Conclusion: Myocardial infarction did not cause cachexia but led to the development of heart failure. The hepatoma animal model as a model for cancer cachexia severely impaired the cardiac function and led to body and cardiac wasting.

Table. Results of studies

	Day 13		Day 56		Day 210	
	Sham	Tumor	Sham	LAD	Sham	LAD
Δ Body weight vs baseline (%)	23.4±2.06	−23±1.08 ***	71.6±3.51	66.3±2.99	142±8.49	178±4.42 ***
Δ Lean mass vs baseline (%)	21.7±1.36	−24.8±1.15 ***	12.13±1.28	12.42±1.10	NA	NA
Weight of heart (mg)	787±15.93	466±12.14 ***	1,132±19.61	1,278±45.3 **	1,220±32.7	1,582±56.4 ***
LV EF (%)	79.7±1.50	50.5±1.39 ***	63.3±1.18	30.74±1.86 ***	72.9±4.85	37.1±1.52 ***
Caspase-3 activity in the heart (nmol/mg/min)	29.3±3.70	99.2±28.9 *	221±76.9	200±33.1	74.9±11.53	81.6±6.88

*$p<0.05$ vs Sham, **$p<0.01$ vs Sham, ***$p<0.001$ vs Sham.

2–23

Atrophy-mechanisms are up-regulated in cardiac tissue of patients with advanced dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is the most common form of cardiomyopathy and patients suffer from typical symptoms of heart failure. Recently, it was shown that wasting of cardiac mass may play a crucial role in heart failure.

The aim of the study was to examine which hypertrophic and atrophic signalling pathways are influenced in cardiac tissue of patients with advanced DCM in comparison to a control group (donors).

Therefore, protein expression of total and phosphorylated Akt (pAkt), p70S6K, 4E-BPI, GSK3-α/β, Myostatin, and LC-3-I/II as well as p62 as autophagosomal-acting proteins were analysed in septum of 20 heart donors and 41 recipients with advanced DCM. Furthermore, apoptotic activity in the septum was measured by fluorometric caspase assay.

Total Akt but not phosphorylated Akt (active) is significantly ($P<0.05$) down-regulated in patients with DCM in comparison to donors. There is also a significant down-regulation of downstream targets of Akt-like phosphorylated p70S6K (active; $P<0.01$), phosphorylated 4E-BPI (inactive; $P<0.001$), and phosphorylated GSK3β ($P<0.05$) and GSK3α ($P<0.001$; inactive) which all might lead to inhibition of protein synthesis and cardiac tissue growth. Furthermore, an up-regulation of myostatin ($P<0.001$) has been observed. The caspase-3 activity was significantly elevated ($P<0.001$) whereas the caspase-6 activity remained unchanged. Hence, apoptotic processes might be elevated in cardiac tissue of DCM patients compared to control group. Finally, autophagy might also influence the process of wasting in cardiac tissue because the expression of LC-3 II was increased ($P<0.001$) whereas the expression of p62 remained unchanged in comparison to the control group.

The results show a down-regulation of hypertrophic pathways as well as an up-regulation of atrophic pathways in cardiac tissue of DCM patients compared to donors. This might lead to a loss of cardiac tissue and therefore contribute to the progression of heart failure and its symptoms.

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Markers of cholestatic liver damage are increased in patients with cardiac cachexia

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Introduction: Total and direct serum bilirubin have been shown to be predictive for decreased survival in patients with chronic heart failure (CHF). Considering liver function tests, cachectic CHF patients frequently present with hypoalbuminemia. However, levels of bilirubin and other liver function parameters in cardiac cachexia have not been investigated yet.

Methods: We prospectively investigated 112 non-cachectic CHF patients (age 66.5±0.9 years; left ventricular ejection fraction [LVEF] 31.2±0.7; NYHA class 2.4±0.1; peak VO_2 16.7±0.5 mL/kg/min, body mass index [BMI] 28.7±0.5 kg/m^2), 15 cachectic CHF patients (age 66.0±2.7 years; LVEF 27.9±2.0; NYHA class 2.8±0.1; peak VO_2 12.0±1.3 mL/kg/min, BMI 22.9±0.9 kg/m^2), and 31 healthy control subjects (age 61.4±2.2 years; LVEF 59.5±0.8; peak VO_2 31.2±1.8 mL/kg/min, BMI 24.8±0.5 kg/m^2). Diagnosis of cachexia was made according to current consensus based criteria. We measured direct and indirect bilirubin, albumin, gamma-glutamyl transferase (GT), alkaline posphatase (AP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), high-sensitive C-reactive protein (hsCRP) and in a subgroup of 77 patients mid-regional pro-adrenomedullin (MR-proADM) in blood. Hepatic vein diameter, central venous pressure (CVP) and systolic pulmonary arterial pressure (PAP) were estimated by abdominal sonography and echocardiography, respectively.

Results: Cachechtic patients had higher serum levels of direct bilirubin, GT, AP and lower serum levels of albumin compared to non-cachectic patients and controls, indicating altered liver function (direct bilirubin 0.39±0.09 vs 0.21±0.01 vs 0.15±0.02 mg/dl; GT 128.6±31.3 vs 69.9±7.3 vs 25.1±2.6 U/L; AP 111.3±15.6 vs 71.8±3.0 vs 56.4±2.5U/L; albumin 33.2±0.9 vs 36.9±0.4 vs 38.0±0.5 g/L, all ANOVA $p\leq0.002$). Cachectic patients, compared to non-cachectic, had lower transaminase levels; however, within the normal range (ALAT 19.3±3.1 vs 27.1±1.2, $p<0.005$; ASAT 23.8±2.2 vs 29.6±1.0, $p=0.024$). Levels of total and indirect bilirubin were similar in cachectic and non-cachectic patients versus controls. Considering all patients, higher levels of direct bilirubin, GT and AP correlated with CVP (direct bilirubin $r=0.58$; GT $r=0.50$; AP: $r=0.52$, all $p<0.0001$) and PAP (direct bilirubin $r=0.43$, $p=0.0002$; GT $r=0.40$, $p=0.0004$; AP $r=0.35$, $p=0.002$). Furthermore, levels of GT and AP correlated with greater hepatic vein diameter ($r=0.49$, $p=0.008$ and $r=0.55$, $p=0.002$). Higher direct bilirubin in these patients correlated positively with prognostic markers such as hsCRP ($r=0.24$, $p<0.01$), MR-proADM ($r=0.4$, $p=0.0006$), and NYHA class ($r=0.32$, $p=0.0004$).

Conclusion: Markers of cholestatic liver damage are elevated in patients with cardiac cachexia and correlate with indices suggestive of right heart congestion. This indicates a role of liver congestion in elevation of these cholestatic markers in cardiac cachexia as a clinical feature of CHF.

2–25

Reduced hepatic arterial blood supply in patients with advanced chronic heart failure (CHF)

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Introduction: Liver dysfunction is a frequent co-morbidity in CHF patients. Blood supply to the liver in these patients has not been investigated yet. We hypothesized that arterial blood flow to the liver gradually declines in patients with CHF with worsening of the clinical status and correlates with abnormal liver function tests.

Methods: We prospectively investigated 15 CHF patients in New York Heart Association (NYHA) Class I–II (age 64.4±2.7 years; left ventricular ejection fraction [LVEF] 33.2.9±1.8; peak VO_2 18.0±1.2 mL/kg/min, body mass index [BMI] 28.5±1.5 kg/m^2) and 18 patients in NYHA III–IV (age 68.2±2.1 years; LVEF 25.5±2.3; peak VO_2 13.6±0.9 mL/kg/min, BMI 26.2±1.0 kg/m^2). Eight of the patients in NYHA class III–IV were cachectic according to current consensus based criteria (age 65.7±4.1 years; LVEF 22.9±3.4; peak VO_2 12.3±1.8 mL/kg/min, BMI 23.8±0.8 kg/m^2). We measured blood flow in the hepatic artery and portal vein by Doppler sonography. LVEF was assessed by echocardiography. Postischemic forearm blood flow was measured by venous occlusion plethysmography. Liver function tests were measured in serum.

Results: Patients with advanced CHF (NYHA III–IV) showed lower systolic and diastolic blood flow in the hepatic artery compared to patients with milder CHF (NYHA I–II) (Table 1). As expected, patients in NYHA III-IV had a higher pulsatility index of portal vein, reflective of higher systemic and liver congestion. However, blood flow volume in the portal vein did not differ significantly between both groups. Among all CHF patients, cachectic patients had the lowest systolic and diastolic flow in hepatic artery (50.5±9.7 vs 84.1±8.2 mL/min, p=0.03 and 68.9±18.2 vs 107.3±11.7 mL/min, p=0.096). In CHF patients, we found a positive correlation between systolic flow in the hepatic artery and LVEF (r=0.47, p=0.01). Diastolic flow in the hepatic artery was positively correlated to postischemic forearm blood flow (r=0.42, p=0.02), a parameter reflective of vascular resistance and endothelial dysfunction. Furthermore, systolic and diastolic blood flow in the hepatic artery correlated negatively with levels of alkaline phosphatase (p<0.05, p=0.26), suggesting a role of reduced arterial flow in liver dysfunction.

Conclusion: Arterial blood supply to the liver is reduced in patients with advanced CHF and may contribute to cholestatic liver damage in these patients.

Table 1 Cardiac and hepatic sonographic measurements in patients grouped by NYHA class

	Mean±standard error			
Factor	All	NYHA I–II (n=15)	NYHA III–IV (n=18)	p value
LVEF (%)	29.2±1.2	31.2±0.7	25.5±2.3	0.008
Hepatic arterial FV (mL/min)	77.6±7.9	92.6±1.4	64.4±10.2	0.075
Hepatic arterial systolic FV (mL/min)	75.1±7.0	94.6±10.8	58.1±7.1	0.007
Hepatic arterial diastolic FV (mL/min)	97.1±10.2	122.9±15.4	74.5±11.2	<0.02
Portal vein diameter (cm)	0.81±0.02	0.79±0.03	0.82±0.03	NS
Portal vein FV (mL/min)	306.2±24.9	319.7±46.4	294.9±25.5	NS
Pulsatility index of portal vein	0.42±0.03	0.33±0.03	0.49±0.05	<0.02

Abbreviations: *NS* not significant, *LVEF* left ventricular ejection fraction, *FV*, blood flow volume

2–26

Common cardiac symptoms in chronic diseases: a comparison between patients with heart failure and colorectal cancer concerning heart rate variability, cardiac function and exercise capacity

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Background: The symptoms fatigue, weight loss, impaired exercise capacity and dyspnea are typically present in patients with chronic heart failure (CHF) as well as in those with colorectal cancer (CRC). We hypothesize that in patients with CRC neuroendocrine activation and autonomic dysfunction may contribute to the typical symptoms of CHF by decoupling of physiologic pathways.

Methods: We prospectively studied 28 patients with CHF (age 62±10 years, 27 male, body mass index [BMI] 28±4 kg/m^2, hemoglobin [Hb] 13.2 g/dl), 28 patients with CRC (60±12 years, 11 male, BMI: 25±4, Hb: 12.0) and 40 healthy controls (CON 60±11 years, 26 male, BMI 25±3, Hb 14.4). An echocardiogram and a 24 h-ambulatory-holter ECG were obtained from each subject to assess parameters of heart function and heart rate variability (HRV). The HRV is a measure of autonomic function. Peak oxygen uptake (Peak VO_2) and breathing efficiency (BE) were obtained to assess exercise capacity by cardiopulmonary exercise testing (spiroergometry).

Results: Patients with CHF and CRC displayed significantly impaired autonomic function as compared to controls when measured as time domain analysis by the standard deviation of normal RR intervals (SDNN, CHF 116.9±29.8, CRC 122.5±32.6, CON 142.6±36.8 ms, both $p<0.05$ vs. CON). A significantly reduced SDNN-Index was also observed in CRC patients (CRC 41.5±14, CON 54±16 ms, $p=0.002$). In the frequency domain analysis, patients with CHF and CRC had a significantly decreased low frequency power (CHF 421±361, CRC 430±303, CON 742±429 ms^2, both $p<0.05$ vs. CON) and an impaired ratio of low-to-high frequency spectra power (CHF 2.6±1.1, CRC 3.8±1.9, CON 5.1±2.2 ms^2, both $p<0.05$ vs. CON). In CRC, patients the very low frequency power was also significantly reduced (CRC 1,353±900 vs. CON 2,268±1,274 ms^2, $p=0.003$). Peak VO_2 and BE were significantly reduced in CHF and CRC patients compared to controls (CHF 17±4/36±7, CRC 20.6±5/32±6, CON 28±6 ml/min/kg/28±4 L, all $p<0.05$ vs. CON). The left ventricular ejection fraction was significantly reduced in patients with CHF (35±8%) and in patients with CRC (59±4%) as compared to controls (63±5%, both $p<0.05$ vs. CON).

Conclusions: Decreased HRV as an indication for predominant sympathetic activation, limited exercise capacity, and disturbance of heart function are present in CHF patients as well as in CRC patients. This may influence quality of live and survival. Further studies are required.

2–27

Lower mean systolic blood flow in the mesenteric arteries and celiac trunk and gastrointestinal symptoms in patients with cardiac cachexia compared to non-cachectic patients and healthy control subjects

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Introduction: Chronic heart failure (CHF) is characterized by reduced circulatory blood flow due to low cardiac output, sympathetic activation and impaired vasodilator capacity. Impaired tissue perfusion may occur as well in the intestinal vascular bed. This hypoperfusion may contribute to intestinal dysfunction. Intestinal arterial blood flow and clinical symptoms have not yet been studied in patients with and without cardiac cachexia.

Methods: We investigated 65 patients with CHF (LVEF 30±1%, peak VO2 15.2±0.6 mL/kg/min, BMI 28.2±0.6) and 26 control subjects of similar age and gender (LVEF 64±2%, peak VO2 27.3±1.4 mL/kg/min, BMI 26.1±0.8). Twelve of the patients were cachectic (LVEF 25±2%, peak VO2 12.7±1.2 mL/kg/min, BMI 25.4±1.5). Intestinal peak systolic blood flow was calculated from peak velocity and vessel diameter of the mesenteric arteries (MA) and celiac trunk (CT) using high-resolution ultrasound and Doppler sonography. We measured bowel wall thickness by transcutaneous sonography. Gastrointestinal symptoms were evaluated by gastrointestinal symptom rating scale.

Results: CHF patients showed a lower mean systolic blood flow in the intestine supplying arteries superior and inferior MA and CT compared to control subjects (351±22 vs. 522±37 mL/min, and 55±4 vs. 93±7 mL/min and 419±33 vs. 672±89 mL/min, all $p<0.004$). The same applied to the peak systolic blood flow in these three main vessels which was again lower in CHF patients compared to controls (1.9±0.1 vs. 2.6±0.2 L/min, and 0.29±0.02 vs. 0.47±0.038 and 1.9±0.1 vs. 2.9±0.4 L/min, all $p<0.004$). Cachectic CHF patients showed lowest mean systolic blood flow in superior and inferior MA and CT compared to non-cachectic patients and control subjects (259±51 vs. 378±24 vs. 522±22, 47±11 vs. 57±4 vs. 93±7, and 287±59 vs. 441±36 vs. 672±89 mL/min, $p=0.0007$). In superior MA, inferior MA and CT, patients compared to controls had lower mean diastolic blood flow, too (266±27 vs. 307±31 mL/min, $p<0.04$; 34±3 vs. 51±6 mL/min $p<0.006$; and 338±27 vs. 582±84 mL/min, $p<0.004$). Cachectic patients showed lowest mean diastolic flow in celiac trunk compared to non-cachectic patients and controls (260±69 vs. 352±29 vs. 582±84, $p<0.05$). Impaired intestinal blood flow in CHF was in accord with greater bowel wall thickness suggestive for bowel wall edema in cachectic and non-cachectic patients compared to controls (all $p<0.0007$). Patients compared to controls had more murmurs from the intestine, burping, feelings of repletion and flatulences (all $p<0.05$). Burping was more often in cachectic vs. non-cachectic patients (5/11 vs. 8/46, $p<0.05$). CHF patients with abdominal discomfort had lower mean systolic flow in celiac trunk (274±36 vs. 480±38 mL/min, $p=0.02$).

Conclusion: Impaired tissue perfusion occurs as well in the intestinal vascular bed in CHF. This is most pronounced in cachectic patients. This mesenteric malperfusion may contribute to intestinal hypoxia and may hence contribute to gastrointestinal dysfunction in patients with cardiac cachexia.

4–20

The myogenic potential is reduced in experimental cancer cachexia

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Background and aims: Cancer cachexia is a syndrome characterized by loss of skeletal muscle proteins, depletion of lipid stores and hormonal perturbations. Muscle wasting is mainly due to a hypercatabolic response;

however, a reduced myogenic potential has been proposed to contribute to the onset of cachexia.

Methods: Balb-c mice were divided into controls (C) and tumor bearers (TB); the latter inoculated s.c. with 5×105 C26 carcinoma cells. The day after, mice received an i.m. injection of 1.2% BaCl2 in the tibialis anterior. They were sacrificed at day 14 of tumor growth. The tibialis was excised and stored at −80°C. Muscle cross-sectional area (CSA) was evaluated after Hematoxylin & Eosin (H&E) staining, while the expression of PAX7, a transcription factor expressed by proliferating satellite cells, and Myogenin (Myog), a late differentiation marker, were analyzed by immunofluorescence (IF) and Western blotting (WB).

Results: When compared to C, C+BaCl2 mice showed a reduced CSA, and centro-nucleated fibers were visible by H&E staining. The percentages of PAX7+ and of Myog+ cells increased in IF and an increment in both these proteins was detected by WB. The growth of C26 tumor causes in mice a marked decrease of both muscle mass and CSA, associated with accumulation of PAX7+ and Myog+ cells. When the muscle of TB is injured by BaCl2, the following regenerative process is far from being complete. Indeed, compared to BaCl2-treated C, H&E staining in TB injured muscles showed a further reduction in CSA, presence of small centro-nucleated fibers and accumulation of interstitial cells; IF analysis revealed an increased percentage of PAX7+ and Myog+ cells.

Conclusions: The accumulation of PAX7 in TB muscles suggested a defective myogenic potential. Muscle injury in C26 mice severely affected the regenerative program, likely because of a marked inflammatory response. Further studies are needed to clarify the relevance of satellite cell accumulation to the onset of muscle wasting and to unravel the role of pro-inflammatory environment.

4–21

Hypogonadism and inflammation in patients with cancer

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Background: Male cancer patients suffer from fatigue, sexual dysfunction, and decreased functional performance and muscle mass. These symptoms are also seen in men with hypogonadism and/or inflammatory conditions. However, the relative contribution of testosterone and inflammation to symptom burden has not been well established.

Aims: To determine the prevalence of hypogonadism and the relationship between testosterone, inflammation, and symptom burden in male cancer patients.

Methods: This cross-sectional study enrolled patients from a tertiary-care center. Patients included males with cancer cachexia (CC, n=45), cancer without cachexia (CNC, n=50), and non-cancer controls (n=45). Total testosterone (TT), bioavailable testosterone (BT), C-reactive protein (CRP), and interleukin (IL)-6 were measured in plasma. Functional performance was assessed by the ECOG (Eastern Cooperative Oncology Group) and Karnofsky performance scales (KPS). Sexual function was evaluated by IIEF (International Index of Erectile Function).

Results: Prevalence of hypogonadism was >70% in CC. TT was lower in CC compared to CNC (p<0.05). Also, CC had lower BT, grip strength, IIEF scores, appendicular lean body mass (aLBM), and fat mass; and higher IL-6 and CRP compared to controls (p≤0.05). ECOG and KPS were lower in CC and CNC compared to controls (p≤0.05). On multiple regression analysis, TT and CRP predicted most symptoms in cancer patients.

Conclusions: Cancer cachexia patients have higher inflammation and lower testosterone, grip strength, functional status, erectile function, fat mass, and aLBM. Inflammation and hypogonadism are associated with heavier symptom burden in this population. Interventional trials are needed to determine if testosterone replacement and/or anti-inflammatory agents benefit cancer patients.

4–22

Autonomic nervous system dysfunction in cancer cachexia patients is predominantly sympathetic

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Background and aims: Cancer cachexia occurs in a majority of patients suffering from incurable solid tumours. It is characterized by loss of muscle tissue and a combination of reduced food intake and catabolism such as systemic inflammation. Recent work, also of patients having cardiac cachexia, suggests the importance of neuro-hormonal mechanism in cachexia. Preliminary empirical studies document autonomic nervous system dysfunction in cancer cachexia; further understanding of its components may guide cachexia treatment.

Methods: Cancer cachexia patients with no current anti-cancer treatment were eligible. Autonomic testing consisted of a time domain-based analysis of heart rate variability under the paradigms of breathing (at rest and deep breath) for the evaluation of the parasympathetic cholinergic (PC) nervous system, blood pressure changes following valsalva manoeuvre (qualitative and quantitative evaluation of the sympathetic noradrenergic (SN) system) and active standing (orthostasis) with the Finometer Pro (FP) device (Finapres Medical Systems, The Netherlands). Sympathetic skin response was done for the evaluation of the sympathetic cholinergic (SC) system. Nerve conduction studies were additionally performed.

Results: Thirteen patients (five NSCLC, three GI, and six other tumours) were included (median age, 66 years; gender, 11 male). Eleven of 13 patients showed pathological results in two categories of which SN (n=6), SC (n=6) and orthostatic hypotension (n=5) were equally affected. Of note, only one patient showed pathological results in PC category. Eight of 13 patients additionally showed subclinical large fibre polyneuropathy, only two of them being previously treated with neurotoxic chemotherapy.

Conclusion: In this small cohort of patients with advanced solid tumours, autonomic dysfunction occurs frequently in the sympathetic but rarely in the parasympathetic cholinergic nervous system. Subclinical polyneuropathy was not associated with this finding. Our results contradict recent publications showing an impairment of parasympathetic function in male patients with advanced solid cancer.

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4–23

Parameters of cardiovascular function in cachectic and non-cachectic patients with pancreatic cancer

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Background: Cachexia, fatigue, and dyspnea are frequently observed among patients with pancreatic cancer (PCA). We hypothesize that the development of the cancer fatigue syndrome is at least partly due to cardiovascular pertubations with the consequence of decreased exercise capacity and reduced quality of life.

Methods: We prospectively studied cardiovascular parameters in 96 patients with PCA (age 58.9±0.9 years; mean±SE; 60 male; body mass index [BMI] 23.2±0.4 kg/m^2; hemoglobin [Hb] 11.3±0.2 g/dl) and 74 healthy controls (CON 59.7±1.3 years; 40 male; BMI, 26.2±0.5 kg/m^2; Hb 13.9±0.2 g/dl) in exercise capacity using symptom-limited exercise test, cardiac function using echocardiography, body composition using dual energy X- ray absorptiometry, heart rate variability using 24-h ECG, and peripheral blood flow using venous occlusion plethysmography in patients and controls. In addition, we studied, serum levels of tumor necrosis factor-a (TNF-α), interleukin-6, and TNF-receptor 1/2 (TNF-R1/2) in a subgroup of 42 patients with PCA and 22 CON. Cachectic and non-cachectic patients were compared.

Results: No significant difference was detected between patients and CON in terms of sex, age, and systolic function. Patients with PCA compared to CON displayed impaired exercise capacity (peak VO_2, 21.1±0.6 vs. 27.2±1.0 mL/min/kg), less total lean mass (68.6±1.3 vs. 78.4±1.6 kg), increased resting blood flow in the leg (4.3±0.4 vs. 2.8.3±0.2 mL/100 mL/min), impaired autonomic function (SDNN 24 h, 100.3±30.8 vs. 134±37.5 ms), and elevated pro-inflammatory markers (IL-6: 5.2±0.8 vs. 1.8±0.2 pg/ mL; TNF-R1: 1,828 ±132 vs. 1,245±69 pg/mL; TNF-R2: 2,811±143 vs. 2,018±120 pg/mL; all $p<0.05$). Autonomic dysfunction in cachectic patients was even worse than in non-cachectic patients (SDNN 24 h 94.6±4.9 vs. 109.9±4.5 ms, $p=0.03$).

Conclusions: Reduced exercise capacity, less lean mass, autonomic dysfunction, increased resting blood flow, and elevated proinflammatory markers are present in patients with PCA. Further studies are required.

4–24

Parameters of cardiovascular function in cachectic and non-cachectic patients with non-small cell lung cancer

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Background: Cachexia, fatigue, and dyspnea are frequently seen in patients with non-small cell lung cancer (NSCLC). We hypothesize that the development of the cancer fatigue syndrome is at least partly due to cardiovascular perturbations with the consequence of decreased exercise capacity and reduced quality of life.

Methods: To investigate the cardiovascular parameters, we prospectively studied 42 patients with NSCLC (age, 60±9 years; mean±SEM, 26 male; body mass index [BMI], 24.8±4.7 kg/m^2; hemoglobin (Hb), 12.2±1.9 g/dl) and 41 healthy controls (CON 61±11 years, 26 male; BMI 25.4±3.5 kg/m^2; Hb, 14.4±1.0 g/dl) in exercise capacity using symptom-limited exercise test, cardiac function using echocardiography, body composition using dual energy X-ray absorptiometry, peripheral blood flow using venous occlusion plethysmography, and heart rate variability using 24-h ECG in patients and controls. A subgroup analysis of non-cachectic (N-CACH; $n=28$) and cachectic (CACH) patients ($n=13$) was performed due to the consensus definition of cachexia.

Results: No significant difference was detected between patients and CON in terms of sex, age, and systolic function. Patients with NSCLC displayed impaired exercise capacity (peak VO_2 in NSCLC 17.6±0.9 mL/min/kg vs. CON 28.3±1.1 mL/min/kg), less total lean mass (NSCLC 47.8±1.8 vs. 53.3± 1.8 kg), lower peak blood flow in forearm (NSCLC 17.4±1.0 mL/100 mL/min vs. CON 22.3±1.4 mL/100 mL/min), and leg (NSCLC 11.0±1.1 mL/100 mL/min vs. CON 15.4±1.4 mL/100 mL/min), and impaired autonomic function (SDNN in NSCLC 97.5±36.5 ms vs. CON 142±36.9 ms; all $p<0.05$). CACH patients showed significantly impaired exercise capacity compared to N-CACH patients (CACH 18.84±0.97 mL/min/kg vs. N-CACH 13.96± 1.4 mL/min/kg, $p=0.03$).

Conclusions: Reduced exercise capacity, less lean mass, worse blood flow, and autonomic dysfunction are present in patients with NSCLC. Symptoms may be due to systemic changes like neuroendocrine activation and inflammation. Further studies are required.

5–22

C-terminal agrin fragment—a serum marker of sarcopenia?

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Sarcopenia is characterized by loss of muscle mass and functionality at old age. The causes of sarcopenia are subject of intensive research but largely poorly understood. A prerequisite for successful treatment of sarcopenia patients is the development of effective diagnosis. Various causes for sarcopenia have been suggested, among which, on the basis of recent data in aged animals, a crucial role of the neuromuscular junction (NMJ), the sole link between motor neurons and muscle fibers, has emerged. As a consequence sarcopenia is commonly referred to as a syndrome of the NMJ. The extracellular matrix protein agrin is essential for the formation and stabilization of NMJs. Agrin is cleaved by the pre-synaptic protease neurotrypsin at two sites thereby losing its NMJ-stabilizing function. Agrin cleavage by neurotrypsin frees a soluble 22-kDa C-terminal agrin fragment (CAF) detectable in blood. Clinical trials were undertaken to test the hypothesis that overactivity of neurotrypsin as revealed by elevated levels of CAF in serum, plays a pathogenic role in the genesis of sarcopenia. Initial normal range studies demonstrated that in a healthy population of Swiss blood donors, CAF is measurable in blood where it shows a narrow range of values that do not vary with aging. Based on these observations, a pilot multicenter, non-randomized, open-label, vertical clinical study was designed. Briefly, 133 informed and consenting elderlies (>65-year-old adults) were recruited and

assigned to a sarcopenia patients group defined according to up to date diagnostic criteria and an aged matched control group. Elevated agrin degradation occurs in a substantial subset of sarcopenia patients and can be used to identify those patients in whom a novel pathogenic target may be therapeutically exploited. Excessive degradation of agrin by neurotrypsin leading to fragmentation of the NMJs appears to be an important process in the pathogenesis of sarcopenia.

5–23

Animal model for agrin-dependent sarcopenia—the SARCO mouse

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Sarcopenia is characterized by loss of muscle mass and muscle function. The causes of sarcopenia are subject of intensive research but largely poorly understood. A requirement to properly address diagnosis and treatment of pathological conditions is the availability of suitable animal models. These models should reproduce the pivotal behavioural and pathological features of the condition they are supposed to mimic. We have recently found that levels of a c-terminal agrin fragment (CAF), exclusively generated from agrin's cleavage by neurotrypsin, are significantly augmented in 40% of sarcopenia patients. Agrin, a synaptically located protein, is a key player during initial formation and maintenance of neuromuscular junctions (NMJs) where it induces acetylcholine receptor assembly and aggregation. Agrin forms a complex with LRP4, a low-density lipoprotein receptor-related protein and MuSK, a transmembrane tyrosine kinase. Once cleaved by neurotrypsin, agrin is inactive leading to dispersal of NMJs. In the cleavage process, a soluble, 22-kD CAF fragment is freed and circulates in body fluids. A transgenic mouse overexpressing human neurotrypsin (termed SARCO) was generated (Bolliger, J Cell Sci, 123:3944, 2010) in order to provide an animal model of sarcopenia to advance knowledge of the pathogenic mechanisms. As expected, CAF levels in SARCO mice are elevated by a factor of 1.5 compared to the WT. Furthermore, SARCO mice share all the essential pathological features of sarcopenia patients which include reduction of muscle mass, irregular fiber size with central nuclei, selective fiber-type loss and altered morphology of the NMJs (Bütikhofer, Faseb J, 2011). In addition, SARCO mice are weak and show significant motor impairment which aggravates with time. These phenotypes prefigure a pathologically altered neuromuscular system. The SARCO mouse represents a valuable model of sarcopenia and offers an ideal in vivo approach to test possible pharmaceutical treatments aimed at this new target.

5–24

The association between sarcopenia and sarcopenic obesity and lipid profile in elderly men

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Background: Sarcopenia is a main cause of the loss of mobility and independence in the elderly. A combination of excess weight and reduced muscle mass and strength is called as sarcopenic obesity. The studies of lipid metabolism in sarcopenic obesity are limited.

Purpose: The aim of this study was to investigate serum lipid profile in sarcopenic and sarcopenic-obese elderly men.

Methods: A cross-sectional study design was used in this study. Subjects of this study were men (n=47) aged 65 years and more. Exclusion criteria were conditions and current use of any medication known to affect muscle and lipid metabolism. DXA was used to measure fat mass, body fat percentage, and lean mass (iDXA, GE Lunar). Sarcopenia was defined condition when appendicular skeletal muscle mass divided by stature squared was 7.26 kg/m^2 and gait speed was >0.8 m/s. In case of the combination of sarcopenia and excess body fat (percentage of body fat greater than 27%), subjects were classified as sarcopenic-obese. Blood samples were obtained between 9 and 11 am after overnight fasting. Total cholesterol, high-density lipoproteins, low-density lipoproteins (LDL), and triglycerides were analyzed.

Results: Of all men investigated, 31 were sarcopenic and 16 were defined as sarcopenic-obese. There was the weak negative association between muscle mass and total cholesterol (r=−0.34, p=0.018) and LDL (r=−0.36, p=0.012) in sarcopenic group, but in sarcopenic obesity group muscle mass was not statistically significant associated with lipid profile.

Conclusions: Sarcopenia is associated with serum lipids: total cholesterol and LDL. Larger cohorts of subjects studied are needed to clarify the significance of our finding.

5–25

Muscle-specific atrophy with aging: the AGES-Reykjavik study

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Background: Aging is associated with loss of skeletal muscle mass and muscle attenuation (i.e., higher muscle fat infiltration). Although most studies have previously used quadriceps muscle, muscle atrophy and attenuation may vary across muscle groups and may relate to the muscle structure-function paradigm of mechanical loading/unloading.

Aims: The purpose of this study is to investigate whether with increasing age there is differential muscle-specific atrophy and muscle attenuation in muscles with different mechanical function.

Methods: Men (n=2,214) and women (n=2,997) from the Age, Gene/Environment (AGES) Reykjavik Study, born in 1907–1935 (age range 66–96 years), and living in Reykjavik participated in this cross-sectional study design. Muscle-specific cross-sectional areas of seven different muscles were manually outlined from two CT images (L4/L5 and mid-thigh). Fatty infiltration (captured by the Hounsfield's unit (HU) of the

muscle tissue) was calculated as the average density in each muscle. Quadriceps, hamstring, sartorius, psoas (primary antigravity/locomotion function) and rectus abdominis, paraspinal, and lateralis (primary postural function) were outlined.

Results: Compared to quadriceps muscle, with increasing age, we observed: (1) greater muscle mass atrophy for paraspinal and rectus in men and women; (2) greater fatty infiltration for rectus, hamstring, paraspinal (female only), lateralis (female only), sartorius (male only); (3) lower muscle mass atrophy in men and women for hamstring, sartorius and psoas (female only); (4) lower fatty infiltration for psoas; and (5) increasing total muscle atrophy with age (i.e., sum of all muscles) with the oldest group (85 +years) having ~78% muscle mass of the youngest group.

Conclusions: Increasing age is associated with heterogeneous muscle-specific atrophy and muscle attenuation. This may relate to the muscle structure–function paradigm of mechanical loading/unloading (e.g., postural versus antigravity/locomotion primary function). Functionally, these results suggest a potential greater loss of mechanical muscle function for postural muscles which may jeopardize to a greater extent older adults' ability to carry out motor tasks involving postural control.

6–19

Branched-chain amino acid oxidation in skeletal muscle—practical importance of its modulation by glutamine and ammonia availability in cachectic illness

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Background and aims: Enhanced oxidation of branched-chain amino acids (BCAA; valine, leucine, and isoleucine) in skeletal muscle is a typical metabolic alteration of cachectic illness associated with activated synthesis of glutamine and development of muscle wasting. It can be hypothesised that modulation of glutamine synthesis by reactant availability may affect BCAA oxidation and protein metabolism in skeletal muscle.

Methods: Two separate experiments were performed using male Wistar rats in which the effect of glutamine synthesis inhibition and glutamine synthesis stimulation on BCAA and protein metabolism was evaluated. In the first study, glutamine synthesis was inhibited via alanyl-glutamine infusion in endotoxemic and intact rats. In the second study, glutamine synthesis was stimulated by infusion of ammonium acetate/bicarbonate mixture. Control animals were infused by the mixture of sodium salts. The parameters of protein metabolism and leucine oxidation were measured under steady state conditions using L-[1-^{14}C]leucine infusion. Statistical comparisons were performed using ANOVA and Bonferroni test.

Results: Infusion of alanyl-glutamine induced a decrease in plasma BCAA levels, a decrease in leucine oxidation, and an improvement of protein balance due to the decrease in proteolysis both in intact and endotoxemic rats. Ammonium infusion induced an increase in ammonia and glutamine, an increase in BCAA oxidation, a decrease in BCAA and alanine levels in blood plasma, a decrease in whole-body protein turnover, and a decrease in protein synthesis in skeletal muscle.

Conclusions: It is concluded that changes in glutamine synthesis induced by alanyl-glutamine and by ammonia infusion are associated with significant alterations in BCAA and protein metabolism. The results also demonstrate that the cause of decreased plasma BCAA levels observed frequently in liver cirrhosis is hyperammonemia.

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6–20

Epidemiological study to assess the prevalence of anorexia–cachexia syndrome in elderly patients

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Objective: The objectives of this study were to determine the prevalence of anorexia–cachexia syndrome (ACS), and to know the clinical profile, nutritional status, and therapeutic approach of ACS in Spanish elderly patients.

Methodology: This is a multicenter epidemiological study in two phases. The study is approved by the Ethics Committee of Hospital General de l'Hospitalet de Llobregat (Barcelona).

The objectives of this study were: for phase A, to determine the prevalence of ACS in elderly, according to the Evans's criteria; and for phase B, to describe the clinical profile of geriatric patients with ACS. The eligible patients had met the following inclusion criteria: age >65 years; comply with the definition of ACS according to Evans et al. (Clin Nutr 27:793–799, 2008) (at least 5% loss of edema-free body weight in the previous 12 months or BMI below 20 kg/m^2); also meet at least three of the following criteria: decreased muscle strength, fatigue, anorexia, low rate free-fat mass, and biochemical abnormalities. All patients had given written informed consent.

Results: Sixty-six centers distributed for Spain participated in the study. Four thousand fifty-three (4,153) patients were included in phase A for the prevalence study. Twenty-four percent (24%) had weight loss >5% in the last 12 months. Of these, 15.8% met the diagnostic Evans's criteria for ACS. The results in phase B were: mean age 83 years and concomitant diseases: neoplasia (15.4%), chronic kidney disease (26.8%), COPD (27.5%), diabetes mellitus (30.9%), and chronic heart failure (45.6%). Nutritional status assessed by the MNA questionnaire indicated that 76.4% of patients were malnourished, 22.3% were at risk of malnutrition. There was a positive correlation between MNA and the five criteria of Evans. The therapeutic approach followed in these patients showed that 71% received dietary counseling, 40.5% received drug treatment, and 82% of them were already being treated with megestrol acetate.

Conclusions: The ACS affects 15.8% of the Spanish elderly population. The ACS is associated with chronic diseases frequent in elderly population such as chronic heart failure, COPD, renal failure, diabetes mellitus, and cancer. There is a positive correlation between nutritional status and the parameters that define the Evans's criteria of ACS, this allows concluding that these criteria can be a useful tool in clinical practice to diagnose and monitoring these patients. Over 80% of ACS patients with pharmacological treatment received megestrol acetate. Under our knowledge, this is the first study that provides data on the prevalence of SCA in the elderly, using the criteria of Evans as a diagnostic tool.

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6–21

Plasma nesfatin-1 concentrations in restricting-type anorexia nervosa

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Background and aims: Restricting-type anorexia nervosa (AN-R) is an eating disorder characterized by severe emaciation with marked caloric reduction secondary to an inordinately strong desire to lose more weight, and pervasive fear of fatness, resulting in sustained low weight. Although previous studies have shown that changes in feeding regulatory peptides such as ghrelin are associated with anorexia, little is known about the relationship between AN-R and nesfatin-1, a novel 82-amino acid peptide identified as a satiety peptide derived from nucleobindin-2. Therefore, we measured the plasma nesfatin-1 levels in AN-R patients to investigate its role in AN-R.

Methods: Fifteen women participated in this study; seven patients with AN-R and eight age-matched healthy controls (average BMI, 13.0±0.3 vs. 21.6±0.5, respectively). After overnight fasting, blood samples were obtained from each subject. The levels of nesfatin-1, acyl ghrelin, and des-acyl ghrelin in the samples were measured.

Results: Plasma nesfatin-1 levels were significantly lower in AN-R group than in control group ($P<0.05$). Plasma acyl ghrelin and des-acyl ghrelin levels were significantly higher in AN-R group than in control group ($P<0.01$ and $P<0.05$, respectively).

Conclusions: Our result indicates that nesfatin-1 is involved in nutrition status.

6–22

The FRAIL scale: a simple scale for diagnosis and predicting outcomes

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The FRAIL scale was developed by the International Academy of Nutrition and Aging as a simple diagnostic tool for frailty. The scale includes five components: fatigue, resistance, ambulation, illness, and loss of weight. FRAIL scale scores range from 0 to 5 (1 point for each component; 0=best to 5=worst) and represent frail (3–5), pre-frail (1–2), and robust (0) health status. In a population of middle-aged adults (ages 49–65) in the African American Health (AAH) study ($N=703$), cross-sectional analyses showed that frail or pre-frail health status were associated with more instrumental activities of daily living (IADLs) disabilities, lower short physical performance battery scores, lower grip strength, and shorter times for the one-leg stand (all $p_s<0.01$) when those with any baseline deficits in activities of daily living (ADLs) were excluded from the analyses. TNFR1 and CRP values were elevated in pre-frail and frail individuals ($p_s<0.05$). Being frail or pre-frail at baseline also significantly predicted incident ADLs and mortality at 9 year follow up (all $p_s<0.05$). The FRAIL scale showed overlap with the Cardiovascular Health Study, Study of Osteoporotic Fractures, and Rockwood et al. frail scales. The FRAIL scale was equivalent or superior to the other scales at predicting incident ADL deficits, incident IADL deficits, and mortality at 9 years. Muscle mass in the frail was 62.32%, pre-frail 61.72%, and non-frail 65.95% ($F=14.78$, $p<0.001$; pre-frail versus non-frail $p<0.001$; frail versus non-frail $p<0.05$). Fat mass in the frail was 37.68%, pre-frail 38.28%, and non-frail 34.05%. We concluded that the FRAIL scale is an excellent tool for identifying frailty. This research was supported by a grant from the National Institute on Aging to Dr. D. K. Miller (R01 AG-10436).

6–23

Low appendicular skeletal mass (ASM) with limited mobility predicts poor outcomes after 6 years in middle-aged African Americans

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The Society of Sarcopenia, Cachexia and Wasting Disorders (SCWD) has defined sarcopenia with limited mobility as a person with a low appendicular skeletal mass (ASM; height corrected, 2 SD below referent adult group ages 20–30) and with a gait speed walk ≤1m/s or with a 6-min walk distance less than 400 m. In the population-based African American Health (AAH) study ($N=998$ at baseline/year 1), muscle mass and mobility were evaluated in a clinical testing center in a subsample of $N=319$ persons (ages 52–68). Muscle mass was measured using dual energy x-ray absorptiometry (Hologic QDR 4,500W; Hologic, Inc., Bedford, MA, USA) and mobility by a 6-min walk test and 4-m gait walk test. Height corrected ASM (9.0±1.5 in $n=124$ males, 8.3±2.2 in $n=195$ females) was computed as total lean muscle mass in arms and legs (kilograms) divided by the square of height (meters). The longitudinal association of low ASM (bottom 25% AAH sample; <7.96 males and <7.06 females) and low ASM with limited mobility (4-m gait walk≤1 m/s or 6-min walk<400 m) with poor outcomes after 6-years was examined for mortality, activities of daily living (ADLs), instrumental activities of daily living (IADLs), injurious falls, and frailty. Sample size was not large enough to define sarcopenia according to the SCWD definition. Longitudinal analyses with

adjustments for age and gender showed that low ASM with limited mobility was associated with increased mortality (p=0.003), the presence of one or more ADL disabilities (p=0.059; marginal significance), the presence of one or more IADL disabilities (p=0.030), and with frailty (p=0.037) but not with injurious falls (p=0.235). Low ASM alone was marginally associated with mortality (p=0.085) but not with any other outcomes (all $p_s \geq 0.10$). We concluded that low ASM with limited mobility is a robust predictor of poor outcomes among African Americans. This research was supported by a grant from the National Institute on Aging to Dr. D. K. Miller (R01 AG-10436).

6–24

Plasma levels of acyl ghrelin, des-acyl ghrelin, and ratio of acyl ghrelin to total ghrelin change in female inpatients with restricting-type anorexia nervosa after treatment

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Anorexia nervosa (AN-R) is characterized by a restrictive eating pattern resulting in severe weight loss, amenorrhea, and distorted perceptions of body weight and shape. Associated psychopathology includes high levels of anxiety and depression, low self-esteem, and interpersonal and familial difficulties. Physical health is also severely compromised due to malnutrition. In patients with AN-R, plasma ghrelin levels were reported to be higher compared with normal-weight control subjects, reflecting a negative energy balance of affected individuals. However, the early progress of these patients and changes in the levels of acyl ghrelin and des-acyl ghrelin during treatment were not reported. The purpose of this study was to determine the changes on ghrelin levels (acyl and des-acyl) during early treatment. A total 15 women participated in the study; 5 patients with AN-R and 10 age-matched healthy controls. As a result, des-acyl ghrelin in AN-R patients is higher than control subjects before therapy, but it is decreasing with treatment. The plasma des-acyl ghrelin in AN-R patients started decreasing more rapidly and in early stage of the hospitalization than ever reported, and after 8 weeks, it is significantly lower than in control subjects. It means that des-acyl ghrelin is sensitive and changeable with their nutrition state. Furthermore, the ratio of acyl ghrelin to total ghrelin is increasing with 8-weeks treatment. These findings may be useful for developing anti-AN drug which increase the ratio of acyl ghrelin to des-acyl ghrelin.

7–17

Atorvastatin modulates lipopolysaccharide induced TNF-alpha secretion from precursors of human skeletal muscle

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Background and aims: Pro-inflammatory cytokines are important mediators in chronic diseases and cachexia development. Skeletal muscle actively participates in cytokine production. Tumor necrosis factor (TNF)-alpha is one of the major inflammation promoters with a central role in sepsis development and chronic diseases progression. Statins have beneficial anti-inflammatory effects and are widely prescribed. We examined TNF-alpha production in human myotubes and the effect of atorvastatin (AT) on constitutive and lipopolysaccharide (LPS)-stimulated TNF-alpha secretion with regard to AT concentration and time-of-exposure.

Methods: Human myotubes were exposed to different AT concentrations ranging from sub- to supratherapeutic (0.1, 1, 10, 100 μM). AT exposure was combined with time-dependant LPS (100 ng/mL) exposure (no exposure, 48-h co-exposure, 24-h pre-exposure, 12-h postexposure) to evaluate for time-of-exposure effects. Constitutive and LPS-induced TNF-alpha production was observed. TNF-alpha concentration was measured using ELISA.

Results: Constitutive TNF-alpha levels were 9.78±1.03 pg/10.000 nuclei. After exposing myotube cultures to increasing AT concentrations, no effect on TNF-alpha secretion was observed. LPS stimulated TNF-alpha secretion (9.8 vs. 24.5 pg/10,000 nuclei; p<0.01). After co-exposing myotube cultures to LPS and AT, inhibitory effect of AT on LPS-induced TNF-alpha secretion was observed, as well as in cultures pre-exposed to LPS before treatment with AT. However, when myotube cultures were first treated with AT and followed by LPS-exposure controversial stimulatory dose dependent effect of AT on TNF-alpha secretion was observed.

Conclusions: AT does not affect constitutive TNF-alpha secretion in cultured human myotubes, but inhibits LPS-stimulated secretion. Controversial pro-inflammatory AT effect was observed in pre-treatment prior to LPS, suggesting a complex AT effects and involvement of different molecular pathways. Concentration and time-of-exposure seem to be of great importance when considering statin-induced effects on TNF-alpha production.

7–18

Treatment of cancer cachexia-induced cardiomyopathy

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Background: The AH-130 Yoshida hepatoma causes a severe cachexia in rats, which then leads to the development of cardiomyopathy. Hence, we wanted to investigate if treatment of tumor-bearing rats with cardiovascular drugs can improve survival, body wasting and heart function.

Study design and methods: We used the AH-130 hepatoma model to induce cachexia in male rats. We assessed body weight and body composition at begin (day 0) and end of the study (day 16). On day 1 we inoculated the rats with 100×10^6 AH-130 cells and started daily with the cardiovascular medication (bisoprolol, spironolactone, imidapril).

Furthermore, we analysed the cardiac function and quality of life on days 0 and 10/11. After sacrificing the rats, we measured the weight of the organs and tested the heart for atrophic mechanisms (UPS-system, caspases) and anabolic signaling (Akt pathway).

Results: Treatment of tumor-bearing rats with bisoprolol or spironolactone ameliorated body wasting by maintaining lean and fat mass. Furthermore, these rats had an improved heart function compared to the placebo group and a better survival (Biso: HR 0.32, p=0.0017, Spiro: HR 0.31, p=0.0007). We could confirm the results by showing less protein degradation and more protein synthesis by treatment (increased activity of Akt, p70S6K). Treatment with imidapril was not beneficial.

Conclusion: The Yoshida hepatoma animal model as a model for cancer cachexia impaired severely the cardiac function and led to body and cardiac wasting. Treatment of cancer cachexia with cardiovascular drugs improved outcome.

Table 1. Cardiovascular treatment in cancer cachexia

	Sham (n=16)	Placebo (n=73)	Bisoprolol, 5 mg/kg/day (n=23)	Spironolactone, 50 mg/kg/day (n=16)
Δ Body weight [g]	59.8±2.1 ***	−53.7±1.77	−21.9±10.55 ***	−21.0±11.03 ***
Δ Fat mass [g]	9.11±0.90 ***	−12.35±0.36	−5.86±1.87 ***	−6.70±2.13 ***
Δ Lean mass[g]	41.7±2.03 ***	−39.8±1.56	−16.71±7.68 ***	−11.86±8.69 ***
Food intake day 11 [g/24 h]	21.3±0.84 ***	4.30±0.48	10.93±2.01 ***	9.81±1.73 **
Activity day 11 (counts/24 h)	67,192±2,847 ***	29,509±1,775	43,755±3,741 **	44,817±5,286 **
LV Ejection fraction [%]	72.8±2.34 ***	51.9±1.99	57.1±4.11	65.9±3.48 **
Fractional shortening	51.6±1.53 ***	30.8±1.57	32.3±3.47	42.8±2.6 **
Δ LV mass [mg]	110±29.1 ***	−101±14.18	−4.52±31.2 **	37.9±12.03 ***
Trypsin-like activity of the heart [nmol/mg protein /min]	488±95.9 **	1,094±118	805±46.1 *	762±7.87 *
Activity of caspase-3 in the heart [nmol/mg protein/min]	35.6±4.453 ***	82.9±10.70	60.2±9.01	40.8±10.23 *

LV left ventricular

*p<0.05, **p<0.01, ***p<0.001 vs placebo

7–19

mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass

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Skeletal muscle atrophy is a common and debilitating condition that lacks a pharmacologic therapy. To develop a potential therapy, we identified 63 mRNAs that were regulated by fasting in both human and mouse muscle, and 29 mRNAs that were regulated by both fasting and spinal cord injury in human muscle. We used these two unbiased mRNA expression signatures of muscle atrophy to query the connectivity map, which singled out ursolic acid as a compound whose signature was opposite to signatures of muscle atrophy. A natural triterpene acid, enriched in apple peels, and ursolic acid reduced muscle atrophy in three distinct mouse models: fasting, denervation, and immobilization. Moreover, when administered to mice in the absence of an atrophy-inducing stress, ursolic acid stimulated muscle hypertrophy and increased grip strength. We found that ursolic acid reduced atrophy and stimulated hypertrophy by enhancing skeletal muscle insulin/IGF-I signaling and inhibiting atrophy-associated mRNA expression. Importantly, ursolic acid's effects on muscle were accompanied by reductions in adiposity, fasting blood glucose, and plasma cholesterol and triglycerides. These findings identify a potential therapy for muscle atrophy and perhaps other metabolic diseases. This work was supported by the Doris Duke Charitable Foundation, NIH, the Department of Veterans Affairs, the University of Iowa Institute for Clinical and Translational Science and the University of Iowa Research Foundation.

7–20

Tissue-protective effect of the non-hematopoetic erythropoietin analogues ARA284 and ARA286 in the treatment of cancer cachexia

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Background: Cancer cachexia is a syndrome characterized by significant loss of muscle and fat tissue. Therefore searching for the compounds protecting tissue during cachexia is of current interest. Erythropoietin (EPO) was shown to be effective in tissue protection, but has side effects like hematopoesis. The aim of our research was to investigate the protective effect of two non-hematopoetic EPO analogues ARA284 and ARA286 on rat tissues during cancer cachexia.

Methods: Young male Wistar Han rats were inoculated with 10^8 Yoshida AH-130 hepatoma cells and treated with 500 or 5,000 U/kg/day EPO or the equivalent dose of the tissue-protective molecules (TPM) ARA284 and ARA286 (0.17 or 1.7 μg/kg/day, respectively). Body weight and body composition were analyzed before the initiation of experiment and after sacrifice and removal of the tumour on day 16. Organs were weighted and stored at −80.

Results: It was shown that ARA284 and ARA286 in high doses (HD) significantly reduced weight loss in comparison with placebo group (p= 0.0058). The loss of fat mass was attenuated (p=0.027) as well as preservation of epididymal fat (p=0.003) and protection of lean mass (p=0.012) compared to placebo. The weight of gastrocnemius muscle increased (p= 0.015) in HD groups and at the same time the levels of biochemical markers of cachexia improved in these samples. Thus, the levels of phosphorylated p38 MAPK and activated myostatin were significantly decreased (p<0.05) in both HD groups, amount of GSK-3β and Akt significantly changed (p< 0.05) in ARA286 group. Low-dose ARA284 also protected the tissues, but to a lesser extent than high dose. ARA286 in low concentration had no effect. No effect was observed on survival using TPMs.

Conclusions: ARA284 and ARA286 were shown to be effective in reducing tissue wasting in rat cancer cachexia model. These compounds should be seen as prospective drugs for human cancer cachexia.

7–21

MT-102, a new "anabolic catabolic transforming agent", reverses effects of sarcopenia in a rat model

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Background: Sarcopenia, an age-related progressive loss of skeletal muscle mass, strength and function, would be a more relevant future health issue within the next years. There are no efficient pharmacological interventions to counteract effects of sarcopenia.

Methods: Body weight and body composition (NMR-scan) of 19-month-old male Wistar Han rats (weight approximately 555 g) were analysed 1 day before starting treatment with 3.0 mg/kg/day MT-102 or placebo as well as once a week of the 31-day protocol. Quality of life parameters (food and water intake, spontaneous activity) were determined 1 day before treatment started and on day 28. Proteasome and caspase activities were measured by a kinetic turnover of fluorogenic substrates. Proteins involved in anabolic and catabolic signalling were analysed by Western blot.

Results: Aged rats lost body weight (−15.5±7.2 g), lean mass (−1.5±4.2 g) and fat mass (−15.6±2.7 g). Food intake was unchanged (+0.66±0.8 g), water intake was decreased (−1.7±1.4 ml) compared to baseline. Animals treated with 3.0 mg/kg/day MT-102 gained body weight (+8.0±6.1 g, p<0.05) and particularly lean mass (+43.4±3.5 g, p<0.001), leading to increased weight of skeletal muscle and heart. Interestingly, animals lost more fat mass compared to placebo (−38.6±3.4 g, p<0.001). Food (+4.8±1.5 g, p<0.01) and water (+2.6±1.9 ml, p<0.05) intake could be increased by 3.0 mg/kg/day MT-102 compared to baseline. A decrease in proteasome and caspase-6 activity was observed by 3.0 mg/kg/day MT-102. FoxO3a and NFkB (p<0.01), key regulator proteins for catabolic signalling, were less expressed, confirmed by less expression of E3-ligases MuRF-1 (p<0.01) and atrogin-1 (p<0.01). Myostatin was less expressed (p<0.001), leading to an increase in MyoD expression (p< 0.01). A reduction in autophagy was observed, indicated by a decreased LC3-II protein expression (p<0.001) and a higher LC3-ratio (p<0.001).

Conclusions: MT-102 (3.0 mg/kg/day) reversed effects of ageing, especially loss of muscle mass and increased fat mass. Hence, it is likely to be also a prospective drug to treat patients suffering from sarcopenia.

7–22

MT-102, a new "anabolic catabolic transforming agent", improves survival by a gain of skeletal muscle in a rat model of cancer cachexia

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Background: MT-102 is being developed to reverse effects of cancer cachexia. The pharmacological profile includes anabolic effects on skeletal muscle, inhibition of lipolysis, stimulation of appetite and reduction in energy expenditure. MT-102 is currently in a phase II clinical trial for treatment of cancer cachexia due to Stage III or IV colorectal cancer or non-small cell lung cancer.

Methods: Young male Wistar Han rats (approximately 200 g) were intraperitoneally inoculated with 10^8 Yoshida AH-130 hepatoma cells. Animals were treated once a day with 0.3 or 3.0 mg/kg MT-102 or placebo. Body weight and body composition (NMR-scan) were analysed 1 day before tumour-inoculation and after sacrifice. Proteasome and caspase activities were measured by a kinetic turnover of fluorogenic substances. Proteins involved in anabolic and catabolic pathways were analysed by Western blot.

Results: A prevention of losing body weight (−0.9±13.1 g vs. placebo −53.7± 1.8 g; p<0.001) and fat mass (−5.87±2.02 g vs. placebo −12.35±0.36 g; p< 0.001) could be achieved by 3.0 mg/kg/day MT-102. Interestingly, 3.0 mg/kg/

day MT-102 led to a gain of lean mass (+1.1±10.3 g vs. placebo −39.8±1.6 g; $p<0.001$). Moreover, survival proportion was improved by 3.0 mg/kg/day MT-102 (HR=0.29, 0.16−0.91; $p<0.0001$). Proteasome and caspase activities were not reduced. Expression of PI3K and Akt in a phosphorylated, activated form was significantly upregulated ($p<0.05$ vs. placebo) by 3.0 mg/kg/day MT-102, whereas expression of activated form of FoxO3a ($p<0.01$ vs. placebo) and FoxO1 ($p<0.05$ vs. placebo), key regulator proteins for catabolic signalling, were significantly downregulated. Phosphorylated, activated form of NFkB ($p<0.05$ vs. placebo) and Smad2 ($p<0.001$ vs. placebo) and activated form of GSK3a ($p<0.05$), proteins involved in catabolic signalling, were significantly less expressed. Autophagic activity was reduced, indicated by a lower expression of LC3-II protein ($p<0.001$ vs. placebo).

Conclusions: MT-102 (3.0 mg/kg/day) implicates a pro-anabolic and anti-catabolic effect, resulting in a gain of lean mass. Importantly, survival was significantly improved in this animal model of severe cancer cachexia.

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MT-102, a new "anabolic catabolic transforming agent", improves heart function in a rat model of cancer cachexia

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Background: Cancer cachexia is associated with impairment in heart function, caused by a progressive loss of heart weight due to a pathologic decrease in size and mass. Atrophy of epicardium with marked diminution and therefore loss of epicardial fat mass is also described, as well as increased pigmentation of myocardium and little or no alteration of endocardium.

Methods: Young male Wistar Han rats (weight approximately 200 g) were intra-peritoneally inoculated with 10^8 Yoshida AH-130 hepatoma cells. Animals were treated once a day with 0.3 or 3.0 mg/kg MT-102 or placebo. Echocardiography of heart was determined 1 day before tumour-inoculation and on day 11 of the 16-day protocol. Body weight and body composition (NMR-scan) were analysed 1 day before tumour-inoculation and after sacrifice.

Results: Heart weight was significantly increased in the group treated with 3.0 mg/kg/day MT-102 (573±32 mg vs. placebo 506±8 mg; $p<0.001$). Heart rate was not significantly affected by 3.0 mg/kg/day MT-102 (326±12 bpm), or 0.3 mg/kg/day MT-102 (327±18 bpm) compared to placebo (366±13 bpm). Left ventricular ejection fraction (64.06±2.50% vs. placebo 51.91±1.99%; $p<0.01$) and fractional shortening (38.91±2.84% vs. placebo 30.75±1.57%; $p<0.05$) were likely to be significantly improved by high dose of MT-102, as well as stroke volume (175.30±16.67 μl vs. placebo 104.93±6.93 μl; $p<0.001$). Left ventricular end-diastolic diameter was significantly larger in both treated groups (0.3 mg/kg/day MT-102, 6.40±0.12 mm; 3.0 mg/kg/day MT-102, 6.27±0.14 mm vs. placebo 5.71±0.11 mm; $p<0.05$), but close to sham level (6.39±0.08 mm). Left ventricular end-diastolic volume was also significantly increased by 3.0 mg/kg/day MT-102 (265.3±17.3 μl vs. placebo 196.6±9.8 μl; $p<0.05$) and with it close to sham level (269.18±10.77 μl).

Conclusions: A daily dose of 3.0 mg/kg MT-102 reversed impaired heart function and stopped cardiac wasting seen in placebo animals in this animal model of cancer cachexia. Hence, it is a prospective drug to treat patients suffering from cancer cachexia particularly if patients show signs of declined cardiac function. Currently, MT-102 is in a phase II cancer cachexia trial.

7–24

Dedifferentiated fat: a potential resource for a cell therapy approach to treat cachexia

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Background and aims: Stem cell therapy is a potential approach to treat loss of skeletal muscle observed in cachexia. Vascular stromal fraction of adipose tissue represents an abundant and accessible source of pluripotent cells that can differentiate into several cell types including myogenic cells. In vitro studies have shown that mature adipocytes are capable of dedifferentiating into proliferating fibroblast-like cells. Dedifferentiated fat (DFAT) cells can differentiate, upon appropriate treatments, into skeletal myocytes. The final aim of this project is to study the myogenic potential of human DFAT cells in a mouse model of cachexia evaluating their contribution to regenerate skeletal muscle tissue.

Methods: We used the ceiling culture method to obtain DFAT cells from human adipose tissue. DFAT cells gene expression was analysed by RT-PCR. Moreover, we labelled DFAT cells with nuclear GFP using a lentiviral transduction system.

Results: We analysed the gene expression profile of DFAT cells in order to characterise their stemness and myogenic potential. To investigate the ability of human DFAT cells to fuse with myocytes in co-culture, we validated a GFP-labelling method based on lentiviral transduction system. Moreover, we plan to improve the myogenic potential of DFAT cells by lentiviral-mediated forced expression of MyoD in vitro.

Conclusions: We propose to evaluate the myogenic potential of human DFAT cells in muscle engraftment approaches in a mouse model of cachexia. In summary, we suggest DFAT cells as a potential novel promising source of pluripotent cells available for a new cell therapy approach designed to treat cachexia.

8–13

A phase I study of the effects of a true human, monoclonal antibody against interleukin 1α on lean body mass, nutritional intake and appetite in advanced cancer patients: preliminary findings

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Background and aims: The proinflammatory cytokine interleukin-1α plays an important role in anorexia–cachexia syndrome. MABp1 is the first true human monoclonal antibody against interleukin-1α. We determined the effect of MABp1 on lean body mass, nutrition intake, and appetite among advanced cancer patients.

Methods: This is an open-label, first-in-man, phase I trial of MABp1 in patients with metastatic/refractory cancers. Patients were given MABp1 intravenously at one of four dose levels every 3 weeks. For anorexia–cachexia assessment, we collected serial data using dual-emission X-ray absorptiometry scan (DEXA), indirect calorimetry, daily nutrition diary, and EORTC QLQ-C30. We compared our findings between baseline and cycle 3.

Results: Baseline characteristics of the 36 enrolled patients were: average age 60, female 20 (56%), colorectal malignancies 14 (40%), median weight 61 kg (range 42–90 kg), median body mass index 24 kg/m^2, median resting energy expenditure 1,915 kcal, and hypermetabolic 92%. MABp1 was well tolerated with no infusion reactions and minimal adverse side effects. Twenty patients completed three or more cycles, and were included in the anorexia–cachexia analysis. Between baseline and cycle 3, the lean body mass increased by a median of 0.32 kg by DEXA (range −1.4–6.9 kg, N=17, P=0.13). Lean body mass change was not associated with tumor response. Median resting energy expenditure remained similar to baseline. The daily average caloric intake increased by a median of 178 kcal (range −985–1,017 kcal). EORTC-appetite significantly improved (median change −33, P=0.02). EORTC-global quality of life improved in 7/20 (36%) and remained the same in 8/20 (40%) patients.

Conclusions: In this phase I study, MABp1 showed preliminary evidence of improving lean body mass, nutritional intake and appetite despite the high baseline hypermetabolic rate. MABp1 was well tolerated with minimal adverse events. Further studies are needed to confirm our findings.

8–14

Effects of nutritional support in patients with colorectal cancer

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Introduction: Cancers of the colon and rectum are the third most common forms of cancer worldwide. The prognosis for survival after disease progression is usually poor. Cancer anorexia–cachexia syndrome is prevalent among advanced cancer patients, and has a large impact on morbidity, mortality, and a patient's quality of life. Early intervention with nutritional supplementation has been shown to halt malnutrition, and may improve outcome in some patients. In our study, we assessed the influence of nutritional support (counseling, nutritional supplements, and megestrol acetate) on the nutritional status and symptom prevalence in patients with colorectal cancer during chemotherapy. The study was designed to investigate whether dietary counseling, oral nutrition (commercial supplements), and megestrol acetate during chemotherapy affected nutritional status and survival in patients with colorectal cancer.

Methods: Six hundred and twenty-eight colorectal cancer patients were included in the study from January 2001 through December 2009, and randomized into one of two groups. Group I consisted of 315 patients (50%) who were monitored prospectively and were given nutritional support. Group II included 313 patients without nutritional counseling or nutritional support, in whom data were collected prospectively during a 9-year period of time. Patients were well balanced between the two groups. After evaluation (Nottingham Screening Tool Score, Appetite Loss Scale, and ECOG PS, weight), all patients in group I received nutritional counseling, oral nutritional food supplements, and megestrol acetate.

Results: After the completion of chemotherapy, there were lower proportions of patients in group I with a BMI<20, NST≥5, loss of appetite, and decreased weight gain. Nutritional counseling, supplemental feeding, and pharmacological support temporarily halted weight loss and improved appetite. This improvement may have implications for patient survival. Patients with early nutritional support lived 19.1 months while patients in the control group had a survival of 12.4 months (p=0.022).

Conclusion: These results encourage further studies with more specific nutritional supplementation in patients with colorectal cancer.