

## Abstracts of the 7th Cachexia Conference, Kobe/Osaka, Japan, December 9–11, 2013 (Part 2)

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1–26

### C-terminal Agrin Fragment as a marker of muscle wasting in patients after acute stroke during early rehabilitation

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**Background:** C-terminal Agrin Fragment (CAF) has been proposed as a potential marker for sarcopenia caused by degeneration of the neuromuscular junction. In patients with ischemic stroke muscle wasting is a common observation. We aimed to evaluate plasma level of total CAF and the sub-fragment AgrinC110 in relation to parameters for physical performance in patients during early rehabilitation after acute stroke.

**Methods:** 101 patients with acute ischemic or haemorrhagic stroke (age  $70 \pm 11$  y, BMI  $26.7 \pm 5.7$  kg/m<sup>2</sup>, all mean  $\pm$  SD) admitted to hospitalized rehabilitation centre and 15 healthy controls of similar age and BMI ( $64 \pm 8$  y,  $25.0 \pm 3.5$ ) were studied. Base line (BL) physical examinations performed at admission ( $23 \pm 18$  days after acute stroke) followed by follow up (FU) examinations ( $27 \pm 5$  days,  $50 \pm 18$  days after acute stroke) included functional assessment (3 m walk test) and maximum hand grip testing and body composition analysis by bioelectrical impedance analysis (BIA). CAF concentrations were measured by Western blotting.

**Results:** In patients serum levels of total CAF and AgrinC110 were elevated at admission and at discharge (total CAF  $545.3 \pm 175.2$  and  $541.8 \pm 159.9$  pM; AgrinC110  $405.5 \pm 133.0$  and  $417.4 \pm 127.5$  pM, respectively) compared to healthy controls (total CAF  $425.5 \pm 77.0$  pM,  $p < 0.01$ , AgrinC110  $316.4 \pm$

$59.6$  pM,  $p < 0.05$ ). AgrinC110 and total CAF levels were associated with hand grip strength of the non-paretic arm ( $r = -0.207$ ,  $p = 0.0381$  and  $r = -0.227$ ,  $p = 0.0227$ , respectively). Lower AgrinC110 and total CAF levels were associated with faster time in 3 m walk test ( $r = 0.448$ ,  $p = 0.0147$  and  $r = 0.445$ ,  $p = 0.0155$ , respectively) in patients who were able to complete the test ( $n = 29$ ). Total CAF and AgrinC110 serum levels were associated with BIA parameters of cellular integrity (reactance:  $r = 0.345$ ,  $p = 0.0005$  and  $r = 0.332$ ,  $p = 0.0008$ , respectively and phase angle:  $r = 0.392$  and  $r = 0.375$ ,  $p \leq 0.0001$  both, respectively).

**Conclusion:** CAF serum levels were elevated at admission and remained high throughout the hospitalized rehabilitation period. CAF related to body composition and functional capacity during post-stroke rehabilitations. CAF serum levels may provide additional information to monitor muscle status and functional recovery in the early phase of post stroke rehabilitation.

1–27

### The relationship between physical fitness and cognitive function on nondemented elderly

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**Aims:** The present study investigated the association between physical fitness ability and cognitive function in community based nondemented elderly. We are aware of the fact that several explanations for the prospective effect of physical activity on cognitive functions have been suggested. However, many studies with regard to physical activity, data were obtained from questionnaires; thus, bias could be

introduces by misinterpretation of the fatigue. In the present study we therefore did actual measurement of physical fitness test, and attempted to determine the extent of physical fitness ability and cognitive levels.

**Methods:** A total of 588 healthy elderly volunteers participate in this study. All subjects live in the Kameoka city, Kyoto prefecture, Japan. They underwent the Mini Mental Status Examination (MMSE) to test for dementia, and we divided into two groups: control ( $\leq 26$ ), and surely cognitive impairment ( $> 26$ ). They also determined with physical fitness ability test.

**Results:** As to the MMSE between the two groups, the surely cognitive impairment group had a significantly lower the chair stepping, the chair stand (5 times), and the chair stand (30 s) test compared with the control group.

**Conclusions:** Aging is associated with a progressive decline of perception, motor behavior, cognition, memory function and physical fitness. Under these conditions, the preservation of everyday life competence and thus the maintenance of independent living are at severe risk. Particularly, impaired physical and cognitive function increase the risk of falling with massive burden for the individual and the society. The present study supports these previous findings and suggests that even surely cognitive impairment group reduced physical fitness ability among actual the physical fitness test. We conclude that the important role of physical fitness in the protection of cognitive functions in community based nondeterment elderly.

1–28

### Cachexia assessed by bioelectrical impedance vector analysis in chronic stable heart failure patients

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**Background:** The use of phase angle and other raw parameter of the Bioelectrical Impedance Vector Analysis (BIVA) have gained attention as an alternative to the assessment of body composition over the last years. BIVA provides information about hydration status and body cell mass and therefore allows assessment of patients with increase or decrease body cell mass. This study explored whether the cachectic state assessed by bioimpedance vector analysis provides additional prognostic information.

**Methods:** We included 76 patients with stable chronic heart failure. BIVA uses the plot of impedance parameters resistance and reactance normalized per height as a bivariate vector in the vectorgraph. Phase angle is calculated directly from resistance (pure opposition of a biological conductor to alternating electric current) and reactance (capacitative resistance). Reference values exist for both phase angle and BIVA, which facilitates interpretation of the data. The shortening or lengthening of the vector indicates hydration status in form of oedema or dehydration, respectively, whereas a migration sideways indicates lean, cachectic or obese status. Cachexia was identified in those subjects who fell in the right lower quadrant of the reference curve of 75 % percentile and outside the resistance/reactance graph [bioelectrical impedance vectorial analysis (BIVA)-cachexia]. Clinical and biochemical data were also evaluated.

**Results:** Patients with BIVA-cachexia ( $n=11$ ) were older (mean age  $73 \pm 5$  years) and had significantly lower ejection fraction (mean LVEF  $35.5 \pm 10.1$  %) compare to patients without BIVA-cachexia (mean age  $66 \pm 10$  years, LVEF  $47.4 \pm 12.1$  %). The frequency of patients with body mass index  $< 20$ , and presence of oedema were higher in patients with BIVA cachexia. Additionally we found that patients with BIVA cachexia had significantly higher New York Heart Association functional class (NYHA  $3 \pm 0$  vs  $2.1 \pm 0.7$ ,  $p < 0.005$ ), as well as higher creatinine levels (mean  $1.4 \pm 0.5$  vs  $0.96 \pm 0.2$ ,  $p < 0.0005$ ) compare to patients without BIVA cachexia. A total of five patients with BIVA cachexia fulfilled the cachexia criteria from Evans et al. (Clin Nutr 2008).

**Conclusions:** BIVA could represent a valuable tool to assess presence of cachexia as changes in body cell mass in heart failure patients because provide information additional to weight loss. Impedance parameters and phase angle are useful for clinical practice and allow the identification of risk patients and are relevant for nutritional assessment.

1–29

### The application of segmental bioelectrical impedance spectroscopy in assessment of sarcopenia in elderly

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**Background and Aims:** Skeletal muscle contains extracellular water (ECW) in vivo which is not related to muscle strength, and thus, it is important to assess not only total skeletal muscle mass but also intra-cellular water (ICW) and ECW compartment of skeletal muscle to examine the actual relationship between skeletal muscle mass and physical functions. Segmental bioelectrical impedance spectroscopy (BIS)

is a unique tool to assess ICW and ECW in the limbs. The aim of the present study is to examine the application of segmental BIS in assessment of sarcopenia in elderly.

**Methods:** A total of 93 elderly men participated in the study (73 healthy elderly and 20 elderly who were requiring care and using ambulatory day-care service in the public long-term care insurance system). The ICW and ECW in the upper and lower legs were assessed by segmental BIS. Gait speeds, grip strength and maximal isometric knee extension strength were measured.

**Results:** The elderly requiring care had significantly lower ICW than healthy elderly in the upper and lower leg ( $P < 0.001$ ). The relative expansion of ECW against total water was observed in the elderly requiring care ( $P < 0.001$ ). Receiver operating characteristic curves showed that the area under of the curve (AUC) for the ICW index had similar to AUC for muscle strength to discriminate the elderly requiring care.

**Conclusion:** The elderly requiring care has relative expansion of ECW in the legs, which may mask actual muscle atrophy. Segmental BIS would be useful for the assessment of skeletal muscle cell mass and sarcopenia in the elderly.

### 1–30

#### Identifying a cut-off point for a frail elderly patients who may return to home after discharge

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**Objectives:** Discharging a frail elderly patients from the hospital to their home have a greater risk of falling than young patient. There is no cut-off point which determines how long a frail elderly patients will be in the hospital. Our research verifies cut-off points which determine whether patients should stay in hospital or go back to their home.

**Measurements:** 37 inpatients (mean age 73) were identified as frail people by means of The Cardiovascular Health Study (CHS) index. Using a measurement of physical function (MPPT, TUG, POMA, Timed chair rise, M-GARS, Gait speed, Berg Balance Scale, Functional Reach Test, Seated Step Test, Short physical performance battery, Grip strength), ADL index (Barthel Index, Rosow-Breslau score, PASE, OARS-IAD, Katz Index, FIM, Nagi scale), and measurement of cognitive function (HDS-R, MMSE, The Montreal Cognitive Assessment) were recorded.

They were divided into two groups. Group A (patients who discharged to home) and B (patients who entered nursing home). We used t tests to compute the statistical significance of differences between two groups.

**Results:** Of all these 22 tests, only a Modified Physical Performance Test (MPPT) has  $P$ -values less than 0.01 ( $p$ -value = 0.000514). Other measurement did not show significantly difference. From MPPT results, we assume cut off point at 9/10. It may distinguish between patients who can return to their home safety and should stay hospital or nursing home because they have a greater risk of fall.

**Conclusion:** MPPT suggests cut off point which determines whether frail older patients should stay or leave from hospital. When they are going back to their home, it is desirable that they score at least ten points. If they scored below nine points, they have a greater risk of fall so that they should stay in hospital or need caregiver.

### 1–31

#### Apoptosis in capillary endothelial cells in the aged mouse skeletal muscle

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**Background and Aims:** The age-related loss of skeletal muscle mass and function is a consistent hallmark of aging. Apoptosis plays an important role in muscle atrophy. The aim of this study was to determine whether apoptosis is restricted to myofibre nuclei (myonuclei) or occurs in satellite cells or stromal cells of the extracellular matrix (ECM).

**Methods:** C57BL6 male mice were used when 2-, 11-, 22- or 25-month old, corresponding to young adult, mature adult, early old or advanced old mice, respectively. Apoptosis in the gastrocnemius muscle was measured by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL). TUNEL coupled with immunostaining for dystrophin, paired box protein-7 (Pax7) or laminin-2 $\alpha$ , respectively, was used to identify apoptosis in myonuclei, satellite cells and stromal cells.

**Results:** Sarcopenia in mouse gastrocnemius muscle was characterized by myofibre atrophy, oxidative type grouping, delocalisation of myonuclei, and ECM fibrosis. In adult muscle, apoptosis was not detected in myofibres, but was restricted to stromal cells. Moreover, the age-related rise in apoptotic nuclei was essentially due to stromal cells. Myofibre-

associated apoptosis nevertheless occurred in old muscle, but represented less than 20 % of the total muscle apoptosis. Specifically, apoptosis in old muscle affected a small proportion (0.8 %) of the myonuclei, but a large part (46 %) of the Pax7<sup>+</sup> satellite cells. TUNEL coupled with CD31 immunostaining further attributed stromal apoptosis to capillary endothelial cells, which was concomitant with altered levels of key angiogenic regulators, perlecan and a perlecan domain-V (endorepellin) proteolytic product.

**Conclusions:** Collectively, our results indicate that sarcopenia is associated with apoptosis of satellite cells and impairment of capillary functions, which are likely to contribute to the decline in muscle mass and functionality in aging.

1–32

### C-terminal Agrin Fragment as a novel diagnostic marker for muscle wasting in patients with chronic heart failure: results from the studies investigating Co-morbidities aggravating heart failure

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**Aims:** Skeletal muscle wasting affects about 20 % of patients with chronic heart failure (CHF) and has significant implications for patients' exercise capacity. The accurate assessment of muscle wasting is technically challenging. Agrin is a synaptically located protein, which is responsible for initial formation and maintenance of neuromuscular junctions. C-terminal Agrin Fragment (CAF) is a proteolytic breakdown product of agrin. Depending on the cleavage site, different breakdown products are released. A smaller fragment, called CAF22 and a big fragment called TotalCAF can be measured in patients' serum. A third fragment, CAF110, can be calculated. Recently CAF has been suggested as a candidate marker for the detection of muscle wasting.

**Methods and Results:** We assessed serum CAF22 in 196 CHF patients and serum TotalCAF as well as CAF110 in 189 patients who participated in the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF, age 67±10 years, New York Heart Association [NYHA] class I/II/III/IV 7/53/38/2 %, median LVEF 35 % with IQR 30–54 %, CAF22 median 98 pmol/L with interquartile range [IQR] 78–123 pmol/L, TotalCAF median 551 pmol/L with interquartile range [IQR] 443–693 pmol/L, CAF110 median 445 pmol/L with interquartile range [IQR] 360–585 pmol/L). Muscle wasting was

identified using Dual-Energy X-ray Absorptiometry (DEXA) as defined by international standards in  $n=38$  (19.4 %). CHF patients with muscle wasting demonstrated higher CAF values than CHF patients without muscle wasting (CAF22  $p=0.01$ , TotalCAF  $p<0.01$ , CAF110  $p<0.01$ ). In Receiver Operator Characteristic (ROC) curve analysis, we calculated the optimal cutoff value to correctly identify patients with muscle wasting as for CAF22 >87.5 pmol/L, for TotalCAF >512.5 pmol/L, and for CAF110 >431.8 pmol/L. CAF fragments show a high sensitivity (CAF22 78.9 %, TotalCAF 84.2 %, CAF110 81.6 %) and a poor specificity (CAF22 43.7 %, TotalCAF 49.7 %, CAF110 52.3 %). Using simple regression analysis, CAF fragments were associated with NYHA class (CAF22  $R=0.32$  and  $p<0.01$ , TotalCAF  $R=0.37$  and  $p<0.01$ , CAF110  $R=0.36$  and  $p<0.01$ ) and muscle wasting parameters like exercise time (CAF22  $R=-0.43$ ,  $p<0.01$ , TotalCAF  $R=-0.43$  and  $p<0.01$ , CAF110  $R=-0.42$  and  $p<0.01$ ) and leg strength (CAF22  $R=-0.31$ ,  $p<0.01$ , TotalCAF  $R=-0.28$  and  $p<0.01$ , CAF110  $R=-0.25$  and  $p<0.01$ ). In addition, we detected associations of serum CAF with kidney and liver function as well as with parameters of iron metabolism and storage. In multivariate logistic regression analysis, sex (female,  $p<0.01$ ), creatinine (mg/dl,  $p=0.02$ ), handgrip strength (kg,  $p<0.01$ ), and each CAF fragment (pmol/L,  $p=0.01$ ) were independent predictors for muscle wasting.

**Conclusion:** Serum values of CAF may be useful to identify patients with CHF and muscle wasting. Whilst its sensitivity is high, its specificity remains poor, but elevated levels should prompt further investigation using DEXA scan.

1–33

### High prevalence of pre-sarcopenia; reduced muscle mass and preserved muscle strength and function, diagnosed with the European Working Group on Sarcopenia in Older People (EWGSOP) Algorithm, in Japanese elder patients with chronic obstructive pulmonary disease undergoing pulmonary rehabilitation

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**Backgrounds:** COPD is chronic systemic inflammatory disease and often associated with sarcopenia leading to frailty. However, information on sarcopenia diagnosed with EWGSOP algorithm in Japanese COPD patients is scarce.

**Methods:** In a cross-sectional study, 43 stable COPD outpatients at pulmonary rehabilitation clinic in the National Center for Geriatrics and Gerontology (NCGG), 40 male and 3 female;  $74.9 \pm 5.9$ , 65–87 years; The Global Initiative for COPD stage I: 8, stage II: 20, stage III: 11, Stage IV: 4, were underwent a comprehensive geriatric assessment including anthropometry and body composition with dual-energy X-ray absorptiometry. The control group (CG) was outpatients without COPD in Department of Geriatric Medicine in NCGG (75 male and 63 female;  $77.4 \pm 6.6$ , 65–96 years).

**Results:** Mean appendicular skeletal muscle mass index (ASMI) was  $6.61 \pm 0.64$  kg/m<sup>2</sup>, 14 had sarcopenia diagnosed with EWGSOP algorithm and 23: pre-sarcopenia, 2: Severe sarcopenia. ASMI was positively associated with nutritional status (MNA:  $r=0.33$ ,  $p=0.03$ ) and grip strength ( $r=0.47$ ,  $p=0.002$ ). Prevalence of pre-sarcopenia in COPD patents (53 %) is higher than CG (pre-sarcopenia: 8 %, sarcopenia: 20 %, ASMI:  $6.75 \pm 0.96$  kg/m<sup>2</sup>) and the data of community-dwelling Japanese elderly people in the literature (pre-sarcopenia: 37 %, sarcopenia: 9 %). Pre-sarcopenic COPD patients had lower health status than COPD patients with preserved muscle mass. With Fried's frailty criteria, 7 were classified as frail, 23 as pre-frail, and 13 as robust. All of 6 frail male COPD patients were diagnosed as sarcopenia. Among pre-sarcopenic COPD patients, 12 were pre-frail and 11 were robust. Among 11 pre-sarcopenic patients in CG, 2 were pre-frail and 8 were robust.

**Conclusions:** Pre-sarcopenia was highly prevalent in elder COPD patients and might have different clinical features from patients without chronic inflammatory co-morbidities and an etiological role in the constellation of frailty, disability and COPD (co-morbidity), which deserve further investigations.

1–34

**Muscle strength and fat mass, but not muscle mass are independent predictors of physical performance impairment with age**

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**Background and Aims:** Sarcopenia may have bi-directional associations with impaired mobility and function. Our aim was to determine the contributions of skeletal muscle mass (SMM), fat mass (FM) and lower limb muscle strength to gait speed and physical function in older adults.

**Methods:** Baseline data from 15 exercise trials were pooled. Habitual and maximal gait speeds, gait speed during a 6-minute or 400-meter walk test (LDGait), chair rise time, stairclimb power (dependant variables) as well as age, sex, leg press (LP) and knee extension (KE) one repetition maximum (1RM), and SMM and FM (independent variables) were transformed into z-scores before pooling. Sequential linear mixed-models were constructed, adjusted for age and sex. Independent variables found to be associated with a dependant variable were then entered into a stepwise linear regression, together with age and sex.

**Results:** Cross-sectional data were pooled from 1,501 adults (49.2 % male,  $70.8 \pm 9.3$  years). Habitual and maximal gait were associated with LP and KE 1RM, FM and SMM ( $p < 0.05$ ). LDGait and chair rise were associated with LP and KE 1RM and FM ( $p < 0.05$ ). Stairclimb power was associated with LP and KE 1RM and SMM ( $p < 0.05$ ).

In stepwise regression models, age ( $r = -0.24$ ,  $r = -0.35$ ,  $r = -0.40$  and  $r = 0.24$  respectively), FM ( $r = -0.13$ ,  $r = -0.19$ ,  $r = -0.34$  and  $r = 0.27$  respectively) and KE 1RM ( $r = 0.21$ ,  $r = 0.23$ ,  $r = 0.33$  and  $r = -0.32$  respectively) were independent predictors of habitual and maximal gait speed, LDGait and chair rise performance. By contrast, only age ( $r = -0.16$ ), sex ( $r = -0.29$ ) and KE 1RM ( $r = 0.16$ ) were independent predictors of stairclimb power.

**Conclusions:** Aging is associated with reductions in gait speed and physical function, which may be offset by maintaining quadriceps strength and minimising obesity. Muscle mass did not contribute to performance after accounting for strength. Interventions targeting increases in lower limb strength as well as reductions in adiposity may preserve function in later life.

1–35

**The tumour suppressor Scrib is a novel mediator of TNF-alpha signalling in muscle stem cells**

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**Background and aims:** Satellite cells are the resident stem cells of adult skeletal muscle, supplying myonuclei for homeostasis, hypertrophy and regeneration. Although TNF- $\alpha$  is one of the pathologically cachexic factors that induce muscle wasting, it also plays a physiological role in muscle regeneration to stimulate proliferation of satellite cells. Recent studies have shown that a tumour suppressor Scrib is associated with TNF signalling in invertebrate.

**Methods:** Using satellite cell-specific Scrib knock-out mice and primary cultured satellite cells, we investigated the role of Scrib protein in TNF- $\alpha$ -mediated myogenic progression.

**Results:** We found that Scrib is weakly expressed in proliferative satellite cells after activation and robustly expressed in committed cells to myogenic differentiation. Overexpression of Scrib disrupted self-renewal and proliferation of satellite cells. However, knockdown of Scrib by siRNA also inhibited proliferation and promoted myogenic differentiation, suggesting that levels of Scrib expression influence on satellite cell fate decisions. Importantly, Scrib acts as a mediator of TNF- $\alpha$  signalling in satellite cells. Indeed, TNF- $\alpha$ -stimulated cell proliferation was impaired in satellite cell-specific Scrib knock-out mice. Scrib knock-out mice exhibited significant defect of muscle regeneration after cardiotoxin-induced muscle injury in vivo due to insufficient satellite cell proliferation.

**Conclusions:** Taken together, our results indicate that Scrib is a novel regulator for muscle regeneration to operate satellite cell population expansion by mediating TNF- $\alpha$  signalling.

1–36

**COPD patients with a 6 min walk distance <350 m have less quadriceps strength but a similar body mass index to those with a walk distance >350 m**

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**Introduction:** Skeletal muscle weakness affects a significant minority, between 30 and 40 %, of patients with patients Chronic Obstructive Pulmonary Disease (COPD)1. A 6 min walk (6 MW) distance of less than 350 m in COPD is associated with an increased 1 year risk of hospitalisation or

death<sup>2</sup>; since skeletal muscle weakness is also associated with an increased risk of death we hypothesised that quadriceps strength would be less in patients with a 6 MW less than 350 m.

**Objectives:** To compare the quadriceps strength, measured as maximal isometric voluntary contraction force, in patients as a function of 6 min walk distance in the ERICA (Evaluating the Role of Inflammation in Chronic Airways Disease) cohort.

**Methods:** ERICA is a multicentre UK study investigating the role of inflammation and the prevalence and significance of cardiovascular and skeletal muscle manifestations in COPD. We present data from the first interim analysis in 395 (49 %) of the planned 800 participants. Baseline measurements include quadriceps maximal voluntary contraction force (QMVC), and 6-minute walk distance (6 MW) as well as standard phenotypic measures for COPD.

**Results:** From a preplanned interim data set we identified 147 patients with a 6 MW <350 m and 207 with a 6 MW  $\geq$ 350 m. Their characteristics are shown in the table as mean with range (min-max) in parentheses.

	6 MW <350 m	6 MW $\geq$ 350 m	Significance between groups
Six min walk distance (m)	238 (20–347)	433 (350–634)	Not tested
Age (year)	68 (44–83)	67 (43–84)	0.23
FEV1 (litre)	1.21 (0.4–2.3)	1.52 (0.6–1.5)	$P < 0.0001$
Body Mass Index (kg/m <sup>2</sup> )	27.8 (16.6–44.0)	26.5 (13.2–38.5)	$P = 0.03$
Quadriceps MVC (kg)	27.1 (10.2–66.5)	33.3 (10.7–60.1)	$P < 0.0001$

**Conclusion:** Patients with COPD at increased clinical risk, as judged by 6 min walk distance, have evidence of locomotor muscle weakness despite a slightly greater body mass index. This suggests that therapies which increase locomotor strength may be beneficial in COPD.

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1–37

### Reduced short physical performance battery scores are associated with type II fibre shift in chronic obstructive pulmonary disease

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**Background:** Chronic Obstructive Pulmonary Disease (COPD) is characterised by locomotor muscle dysfunction in which there is a shift from type I to type II fibres (Natanek et al. 2013: 48; 488–97). The Short Physical Performance Battery (SPPB) is commonly used in gerontology, however, the value of the SPPB as a simple assessment tool of lower limb function in COPD has not yet been established. Furthermore, it is not known whether SPPB score reflects fibre shift. We sought to identify whether the SPPB detects functional and structural adaptations of the quadriceps in COPD. **Methods:** In 31 stable COPD patients we measured SPPB score, spirometry, 6 min walk distance, quadriceps strength, rectus femoris cross-sectional area (RF<sub>CSA</sub>) and fat free mass. A vastus lateralis biopsy was also performed with subsequent immunohistochemistry to identify type I and II fibre preponderance, fibre cross-sectional area (CSA) and muscle capillarity (C:Fi). The phenotypic characteristics of patients stratified according to SPPB score were identified (SPPB<10: mild or major impairment and SPPB 10–12: minimal impairment).

**Results:** Stratifying patients according to SPPB score identified those with locomotor muscle atrophy, impaired strength and reduced exercise capacity (see table). Patients with lower SPPB scores also had a higher proportion of type II fibres, 71 (14) v 58 (15) %;  $p=0.04$ , although there were no differences in C:Fi or fibre CSA.

**Conclusions:** The SPPB is a valid and simple assessment tool that may detect a phenotype with functional impairment, loss of muscle mass and structural muscle abnormality in stable COPD patients.

**Table 1:** The demographic and phenotypic characteristics of patients stratified according to SPPB score (SPPB

10–12: minimal impairment, SPPB<10 at least mild impairment).

	SPPB 10–12 (n=22)	SPPB<10 (n=9)	p-value
Age (years)	63 (11)	65 (9)	0.55
Gender (m:f)	12:10	4:5	0.61
Height (cm)	166 (10)	161 (10)	0.22
FEV <sub>1</sub> %pred	56 (25)	42 (22)	0.16
BMI (kg/m <sup>2</sup> )	28.3 (7)	24.7 (8)	0.22
FFM (kg)	50 (12)	41 (11)	0.06
FFMI (kg/m <sup>2</sup> )	18.0 (3)	15.8 (4)	0.06
QMVC (kg)	33.1 (11)	20.6 (7)	<b>0.001</b>
QMVC%pred	73 (14)	53 (13)	<b>0.001</b>
QMVC/BMI	1.22 (0.4)	0.87 (0.3)	<b>0.02</b>
6 MW (m)	437 (81)	274 (113)	<b>0.0002</b>
Rectus Femoris <sub>CSA</sub> (mm <sup>2</sup> )	529 (106)	332 (140)	<b>0.0002</b>
SPPB score	11.4 (0.7)	7.2 (1.9)	<b>&lt;0.0001</b>
% Type II fibres	58 (15)	71 (14)	<b>0.04</b>
Type I fibre CSA (µm <sup>2</sup> )	4324 (865)	4378 (1342)	0.66
Type II fibre CSA (µm <sup>2</sup> )	3395 (894)	3132 (1291)	0.54
Combined type I and II fibre CSA (µm <sup>2</sup> )	3860 (988)	3755 (1425)	0.81
C:Fi	1.6 (0.3)	1.4 (0.4)	0.23

1–38

### Comparison of two methods for determination of fat-free mass index in cachexia

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**Introduction:** According to consensus on cachexia definition, one of the criteria for cachexia diagnosis is low fat-free mass index (FFMI), which can be determined by full body dual-energy X-ray absorptiometry (DEXA) scan or by measurement of upper arm circumference (UAC). We aimed to compare both methods of FFMI assessment and their ability to identify patients with low FFMI.

**Methods:** We included 41 chronic heart failure patients (60 % men, mean age 72 years, 93 % NYHA II/III). Body composition was determined by DEXA and appendicle skeletal muscle index (ASMI) was calculated. If ASMI was <5.45 in females and

<7.25 in males, the criterion for low FFMI was fulfilled. UAC was measured and compared to normative values. If the value was lower than 10th percentile for age and gender, low FFMI was diagnosed. Independent sample *t*-test was used to compare body mass index (BMI) among the following patients' groups: (1) normal ASMI and normal UAC, (2) disagreement between the two methods, (3) low ASMI and low UAC.

**Results:** The methods were in agreement in 27 patients (67.5 %). UAC managed to identify 15 patients (37.5 %) with low FFMI, while ASMI identified only 10 patients (25 %). In 10 patients (25 %) with disagreement between methods, UAC identified low FFMI although ASMI was normal. BMI was significantly higher in group (1) when compared to group (2) (32.8 vs. 25.9,  $p < 0.001$ ). There was no difference between BMI in group (2) and (3) (25.9 vs. 24.6,  $p = 0.611$ ).

**Conclusion:** Low FFMI was more common with UAC than with DEXA, which can lead to overestimation of cachexia prevalence using the UAC. Patients with lower BMI are more prone to disagreement between methods.

1–39

### Glucocorticoid receptor signaling contributes to muscle atrophy caused by acute inflammation

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Skeletal muscle atrophy accompanies acute inflammatory conditions including ARDS, COPD exacerbations and sepsis, and closely correlates with increased mortality. Increased circulating glucocorticoid (GC) levels have been linked to muscle atrophy. Nevertheless, the contribution of glucocorticoid receptor (GR) signalling to muscle atrophy caused by acute inflammation remains to be elucidated. To investigate this, acute pulmonary inflammation was induced by intra-tracheal lipopolysaccharide (IT-LPS) installation, in muscle-specific GR knockout (MLC-Cre<sup>+/-</sup> GR-LoxP) and control (GR-LoxP) mice. Circulating corticosterone levels were significantly increased in response to IT-LPS. Increased expression of mRNA transcripts encoding the GC-sensitive genes Glul, REDD1, KLF-15 and myostatin, as well as the well-established “atrogenes”, atrogen-1 and MuRF1, was detected in skeletal muscle of control

mice following IT-LPS, suggestive of GR- and atrophy signalling. Importantly, the reduction in body and muscle weight following IT-LPS instillation observed in GR-LoxP control mice, was strongly attenuated in muscle-specific GR knockout mice. Concomitantly, the induction of the GC-sensitive genes and the E3 ubiquitin ligases, atrogen-1 and MuRF1 was completely blunted or markedly reduced in GR knockout muscle in response to LPS. Interestingly, upon LPS-treatment loss of muscle GR expression reduced the mRNA and protein abundance of FoXO, a crucial transcription factor regulating atrogen expression.

These data that muscle GR-signaling is required for muscle atrophy following acute (pulmonary) inflammation.

1–40

### Changes of oedema-free body mass and prognosis in patients with heart failure

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**Background and Aims:** Obese and overweight heart failure (HF) patients live longer than normal and underweight. Weight loss (WL) and lower weight before HF has developed is associated with worse outcome while the impact of weight gain (WG) on prognosis remains unclear.

We intended to show associations between oedema-free weight change during HF and all-cause mortality in patients treated according to current guidelines.

**Methods:** In 951 HF patients (age: 53±10 years, 13 % female, 62 % ischemic etiology, NYHA: 2.6±0.8, LVEF: 24±7 %) we have calculated oedema-free weight changes from the onset of HF to the index date and expressed them as percentage of weight before HF. Patients were categorised into 6 groups: 1 – WG >7.5 %, 2 – WG 2.5–7.5 %, 3 – stable weight (±2.5 %) taken as a reference, 4 – WL 2.5–7.5 %, 5: - WL 7.5–15 %, 6 - WL >15 %. Cox proportional hazard model was used to obtain HR±95%CI for weight change categories relative to group 3 in models adjusted for age, gender, BMI in 24-months observation. The frequencies of study outcome were calculated by groups.

**Results:** In the follow-up of 24 months 239 (25.2 %) patients died. BMI dropped from group 3 to 6 and increased from 3 to 1 group. The risk of death was significantly higher only in group 6: HR 2.25 (1.4–3.7),  $p=0.0001$  after adjustment for age, gender, BMI; standard mortality ratio 2.06 (1.6–2.51). The effect of weight gain was completely neutral.

**Conclusion:** Weight gain exerts neutral effect on the risk of death in heart failure.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007–2013] under grant agreement n° 241558 (SICA-HF).

1–41

### Changes of oedema-free body mass and prognosis in patients with heart failure and diabetes

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**Backgrounds and Aims:** Obese and overweight heart failure (HF) patients live longer than normal and underweight. Both, weight loss and lower body weight before HF are associated with worse outcome. Obesity paradox does not exist in HF patients with diabetes (DM). The impact of weight loss on prognosis in HF patients with DM has not been assessed.

We intended to show associations between oedema-free weight change during HF (WC) and all-cause mortality in HF patients with and without DM.

**Methods:** In 951 HF patients (age:  $53\pm 10$  years, 13.6 % female, 62 % ischemic etiology, NYHA:  $2.6\pm 0.8$ , LVEF:  $24\pm 7$  %) we have calculated oedema-free weight changes from the onset of HF to the index date and expressed them as percentage of weight before HF (WC). Cox proportional hazard model (stepwise regression, inclusion for  $p<0.05$ , exclusion for  $p>0.2$ ) was used to obtain HR $\pm$ 95%CI for whole population, DM and non-DM groups for weight change, age, gender, BMI before HF, BMI index, NT-proBNP and LVEF in the 2-year observation.

**Results:** During follow-up of 24 months after index date 239 (25.2 %) patients died. DM was recognized

in 283 patients (29.8 %). WC was an independent predictor of 2-year mortality only in the whole HF population and non-DM group (HR 1.08 (1.02–1.14),  $p=0.005$  and HR 1.1 (1.04–1.16),  $p=0.003$  respectively).

**Conclusion:** In HF weight change was an independent risk factor of 2-year mortality in non-DM patients, but not in those with DM.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007–2013] under grant agreement n° 241558 (SICA-HF).

1–42

### Improvement in lean body mass is associated with prolonged survival in refractory colorectal cancer patients

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**Background:** Energy balance is regulated by the central nervous system (CNS), via the hypothalamic-pituitary-adrenal axis (HPA). Interleukin-1 (IL-1) signaling has been implicated as a trigger for cachexia via IL-1 receptors located in the hypothalamus. Interactions between platelet IL-1 $\alpha$  and the vascular endothelium of the CNS may be the source of tumor related IL-1 signaling that drives cachexia in advanced cancer patients.

**Methods:** 42 subjects with metastatic cancers were treated with MABp1, a True Human™ monoclonal antibody targeting IL-1 $\alpha$ , intravenously every 3 weeks until evidence of tumor progression. Objectives were safety, tumor response, change in IL-6, quality of life, and change in lean body mass (LBM) as measured by DEXA. The colorectal carcinoma (CRC) cohort was also evaluated for overall survival (OS).

**Results:** 30 patients had baseline and follow up DEXA, 21 of 30 (70 %) had increases in LBM (mean=1.0 kg;  $p=0.02$ ). Plasma IL-6 trended downwards (median: 12.8 to 7.6 pg/ml,  $n=42$ ,  $p=0.08$ ); and platelet counts stabilized ( $p<0.001$ ). There was a decrease in fatigue and pain ( $p=0.008$  and  $0.025$ ) and improvement in appetite ( $p=0.02$ ) (baseline to week 8; EORTC-QLQ-C30 quality of life tool;  $N=33$ ). 14 CRC patients were treated, 5 (36 %) increased LBM, one patient achieved a partial response. Those with increases had a median overall survival of 19.3 months versus 6.6 months for those with LBM loss (log-rank  $p=0.098$ ; HR=0.33, 95 % CI 0.09 to 1.29).

**Conclusions:** HPA mediated muscle wasting observed in cancer induced cachexia appears to be mediated by IL-1 $\alpha$  signaling, the source of which may be circulating platelets and tumor cells. The association of increasing LBM and improved survival suggests a potential important anti-neoplastic activity for IL-1 $\alpha$  blockade in these patients, which is independent of radiographic tumor response.

1–43

### Wasting processes in the myocardium of patients with end-stage dilative cardiomyopathy include up-regulation of myostatin

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Heart failure is an important cause of morbidity and mortality in many industrial countries, and dilated cardiomyopathy (DCM) is one of its major causes. DCM is considered to be the most common of the cardiomyopathies and it is characterized by an increase in both myocardial mass and volume. However, the ventricular walls become thin and stretched, thereby impairing cardiac contractility resulting in poor left ventricular function. Catabolic mechanisms were characterized in tissue from the free LV wall from end-stage DCM patients ( $n=46$ ) was obtained during cardiac transplantation surgery. Control LV tissue was obtained from donor hearts ( $n=18$ ). All procedures were approved by the local ethics committee.

Using fluogenic substrates in an enzyme kinetic assessment, an up-regulation of capase-3 and -6 (160 % and 52 %, respectively, both  $p<0.01$  vs donor) was seen. The protein expression of the negative growth regulator of muscle mass, myostatin, was increased by 173% in DCM, as was the activation of its intracellular mediator SMAD-2 (both  $p<0.001$ ). The markers of autophagy, LC-3, processed LC-3 and p62, were up-regulated by approx 50 %, all  $p<0.05$  compared to control. GSK3a/b were both up-regulated (both  $p<0.01$ ) and showed reduced phosphorylation levels (=inhibition of its activity, both  $p<0.05$ ), while the Akt-pathway was down-regulated, as shown by reduced phosphorylation of

GSK, 4E-BP1 and p70S6K. Moreover, IGF-1 mRNA expression was down-regulated by 84 %,  $p=0.02$ . In conclusion, a strong activation of catabolic processes was observed in human DCM, while at the same time, the anabolic pathways were impaired, suggesting a net loss of proteins, cell organelles and possibly cells, contributing the progression of DCM.

2–15

### Transcriptomic and proteomic analysis of livers from cachectic mice reveals dysfunctional metabolism

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**Background:** Cancer cachexia is a catabolic condition characterized by progressive weight reduction and energy imbalance associated with systemic inflammation, elevated CRP & cytokines. While muscle and fat loss are obvious manifestations of cachexia, it is likely that the pivotal role of the liver in nutrient uptake, metabolism and redistribution contributes to dysregulated metabolism of cachexia.

**Methods:** Utilising a multi-platform approach including microarray and MS-based iTRAQ analysis, as well as novel ATP-binding protein enrichment technology coupled with label free MS-quantitation, we have profiled gene and protein expression patterns of livers from C26 tumor-bearing mice displaying cachexia.

**Results:** The transcriptomic and proteomic datasets revealed high correlation between the three approaches, with very few instances of incongruity. Pathway analysis utilizing several software packages indicated that central metabolic processes including lipid handling, glycolysis/gluconeogenesis, amino-acid metabolism, TCA cycle and mitochondrial electron transport chain are reduced in cachectic mice. Linking these metabolic pathways to upstream regulatory events, transcriptional activation is reduced within the RXR canonical pathway (e.g. CAR, LXR, FXR, TR, PPAR $\alpha/\beta/\gamma$ ), associated with cytokine signalling through activated JAK/STAT pathway, SOCS3 and IL-1/LPS-BP signalling. Repressed expression of genes and proteins in key energy generation pathways is counter-intuitive to the expected role of the liver in settings of food restriction/weight-loss - ie to adaptively utilize amino acids, carbohydrates & fatty-acids and activate ketone body production & gluconeogenesis. As a counterpoint to this dramatic

disruption in metabolic pathways, we see enhanced acute phase protein production and a concomitant increase in protein translation, potentially mediated through phosphorylated 4E-BP downstream of mTOR.

**Conclusion:** Chronic stimulation of cytokine-signalling in the liver by distal tumours disrupts metabolic pathways responsible for maintaining energy homeostasis. The net outcome of impaired hepatic processing & supply of nutrients to muscle, fat & other organs would contribute to the devastating effects of cachexia.

2–16

### Association between adiponectin isoforms and anorexia nervosa

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**Background and Aims:** Anorexia nervosa (AN) is a serious disorder affecting adolescents and young adults, and it decreases the quality of life of affected individuals for prolonged periods. Despite various treatments, AN continues to be a refractory disease because of its unknown pathogenesis. Adiponectin is a protein hormone produced almost exclusively in adipose tissue. Its circulation levels are decreased in human obesity. The role of adiponectin in AN has not been clarified. Moreover, few reports have described the relations between adiponectin isoforms and AN in the physical and psychological states. Therefore, we examined the role of adiponectin and adiponectin isoforms in AN.

**Methods:** Eighteen women participated in this study: nine patients with AN and nine age-matched healthy controls. We examined plasma adiponectin and its isoforms levels in all subjects and administered three types of psychological test to patients with AN: the Eating Disorders Inventory–2, the Maudsley Obsessional–Compulsive Inventory, and the Beck Depression Inventory–2.

**Results:** We found that the percentage of high-molecular-weight (HMW) to total adiponectin (%HMW) was significantly low and the percentage of low-molecular-weight (LMW) to total adiponectin (%LMW) was significantly high in the AN group compared with the control group. The %HMW positively and the %LMW negatively correlated with body mass index in the entire study population. The %HMW was also positively correlated with psychological symptoms such as social insecurity or cleaning evaluated with the Eating

Disorders Inventory–2 or the Maudsley Obsessional–Compulsive Inventory.

**Conclusions:** Our study indicates that all adiponectin isoforms should be evaluated in patients with AN in addition to total adiponectin. The decreased %HMW and the increased %LMW that were correlated with the body mass index and some components of psychopathology in our patients may indicate a complex role of adiponectin isoforms in maintaining energy homeostasis and emotion during extreme malnourishment.

2–17

### Cancer-related gastrointestinal dysmotility is mediated by the decrease in ghrelin signaling via serotonin 2c receptor in the hypothalamus

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**Background and Aims:** Gastrointestinal (GI) dysfunction is closely related to anorexia and can lead to cachexia syndrome in cancer patients. Our previous report showed that the hypothalamic serotonin 2c receptor (5-HT<sub>2c</sub>R) plays a major role in cancer anorexia. However, the pathophysiological mechanism underlying GI dysfunction through a brain-gut interaction in cancer remains unclear. We examined whether hypothalamic serotonin and the orexigenic ghrelin-neuropeptide Y (NPY) system are related to GI dysfunction in tumor-bearing rats.

**Methods:** Male Wistar rats were intraperitoneally inoculated with AH-130 hepatoma cells. The 5-HT<sub>2c</sub>R antagonist SB242084 (5 mg/kg, p.o.) and the ghrelin signal potentiator rikkunshito (RKT; 1,000 mg/kg, p.o.) were administered to tumor-bearing rats and fenfluramine-treated rats. Gene expression was quantified by real-time RT-PCR. GI motility was measured in rats under free-moving conditions by a strain gauge force transducer method.

**Results:** GI dysmotility was observed on day 5 after injection of the tumor cells. The fasted motor activities in both the antrum and duodenum were disrupted in tumor-bearing rats. Hypothalamic prepro-ghrelin mRNA expression but not plasma ghrelin concentration was decreased in tumor-bearing rats in the 24-h food-deprived state. SB242084 administration increased prepro-ghrelin and NPY mRNA expression in the hypothalamus and improved GI dysmotility in tumor-bearing rats. The activation of central 5-HT<sub>2c</sub>R signaling

by fenfluramine administration decreased fasted GI motor activity in 24-h food-deprived rats, but this effect was inhibited by treatment with SB242084 (20 micrograms, i.c.v) and NPY (1 nmol, i.c.v.). RKT successfully increased fasted GI motor activity in fenfluramine-treated and tumor-bearing rats.

**Conclusions:** This study showed that central 5-HT<sub>2c</sub>R signaling induced cancer-related GI dysmotility, which is mediated by a decrease in ghrelin receptor signaling in the NPY neurons of the hypothalamus. The enhancement of hypothalamic ghrelin signaling may be effective therapy for cancer patients with GI dysfunction.

## 2–18

### Activation of catabolism in skeletal muscle in a MCAO-stroke mouse model and potential interventions

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**Background:** Muscle tissue wasting is a common complication after stroke that impairs post-stroke recovery and long term outcome. Little is known about the acute and subacute catabolic activation contributing to muscle wasting after stroke. Herein we investigate acute mechanisms of local and systemic catabolic activation in skeletal muscle in relation to the size of brain damage.

**Methods:** We studied body composition changes and muscle tissue catabolic activation in a model of acute focal cerebral ischaemia produced by temporal occlusion of the mouse middle cerebral artery (MCAO). Global body composition (fat and lean tissue) was assessed by NMR and signals of muscle apoptotic activation (Catabolic activity (Caspase 3 and 6), proteasome activity and myostatin levels of the skeletal muscle (gastrocnemius) were assessed in the paretic and non-paretic leg. Factors such as feeding, activity levels, energy expenditure, sympathetic activation and inflammation were taken into account. Apparent targets for interventions to attenuate the catabolic stimulation such as sympathetic inhibition (propranolol), antibiotic therapy (gyrase inhibitor) and high caloric feeding were tested.

**Results:** Severe weight loss was seen in stroke but not in sham operated or control animals. Weight loss (–23 %) was driven by wasting of muscle tissue (–12 %) and fat tissue (–37 %).

Muscle wasting in the gastrocnemius occurred of the contralateral (–20 %) and the ipsilateral leg (–19 %, both  $p < 0.01$ ) and also in the myocardium (–13 %,  $p < 0.05$ ). Activity of caspase 3 and caspase 6, as well as the proteasome were up-regulated after stroke compared to control (all  $p < 0.05$ ). Myostatin expression was increased by 80 % vs control ( $p < 0.01$ ). Energy expenditure was lower in stroke animals but activity level and feeding were not different to controls and sham. Antibiotic treatment, high caloric feeding, or beta blockade were not effective to prevent proteolytic activation and muscle wasting.

**Conclusion:** Increased apoptosis accounts for skeletal muscle wasting after stroke. While global muscle degradation may result from systemic signals, denervation may trigger local signals toward increased apoptosis in the paretic leg that are not seen in the non-paretic leg.

## 2–19

### Cardiac expression of NGAL is up-regulated in experimental cancer cachexia

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NGAL is commonly seen as a marker of kidney injury; however, it has also been associated with critically ill patients (heart failure, sepsis, multi-organ failure) and is thought to play a role in cancer cell motility. Here we compared the mRNA expression of NGAL in the heart of cachectic rats bearing the Yoshida hepatoma ( $n = 16$ ) to that of the aldosterone antagonist spironolactone-treated (5 or 50 mg/kg/d,  $n = 11$  and 9, respectively) rats as well as healthy controls ( $n = 10$ ). Plasma levels of NGAL and aldosterone were assessed by ELISA.

Tumor bearing rats lost  $45 \pm 4$  g body weight, while controls gain  $61 \pm 3$  g ( $p < 0.001$ ) after 16 days. Five mg/kg/d spironolactone reduced wasting ( $-25 \pm 10$  g) and 50 mg/kg/d stopped weight loss ( $+0.5 \pm 16$  g, both  $p < 0.05$ ). Cardiac NGAL mRNA expression was up-regulated by 93 % compared to controls ( $p < 0.05$ ) and was reduced to control levels by 50 mg/kg/d spironolactone ( $p > 0.05$ ), while the 5 mg/kg/d dose was not effective. Aldosterone was up-regulated from  $337 \pm 7$  pg/mL in controls to  $591 \pm 31$  pg/mL in the

placebo group ( $p < 0.001$ ) and reduced to  $396 \pm 22$  pg/mL in animals treated with 50 mg/kg/d spironolactone ( $p < 0.01$ ). Plasma levels of NGAL were increased in tumor-bearing rats ( $1,462 \pm 360$  g/L) compared to controls ( $93 \pm 6$  g/L,  $p < 0.001$ ). High dose spironolactone reduced NGAL levels to  $530 \pm 77$  g/L ( $p < 0.05$  vs placebo). Cardiac function assessed by echocardiography was markedly improved by high dose spironolactone. Cardiac output on day 11 was decreased in the placebo group compared to control  $49 \pm 7$  mL/min vs  $80 \pm 7$  mL/min, respectively ( $p < 0.01$ ). This functional impairment was reduced by high dose spironolactone ( $79 \pm 7$  mL/min,  $p < 0.01$  vs placebo), which may functionally reflect the reduction of NGAL mRNA in the heart and protein in plasma. This may suggest that NGAL could potentially be used as a biomarker to assess cardiac impairment in cancer cachexia.

## 2–20

### MegaceES and progesterone improve survival in cancer cachexia, but progesterone has no effect on wasting

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The use of the appetite stimulant megesterol acetate (megace) has been widely discussed as a treatment for cachexia and clinical trials have shown positive effects on body weight in cancer cachexia. However, the mechanism of action of megace in cachexia is far from clear. Megace acts predominantly as a potent agonist of the progesterone receptor (PR) to exert its effects. It is also an agonist of the glucocorticoid receptor, but with less affinity in comparison to the PR (about 37 %). In this study the effects of 100 mg/kg/d MegaceES (an advanced formulation of megace,  $n = 10$ ) and of 0.5 ( $n = 16$ ), 5 ( $n = 15$ ) or 50 mg/kg/d ( $n = 15$ ) progesterone were compared to placebo-treated male 200 g Wistar rats ( $n = 44$ ) using the Yoshida hepatoma cancer cachexia model (16 day study period).

MegaceES significantly improved survival compared to placebo (HR: 0.44; 95%CI: 0.20–1.00;  $p = 0.0486$ ). Progesterone displayed a trend at 0.5 mg/kg/d (HR: 0.56; 95%CI: 0.28–1.11;  $p = 0.0095$ ) and significantly improved survival at 5 mg/kg/d (HR: 0.48; 95%CI: 0.24–0.95;  $p = 0.0356$ ), while the 50 mg/kg/d dose was not effective (HR: 1.05; 95%CI: 0.50–2.21;  $p = 0.91$ ). However, only MegaceES reduced

attenuated loss of body weight ( $-19.2 \pm 12.1$  g) compared to placebo ( $-51.3 \pm 1.9$  g,  $p < 0.001$ ), while progesterone-treated animals showed the same extent of wasting ( $-63.0 \pm 3.6$  g ( $p < 0.01$  vs placebo),  $-56.5 \pm 2.5$  g ( $p = 0.15$  vs placebo) and  $-48.9 \pm 3.2$  g ( $p = 0.53$  vs placebo) for 0.5, 5 and 50 mg/kg/d, respectively). MegaceES reduced wasting of lean body mass ( $-11.8 \pm 6.3$  g) and fat mass ( $-4.2 \pm 3.1$  g) compared to placebo (lean:  $-33.1 \pm 1.9$  g, fat:  $-12.7 \pm 0.5$  g, both  $p < 0.001$ ). Progesterone at 0.5 mg/kg/d increased loss of lean mass compared to placebo ( $-46.6 \pm 2.9$  g;  $p < 0.001$ ) while 5 or 50 mg/kg/d had no effect on lean mass. The loss of fat mass was not affected by any dose of progesterone.

Our results demonstrate that the beneficial effects of megace in cancer cachexia are only partly mediated by its progesterone receptor agonist properties. The effects of progesterone itself seem to be limited to a survival benefit at the 5 mg/kg/d dose. As both agents used in this study have anti-androgenic properties, as similar study in female rats should be considered.

## 2–21

### Low active ghrelin ratio correlated with appetite loss in patients with advanced pancreatic cancer

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**Background and Aims:** Ghrelin is an appetite stimulant and is one of promising medications for cancer cachexia. In patients with cancer cachexia, the elevation of acyl ghrelin (AG) and active ghrelin ratio (AGR) was previously reported. However, the plasma level of ghrelin and which ghrelin related indexes correlate with appetite loss in patients with advanced pancreatic cancer (PC) are not fully understood. The aim of this study was to elucidate the plasma level of ghrelin and the ghrelin related index which was associated with appetite loss in advanced PC patients.

**Methods:** Patients with treatment-naïve advanced PC were eligible into this study. Ghrelin related indexes including AG, des-acyl ghrelin (DAG), total ghrelin (Total-G) and AGR were measured. Additionally, score of appetite loss was rated numerically from 0 (none) to 10 (worst). Ghrelin related indexes were categorized by their median value. The association with ghrelin related indexes and the score of appetite loss was analyzed by Wilcoxon test.

**Results:** Sixty four patients were analyzed. The median level (25–75 percentile) of AG, DAG, Total-G and AGR were 36.4 (10.6–74.8) pg/mL, 155.0 (83.8–297.4) pg/mL, 195.4 (108.6–356.1) pg/mL and 0.17 (0.06–0.27). The mean score (mean + SD) of appetite loss was 3.3+3.28. AG, DAG and Total-G had no correlation with appetite loss ( $p=0.26$ , 0.54 and 0.28, respectively). However, the score of appetite loss (4.1+3.24) in low group of AGR was higher than the score in high group of AGR (2.5+3.19) ( $p<0.05$ ).

**Conclusions:** AG and AGR were low in advanced PC, compared with previously reports in patients with cancer cachexia. Low AGR was associated with appetite loss in patients with advanced PC. Further study about clinical features of low AGR in advanced PC is currently in progress.

2–22

### Hypermetabolism in advanced cancer patients with cachexia

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**Background:** Elevated resting energy expenditure (REE) may contribute to weight loss and symptom burden in cachectic patients with advanced cancer.

**Aims:** To compare the velocity of weight loss, symptom burden (fatigue, insomnia, anxiety, and anorexia combined score as measured by the Edmonton Symptom Assessment Score), C-reactive protein (CRP), and survival among cachectic cancer patients who have elevated REE to patients with normal REE.

**Methods:** A retrospective analysis of 60 advanced cancer patients evaluated in a cachexia clinic who underwent an indirect calorimeter to measure REE were analyzed. Patients were dichotomized to either elevated or normal REE. Descriptive statistics were generated and two-sample student t tests were used to compare the outcomes between the groups. Kaplan-Meier and Cox regression methodology were used to examine the survival times between groups.

**Results:** 37 patients (61.7 %) were male, 41(68.3 %) were white, 59 (98.3 %) solid tumors, predominantly 23 gastrointestinal cancers (38.3 %), with a median age of 60 (95 % CI 57.0–62.9). 35 patients (58.3 %) were hypermetabolic. Non-Caucasian patients were more

likely to have high REE (OR=6.17 [1.56, 24.8],  $p=0.01$ ). No statistical difference regarding age, cancer type, gender, active treatment with chemotherapy and/or radiation between hypermetabolic and normal REE were noted. The velocity of weight loss (–8.5 kg vs. –7.2 kg,  $p=0.68$ ), CRP (37.3 mg/L vs. 55.6 mg/L,  $p=0.70$ ), symptom burden (4.2 vs. 4.5,  $p=0.54$ ), and survival (288 days vs. 276 days,  $p=0.68$ ) were not significantly different between high versus normal REE, respectively.

**Conclusion:** Hypermetabolism is not uncommon in cancer patients with weight loss and noted to be more frequent in non-Caucasian patients. No association among velocity of weight loss, symptom burden, CRP, and survival was noted in cachectic advanced cancer patients with elevated REE.

2–23

### Cachexia in chronic heart failure patients - a longitudinal study

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**Background:** Cachexia is common in chronic heart failure patients. We used new cachexia definition to assess prevalence and incidence of cachexia in chronic heart failure patients.

**Methods:** We included 49 heart failure patients (mean age 73 years, 57 % men, 6 % NYHA I, 76 % NYHA II, 18 % NYHA III) who were screened for cachexia according to Evans et al. Anorexia was assessed with Simplified Nutritional Appetite Questionnaire and fatigue via Functional Assessment of Chronic Illness Therapy-Fatigue questionnaire and NYHA class. Handgrip strength was measured with Jamar dynamometer and fat-free mass index was determined with dual-energy X-ray absorptiometry. Same procedures were repeated during follow-up of at least 6 months.

**Results:** 41 patients completed the study (3 deaths, 5 early terminations). Mean follow-up was 8 months (min. 6 months, max. 17 months). At baseline, 10 patients fulfilled weight loss/low body mass index criterion for cachexia, but only 4 of them complied with cachexia definition (Table). During follow-up, 6 patients developed weight loss/low body mass index criterion. At follow-up

visit, 12 patients met weight loss/low body mass index criterion, but only 3 actually were cachectic (2 already cachectic at inclusion and 1 developed cachexia during follow-up)

		Baseline	During follow-up (new cases)	After ≥ 6 months
<b>Basic criterion</b>	<b>Weight loss &gt; 5 % body weight or body mass index &lt; 20</b>	<b>10/49 (20 %)</b>	<b>6/41 (15 %)</b>	<b>12/41 (29 %)</b>
1	<b>Biochemistry</b>	<b>5/10</b>	<b>4/6</b>	<b>8/12</b>
	• Hemoglobin < 120 g/L	3/10	1/6	3/10
	• Serum albumin < 32 g/L	0/10	0/6	0/10
	• Serum CRP < 5.0 mg/L	5/10	3/6	5/10
2	<b>Fatigue</b>	<b>2/10</b>	<b>1/6</b>	<b>4/10</b>
3	<b>Anorexia</b>	<b>4/10</b>	<b>3/6</b>	<b>6/10</b>
4	<b>Fat-free mass index</b>	<b>3/10</b>	<b>1/6</b>	<b>2/10</b>
5	<b>Muscle strength</b>	<b>6/10</b>	<b>2/6</b>	<b>6/10</b>
	<b>Cachexia</b>	<b>4/49 (8 %)</b>	<b>1/6</b>	<b>3/41 (7 %)</b>

**Conclusion:** Weight loss or low BMI is common in chronic heart failure, but cachexia criterion was met by about 10 % of heart failure patients.

## 2–24

### Disease severity and prevalence of co-morbidities in patients with cardiac cachexia: Results from the Studies Investigating Comorbidities Aggravating Heart Failure (SICA-HF)

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**Background:** The prevalence of cachexia varies according to disease and ranges from 5 to 20 % in chronic heart failure to 60 % in chronic obstructive pulmonary disease, and is as high as 85 % in advanced cancer. This makes it important to figure out the basic characteristics of this group of patients.

**Methods:** Between February/2010 and September/2013, we recruited 188 out-patients with stable chronic heart failure with reduced ejection fraction (left ventricular ejection fraction [LVEF] < 40 %) at the Charité Medical School, Campus Virchow-Klinikum, Berlin, Germany as part of the ongoing SICA-HF study. Cachexia was defined as loss of ≥ 5 % of weight in 1 year or less. Twenty-six of these patients (13.8 %) were cachectic (mean ± SD, age 62 ± 12, 14.8 % female, body mass index [BMI] 26.2 ± 5.8 kg/m<sup>2</sup>, LVEF 29.1 ± 8.4, New York Heart Association class 2.5 ± 0.7), the remainder were not cachectic (mean ± SD, age 67 ± 11, 16 % female, BMI 29.0 ± 5.4 kg/m<sup>2</sup>, LVEF 31.6 ± 7.4, NYHA 2.4 ± 0.6). Assessments included spirometry, hand grip, lab values and Dual Energy X-ray Absorptiometry. **Results:** 18 (9.6 %) of patients died during a mean follow-up of about three and half years, 4 of whom were cachectic. Patients with cachexia had worse values for peak oxygen consumption (14.8 ± 5.7 vs. 16.3 ± 4.8 ml/min/kg, *p* = 0.2), hand grip strength (34.2 ± 14.6 vs. 37.5 ± 11.9 kg, *p* = 0.2), muscle mass in the legs (16.7 ± 3.4 vs. 17.7 ± 3.3 kg, *p* = 0.1), and a higher prevalence of comorbidities than non-cachectic patients: atrial fibrillation (65 % vs. 39.5 %, *p* < 0.05), anaemia (Haemoglobin 12.4 ± 1.9 vs. 13.6 ± 2.1 mg/dL, *p* = 0.006), coronary artery disease (64.3 % vs. 67.9 %, *p* = 0.8).

**Conclusions:** Patients with cachexia tend to present with worse disease as evidenced by lower values for LVEF, NYHA class, peak VO<sub>2</sub>, and hand grip strength. The prevalence of co-morbidities is significantly higher than among non-cachectic subjects.

## 2–25

### "Hungry" bone syndrome in heart failure—fact or illusion?

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**Background and Aims:** Decrease of parathyroid hormone (PTH) blood level after parathyroidectomy causes hypocalcaemia due to rapid calcium apposition in bones.

The phenomenon is called hungry bone syndrome and diagnosed when postsurgical decrease of PTH is associated with hypocalcaemia without increased calciuria. In heart failure (HF) PTH is increased in parallel to HF severity. Standard treatment may ameliorate HF severity but the effect of treatment on PTH and calcium level remains unknown. We tested hypothesis that improvement of NYHA class following standard pharmacotherapy is associated with laboratory signs of hungry bone syndrome.

**Methods:** In patients with HF admitted to our ambulatory department in whom pharmacotherapy was first initiated and up titrated during consecutive visits we extracted data on baseline NYHA class (bNYHA). NYHA class was reassessed after 3–6 months (indexNYHA) and response to therapy was calculated as indexNYHA – bNYHA. In 1,029 patients (12 % female, NYHA –  $2.7 \pm 0.9$ , LVEF –  $25 \pm 7$  %, 67 % - ischaemic) at index date we measured albumin-corrected serum calcium, PTH and fractional urinary excretion of calcium. Using ANOVA we compared these parameters in patients with different categories of response pharmacotherapy.

**Results:** Worsening of clinical status was confirmed in 25 patients (delta NYHA 1), stable status in 332 patients and improvement in 682 persons (517, 160 and 5 for delta NYHA -1, -2 and -3 respectively). Serum calcium and PTH levels dropped according to improvement of clinical status ( $p$  for trend 0.04 and  $<0.0001$  respectively). There were no changes in fractional urinary excretion of calcium ( $p=0.87$ ).

**Conclusion:** Improvement of clinical status in HF is associated with lower serum calcium and PTH consistent with hungry bone syndrome.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007–2013] under grant agreement n° 241558 (SICA-HF).

2–26

### Serum phosphorus and all-cause mortality in chronic systolic heart failure

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**Background and Aims:** Epidemiological studies in diverse populations have found association of higher normal serum phosphorus (sP) with adverse clinical outcome in patients with normal kidney function. Different potential mechanisms including direct sP toxicity, accelerated calcifications and indirect hormonal effects are discussed. In more advanced stages of heart failure (HF) sP level increase independently of GFR. The impact of sP on mortality has never been examined in HF. We wanted to assess whether sP was independently associated with all-cause mortality in patients with stable systolic HF.

**Methods:** In 1,029 patients with HF (12 % female, NYHA –  $2.7 \pm 0.9$ , LVEF –  $25 \pm 7$  %, 67 % - ischaemic) we measured serum phosphorus. During 18 months 180 (17.5 %) have died. Patients were divided into quintiles of sP and Cox proportional hazard models were constructed to estimate the risk of all-cause mortality for quintiles of sP where Q2 was taken as reference. Raw model and models adjusted for age, gender, BMI, GFR and weight loss were calculated.

**Results:** The risk of 18-month mortality adjusted for age, gender, BMI, eGFR and weight loss was higher in Q4 of sP (HR 1.75 (1.05–2.90),  $p < 0.05$ ) and Q5 of sP (HR 1.77 (1.05–2.98),  $p < 0.05$ ).

**Conclusion:** In HF higher serum phosphorus is independently associated with worse prognosis.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007–2013] under grant agreement n° 241558 (SICA-HF).

2–27

### Mineralocorticoid receptor modulates autophagy and cellular differentiation in the adipose organ and skeletal muscle

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It is known that macroautophagy plays a pivotal role in cell differentiation. In particular autophagy has been shown to be a critical determinant of adipose tissue differentiation and function. We recently investigated the role of MR in the modulation of the autophagic rate in the adipose organ: we analysed responses to the MR antagonists spironolactone (SPIRO) and drospironone (DRSP) in female mice fed a high fat (HF) diet for

90 days. MR antagonism reduced autophagy rate and induced brown adipose tissue (BAT) markers in white adipose tissue (WAT) depots and in cultured adipocytes, providing evidence that MR modulates autophagic rate and, in turn, regulates brown-like remodelling of WAT. Interestingly, a critical role of autophagy has been recently shown also in skeletal muscle development. Therefore we investigated a possible involvement of MR in regulating macroautophagy in skeletal muscle cells. We first characterised the ontogenesis of MR in a murine myoblast cell line (C2C12) by Real Time analysis observing a 2.5 fold-increase in basal MR mRNA expression in skeletal myoblasts compared with myotubes after 72 h of differentiation. To evaluate the effects of MR activity on C2C12 cells we treated myoblasts and myotubes with aldosterone (Aldo, 10–8 mol/L) for 24 h and observed a marked increase in LC3 mRNA expression suggesting an increased autophagic rate. This effect was reverted by treatment with MR antagonist spironolactone (Spiro, 10–5 mol/L). However, protein levels of LC3 were not affected by any treatment, suggesting that longer treatments could be necessary to reveal an effect. Moreover we observed that Aldo significantly reduced in Heavy Chain Myosin (MHC) mRNA levels, suggesting that MR activity represses muscle cell differentiation. Such effect was also MR dependent, given that spiro was able to revert such effect.

These data suggest a potential role for MR in modulating skeletal muscle differentiation and autophagic rate.

## 2–28

### Diet—induced obesity exacerbates anorexia and weight loss in response to endotoxin

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Recent evidence suggests that in addition to its involvement in cachexia, inflammation may be a common underlying cause of many obesity-associated conditions. Animal and humans who acquire excess body weight during exposure to high energy diet (HED), may subsequently defend a new and apparently permanent obese body weight (DIO-D). To test whether obesity alters the response to a model inflammatory stimulus, we investigated its effects on anorexia and weight loss in response

to intraperitoneal injection of bacterial lipopolysaccharide (LPS *E. coli* (500 ug/kg).) We compared Sprague–Dawley rats exhibiting DIO-D, with littermates which showed resistance to weight gain when exposed to HED (diet resistant, DR) and also HED-naïve littermates.

Twenty-four hours after LPS treatment, food intake was decreased in DIO-D, DR and HED naïve animals, but was differentially affected. Cumulative 24 h food intake in HED-naïve animals was reduced by 51±8.2 %, and in DIO-D rats by 69±6.3 % ( $N=12$  and  $17$ , respectively, NS) while in DR animals, it was reduced by only 35±5.3 % ( $N=17$ ,  $P<0.01$  vs DIO-D, NS vs HED-N). Also at 24 h post LPS, body weight loss was consistent with the food intake results, with the DR animals losing significantly less weight than DIO-D animals (11±2 vs 25±3 g  $P<0.01$ ). A plot of the reduction in food intake vs absolute body weight indicated that animals designated DR were found to have the smallest reductions in weight and food intake while DIO-D animals surprisingly did not differ from the HED-naïve animals. The relationship between body weight and LPS-induced weight loss ( $R^2=0.45$ ) was significant ( $<0.0001$ ) for the HED-treated animals, while it was not in the HED-naïve population ( $R^2=.055$ ,  $P>0.45$ ).

Examination of whole hypothalamic markers of inflammation revealed that IL-1 $\beta$  mRNA levels in LPS-naïve animals were lower in DR than DIO-D rats, while the IL-1 receptor was unchanged. 24 h after treatment with LPS, levels of IL1 $\beta$  mRNA were elevated considerably in all 3 groups. A detailed analysis is underway to determine differences in a spectrum of inflammatory signaling pathways, to identify ones that may be involved in conferring these differences. .

**Discussion:** These results suggest that exposure to HED unmasks changes in the anorexia accompanying the inflammatory response to systemic bacterial endotoxin. I. Surprisingly, while these findings link the expected vulnerability to DIO-D as associated with the greatest anorexic effects of LPS treatment, the potentially more interesting finding is that animals most resistant to the obesogenic effects of the HED are also the least affected by the anorexic actions of LPS. Implications for cachexia: DR animals are both resistant to weight gain when exposed to HED and remarkably resistant to weight loss induced by LPS, possibly reflecting a tight regulation of body weight. By contrast DIO-D is associated with larger amplitude of gain and loss of body weight, which associates with inflammation. Studies are now underway to examine whether mutations in genes associated with resistance to cachexia are also seen in the DR rats.

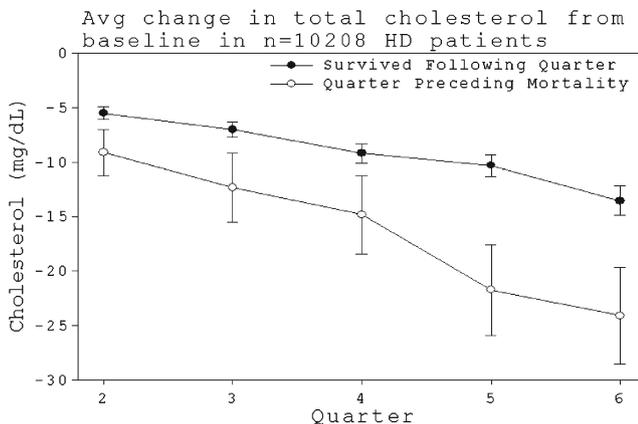
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2-29

### Change in total cholesterol over time and mortality among incident hemodialysis

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Patients who begin hemodialysis (HD) therapy develop wasting syndrome and cachexia over time including body fat loss and lipoprotein pool contraction. To further understand the significance of these trends over time, we examined the relationship between differences in total cholesterol over time and patient mortality and hypothesized that the direction and magnitude of change from baseline in cholesterol is related to patient outcomes in incident (new) HD patients. A 2-year (7/2004–6/2006) cohort of 23,191 incident HD patients were studied for 6 patient quarters (18 months) in a USA based dialysis chain with lipid profile measurements. Using a Cox regression model, the adjusted death hazard ratios (HR) were estimated. Cholesterol dropped over time in both surviving and deceased patients. A higher drop in cholesterol was independently associated with a higher death risk in incident HD patients (HR, 1.08; 95 % CI, 1.05–1.11). Hence, a more prominent contraction of lipoprotein pool over time appears associated with worse survival in HD patients.

3-27

### Nervous system reaction to neural invasion leads to cachexia in pancreatic cancer

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**Background and Aims:** The nervous system is damaged by neural invasion (N-inv), which is linked to muscle atrophy, pain, and astrocytic activation in the connected spinal cord. The reaction of the nervous system to N-inv may lead to cachexia and pain in pancreatic cancer (PCa). The interaction between cancer-nervous system reaction and systemic deterioration was tested using a murine N-inv model and human PCa cells.

**Methods:** The N-inv model was created by injecting Capan-1 cells into the left sciatic nerve of severe combined immunodeficiency mice. The N-inv model was compared to a sham operation group and a subcutaneous tumor group. Propentofylline (PPF), an inhibitor of astrocytic activation, was administered daily into the abdominal cavity. Allodynia with the von Frey test and body weight (BW) were measured weekly. Mice were euthanized 4 or 6 weeks after surgery. Expression of the astrocyte marker glial fibrillary acidic protein (GFAP) in the spinal cord and the murine interleukin-6 (mIL-6) level in serum as an index of systemic inflammation were evaluated at the end of the experiment. Clinical data and autopsied tissue samples from advanced PCa patients were evaluated to test the relationship between N-inv and cachexia, pain, and spinal astrocytic activation.

**Results:** Pain and astrocytic activation were observed 2 weeks after the start of N-inv and maintained throughout the experiment. Loss of BW began at 4 weeks and was worse at 6 weeks. The mIL-6 serum level was not elevated at 4 weeks, but was increased at 6 weeks. PPF treatment inhibited pain, astrocytic activation, mIL-6 elevation, and BW loss in the N-inv model, but did not affect tumor weight. Clinical data and human samples revealed a relationship between N-inv and cachexia, pain, and spinal astrocytic activation.

**Conclusion:** The reaction of the nervous system to N-inv leads to cachexia in PCa.

3–28

### Modeling human cancer cachexia in colon 26 tumor-bearing adult mice

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Muscle wasting is one of the most profound side effects of advanced cancer and is associated with decreased patient quality and survival. At current, no clinical therapies exist to treat cancer-induced muscle wasting. While cancer cachexia generally impacts older individuals, animal models used to study cachexia utilize juvenile mice. The purpose of this study was to attempt to better model human cancer cachexia by comparing the effects of colon 26 (C-26) tumors on young and adult mice. After tumor development, young and adult mice demonstrated no differences in tumor size and had lost significant body mass, muscle, and adipose tissue. Muscles from both young and adult mice tumor-bearing mice had increased proteasome and autophagy signaling. Additionally, tumors increased muscle Pax7 protein abundance and damaged the sarcolemma in both young and adult mice. In conclusion, C-26 tumors have similar effects on juvenile and adult mice, suggesting that young animals are appropriate models to study cachexia even though cancer-induced muscle wasting generally occurs in older adults.

3–29

### The excessive hypothalamic interaction of serotonin with corticotropin-releasing factor induces ghrelin insufficiency and resistance in cancer cachexia syndrome

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**Background and Aims:** Anorexia-cachexia is a major source of the increased mortality in cancer patients. Ghrelin plays an important role in stimulating hunger and maintaining energy homeostasis. However, an attenuation of the adaptive feeding response by the peripheral administration of ghrelin under cachectic conditions has been reported. Thus, we examined the pathophysiological mechanism underlying dysfunction of the ghrelin system in cancer cachexia.

**Methods:** Wistar rats were intraperitoneally inoculated with AH-130 hepatoma cells. The serotonin 2c receptor (5-HT<sub>2c</sub>R) antagonist SB242084 (5 mg/kg, p.o.), the corticotropin-releasing factor (CRF) antagonist alpha-helical CRF (50 micrograms, i.c.v.), and the ghrelin signal potentiator rikkunshito (1,000 mg/kg, p.o.) were administered to tumor-bearing rats. The plasma ghrelin level was determined by enzyme immunoassay. Gene expression was quantified by real-time RT-PCR.

**Results:** Decreased food intake, muscle wasting, hypercytokinemia, and elevated ghrelin in the plasma were observed on day 7 after the injection of tumor cells in rats. Compared with pair-fed normal rats, the plasma ghrelin concentration and the hypothalamic gene expression of neuropeptide Y (NPY) and agouti-related peptide were decreased in tumor-bearing rats. CRF administration (1.5 nmol, i.c.v.) induced a decrease in plasma ghrelin concentration in fasted rats. SB242084 decreased hypothalamic CRF levels and increased hypothalamic prepro-ghrelin and NPY mRNA expression in tumor-bearing rats. Ghrelin administration (3 nmol, i.v.) increased food intake; however, the responses were attenuated in tumor-bearing rats compared with normal rats, suggesting ghrelin resistance. SB242084, alpha-helical CRF, and rikkunshito increased food intake in tumor-bearing rats.

**Conclusions:** This study showed that the adaptive response to starvation was attenuated by decreased peripheral ghrelin secretion, hypothalamic ghrelin downregulation, and ghrelin resistance in cancer cachexia. The decrease in ghrelin signaling was mediated by the excessive hypothalamic interaction of 5-HT with CRF through 5-HT<sub>2c</sub>R. The disruption of ghrelin function may play a pathogenetic role in cancer cachexia.

3–30

### NF-κB dependent Pax7 deregulation in the muscle microenvironment promotes cancer cachexia

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**Background and Aims:** Cachexia is a wasting condition associated with multiple chronic diseases and characterized primarily by the loss of skeletal muscle. Cachexia not only diminishes the quality of life of cancer patients, but also is positively related to cancer mortality. Patients with cachexia are more susceptible to dose-limiting toxicity in chemotherapy. Mechanism regulating cancer cachexia has been extensively studied inside the myofibers. However, little is known about events outside the myofibers, in the muscle microenvironment, and their potential significance in regulating wasting in cancer cachexia.

**Methods:** Both cachectic mice models and muscle biopsies from pancreatic cancer patients with cachexia were used. To examine the abnormalities in cachectic muscles, cellular and histological analysis, including electron microscope, H&E and immunofluorescence was performed. Biochemical analysis, including western blotting and PCR, was used to study the signaling. FACS and cell sorting was combined with nanostring and other bioinformatics analysis to study specific cell populations. Genetic approaches, such as mutant mice, reporter mice, siRNA interference, were used to track and manipulate genes of interest.

**Results:** An abnormal accumulation of interstitial cells was observed in cachectic muscles. These cells were identified as activated muscle stem cells. However, cancer cachexia is associated with an impaired regeneration program, which is due to compromised differentiation from satellite cells and other myogenic progenitors. The self-renewing transcription factor, Pax7, under the control of classical NF- $\kappa$ B signaling, becomes deregulated and is responsible for the block of myogenic differentiation and promoting muscle wasting. Down regulation of NF- $\kappa$ B or Pax7, or overexpression of Pax7's downstream target, MyoD, rescued muscle wasting in cancer cachexia.

**Conclusions:** This study provides new insights into the mechanisms of cachexia, highlighting the relevance and importance of events that take place in the muscle microenvironment in regulating muscle wasting in cancer cachexia.

3–31

### The site of cancer implantation could influence the development of cancer cachexia

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**Background and Aim:** The incidence of cancer cachexia varies greatly according to tumor types. Although certain tumor types are more commonly associated with cachexia, even with the same tumor type there are variations in the extent to which patients exhibit cachexia. It has been considered that a variety of interactions between the host and the tumor could influence on the abnormal metabolism of cancer cachexia. The influence of the site of tumor growth on the development of cancer cachexia remains unclear. Thus, we investigated the influence of the different site of cancer cell implantation on the development of cancer cachexia using mouse model.

**Methods:** The same number of colon26 cells was implanted subcutaneously or intraperitoneally in BALB/c mice. Two weeks after the tumor implantation, the weight of adipose tissue (around epididymis), gastrocnemius muscle and heart were measured. 23 plasma cytokines were determined by using the Bio-Plex array system and plasma Myostatin and Activin A levels were measured by ELISA. Expression of MuRF-1 and Antrogin-1, which are required for muscle atrophy, in gastrocnemius muscle or heart was assessed by RT-PCR.

**Result:** The weights of body, adipose tissue, gastrocnemius muscle and heart significantly decreased in tumor-bearing mice compared with control mice. All of these weights were significantly lower in intraperitoneal tumor model compared with subcutaneous tumor model. The marked increase in the expression of MuRF-1 and Antrogin-1 was observed in intraperitoneal tumor model. As for plasma cytokine levels, several cytokine levels including IL-6 and IL-10 of tumor-bearing mice were significantly higher than those of control mice.

**Conclusion:** In this study, not only weights of adipose tissue and gastrocnemius muscle but also the weight of heart significantly decreased in intraperitoneal tumor model. These data suggest that the site of metastasis could influence the development cancer cachexia.

### Altered regulation of circadian rhythm and lipid metabolism associated with inflammatory signalling in white adipose tissue (WAT) in cancer cachexia

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**Background:** Involuntary weight loss in patients with cancer is the hallmark of cancer cachexia. The aetiology of cachexia is multifactorial involving loss of skeletal muscle and adipose tissue associated with high systemic levels of acute phase proteins and inflammatory cytokines. While muscle wasting overtly impacts on cancer patient quality of life, depletion of lipid depots represents a sustained energy imbalance. Circadian rhythm is important for the integration of environmental cues, nutritional intake and physiological activities of organs such as adipose tissue. In the present study we investigated the impact of the murine cachectic Colon 26 (C26) carcinoma on white adipose tissue (WAT).

**Results:** Microscopic examination of WAT revealed reduced size of white adipocytes accompanied the depletion of fat depots and elevated circulating free fatty acids in cachectic C26 tumour-bearing mice. Perturbed diurnal rhythmic expression patterns of *Rev-erb $\alpha$* , *Bmal1*, *Per2*, *Cry1*, *Ppar $\delta$* , *Ppar $\gamma$* , *C/ebp $\alpha$*  and associated genes *Pbe*, *Fas*, *Lpl* and *Perilipin*, indicate altered circadian regulation of lipid metabolism during the development of cachexia. Furthermore, lipid catabolism did not appear to be stimulated through classical hormone-induced PKA activation of hormone sensitive lipase, but via adipose tissue triglyceride lipase (ATGL). These changes are accompanied by activation of cytokine signalling mediated primarily through phosphorylation of STAT3 and p38 MAP kinase rather than ERK1/2. In addition, the key sensor of low energy status – AMPK is activated while downstream mTOR/4EBP1 signalling was inhibited, implicating suppression of lipogenesis alongside enhanced lipolysis from WAT.

**Conclusion:** Taken together, these findings indicate that during cancer cachexia there is increased cytokine signalling potentially affecting diurnal regulation of WAT and lipid metabolism. Future intervention studies to prevent cachexia should consider the interplay between circadian rhythm and energy metabolism pathways in adipose tissues.

### Validation of the consensus definition for cancer cachexia and evaluation of a classification model—A study based on data from an international multicentre project (EPCRC-CSA)

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**Abstract:** Weight loss limits cancer therapy, quality of life and survival. Common diagnostic criteria and a framework for a classification system for cancer cachexia were recently agreed upon by international consensus. Specific assessment domains (Stores, Intake, Catabolism and Function) were proposed. The aim of this study is to validate this diagnostic criteria (two groups: Model 1) and examine a four group (Model 2) classification system regarding these domains as well as survival.

**Methods:** Data from an international patient sample with advanced cancer ( $n=1,070$ ) was analysed. In model 1 the diagnostic criteria for cancer cachexia (weight loss/BMI) were used. Model 2 classified patients into four groups (I–IV) according to weight loss/BMI as a framework for cachexia stages. The cachexia domains, survival and sociodemographic/medical variables were compared across models.

**Results:** 861 patients were included. Model 1 consisted of 399 cachectic and 472 non-cachectic patients. Cachectic patients had significantly higher levels of inflammation, lower nutritional intake and performance status and shorter survival. In

model 2, differences were not consistent; appetite loss did not differ between group III&IV, and performance status not between I&II. Survival was shorter in group III and IV compared to other groups. By adding other cachexia domains to the model, survival differences were demonstrated.

**Conclusion:** The diagnostic criteria based on weight loss and BMI distinguish between cachectic and non-cachectic patients concerning all domains (Intake, Catabolism and Function) and is associated with survival. In order to guide cachexia treatment a four-group classification model needs additional domains to discriminate between cachexia stages.

3–34

### Prevalence of pre-cachexia and cachexia in surgical cancer patients

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**Introduction:** Cachexia is a devastating condition resulting from the complex interplay between the presence of a tumor, the inflammatory response and the metabolic alterations in the host. With the recent availability of criteria for classification and staging of cancer cachexia, the attention has focused on early detection and prompt interventions aimed at prevention and early treatment of cancer cachexia. The aim of this study was to assess whether the classification of cancer patients according to the new criteria is reflected by changes of Phase angle (PA) and other Bio-Impedance Analysis (BIA) measurements.

**Methods:** After written informed consent was obtained, forty-two consecutive surgical cancer patients (24M18F; age 68.69 ± 11.13 years) were enrolled. Immediately before surgery, all patients were evaluated for the presence of pre-cachexia (Muscaritoli et al.) or cachexia (Fearon et al.). According to the Fearon's criteria, weight loss (WL) and BMI were sufficient to classify patients as cachectic in this cohort. Body weight, BMI, BIA and serum C-reactive protein (CRP) were measured in all patients. Standardized phase angle (SPhA) was calculated according to literature evidences. Handgrip muscle strength (HGS) was also assessed in 20 patients. As appropriated, statistical analysis was performed.

**Results:** Pre-cachexia was present in 6 patients (14.3 %), cachexia in 15 patients (35.7 %), while 21 (50.0 %) patients

did not match either criteria. BMI was decreased in cachectic patients with respect to no pre-cachectic/cachectic ( $p < 0.001$ ). Fat-free mass (FFM) and fat-free mass index (FFMI) were decreased in cachectic vs pre-cachectic and no pre-cachectic/cachectic patients ( $p = 0.005$  and  $p < 0.001$ , respectively). HGS was negatively correlated with age ( $r = -0.739$ ;  $p < 0.001$ ) and CRP ( $r = -0.686$ ,  $p < 0.01$ ), while it was positively correlated with FFM ( $r = 0.516$ ,  $p < 0.05$ ) and SPhA ( $r = 0.468$ ,  $p < 0.05$ ). PA was inversely correlated with age ( $r = -0.436$ ;  $p < 0.01$ ).

**Conclusions:** This study suggests that pre-cachexia and cachexia are highly prevalent in pre-surgical cancer patients. Diagnostic criteria, for pre-cachexia and cachexia allow to identify patients with decreased FFM and FFMI. Changes in PA and its standardized value (SPhA) did not correlate with stages of cachexia, whereas a significant association was found with functional assessment.

3–35

### Establishment of novel animal models of cancer cachexia by implantation of human gastric cancer cell lines

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**Background and Aims:** Cancer cachexia (CC), a syndrome characterized by anorexia and body weight loss due to low fat-free mass levels including reduced musculature, markedly worsens patient quality of life. Although gastric cancer patients have the highest incidence of cachexia, few experimental models for the study of gastric CC have been established. The aims of the present study are to establish experimental CC models by implantation of human gastric cancer cell.

**Methods:** Novel two cell lines (MKN45clone85 and 85As2) were originally established from human gastric cancer cell line

MKN-45 by repeated implantation to mice followed by sorting of the clones causing significant weight loss in mice. To establish CC models, both of cells were subcutaneously inoculated into nude rats. We also extensively evaluated the effects of oral administration of rikkunshito, a traditional Japanese medicine, for 7 days after the onset of CC in the model.

**Results:** Both CC models showed marked weight loss, anorexia, low fat-free mass reduced muscle strength and musculature with increased mRNA expression of atrogene and increased plasma inflammatory markers. CC developed earlier and was more severe in rats implanted with 85As2 than in those implanted with MKN45clone85. Leukemia inhibitory factor (LIF), a known cachectic factor, was increased in the model and also detected in the cell culture supernatant. Surgical removal of the tumor not only abolished cachexia symptoms but also reduced both plasma LIF levels to below detectable limits and mRNA of atrogene expression to control levels. Rikkunshito significantly ameliorated severe cachexia symptoms in the 85As2-induced CC model.

**Conclusions:** We established novel CC models by implantation with novel two cell lines. LIF is more likely to participate in the CC model as a candidate cachectic factor. These models are useful for the study of cancer cachexia including the pathogenesis and potential therapies.

### 3–36

#### MT-102, a new Anabolic Catabolic Transforming Agent improves heart function in a rat model of cancer cachexia

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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 the epicardium with marked diminution and therefore loss of epicardial fat mass is also described.

Young male Wistar Han rats were intra-peritoneally inoculated with 10<sup>8</sup> Yoshida AH-130 hepatoma cells. Animals were treated once a day with 0.3 or 3.0 mg/kg/d MT-102 or placebo. Food intake, locomotor activity over a period of 24 h and

echocardiography of the heart were determined 1 day before tumor-inoculation and on day 11 of the 16-day protocol. Body weight and body composition were analysed per NMR-scan 1 day before tumor-inoculation and after sacrifice.

Heart weight was significantly increased in the group treated with 3.0 mg/kg/d MT-102 compared to placebo (573±32 mg vs. placebo 506±8 mg;  $p<0.001$ ). The heart rate was not significantly affected by 3.0 mg/kg/d MT-102 (326±12 bpm), or 0.3 mg/kg/d 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 significantly improved by high dose MT-102, as was 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/d MT-102: 6.40±0.12 mm, 3.0 mg/kg/d MT-102: 6.27±0.14 mm vs. placebo 5.71±0.11 mm;  $p<0.05$ ), but was close to the sham group level (6.39±0.08 mm). Left ventricular end-diastolic volume was also significantly increased by 3.0 mg/kg/d 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).

A daily dose of 3.0 mg/kg MT-102 reversed impaired heart function and stopped the cardiac wasting seen in placebo animals in this animal model of cancer cachexia. We conclude MT-102 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.

### 3–37

#### Experimental cancer cachexia in Sorafenib-treated tumor-bearing mice

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**Background and Aims:** During the last decades several experimental models were established to study cancer cachexia. However, tumor-injected animals as substitute for cancer patients have a major drawback in the omission of anti-tumor treatments, that frequently complicates patient management. Such treatments might improve, do not affect or even worsen cachexia, according to their effectiveness in getting rid of the tumor or to secondary adverse effects. Aim of the present study was to characterize the cachectic phenotype of three

commonly used experimental tumors in mice treated with Sorafenib (Sor), a multi-kinase inhibitor which targets receptor tyrosine and serine/threonine kinases involved in tumor progression and angiogenesis.

**Methods:** C57BL6 or Balb/c mice were divided into controls (C) or tumor bearers (TB). These latter were injected with B16, or LLC, or C26 cells. Both C and TB were then divided into treated (Sor, 90 mg/kg starting from day 4 of tumor growth) or untreated (vehicle alone). Locomotor activity was evaluated by Actitrack. The animals were sacrificed 2 weeks after transplantation, tumor mass was measured and the occurrence of cachexia evaluated (body and tissue weight).

**Results:** Sor administration nearly abrogated B16 growth, slightly reduced LLC tumor mass and had negligible effects on C26 growth. The assumption that smaller tumors mean less cachexia partially proved wrong. Indeed, despite a very residual tumor, Sor-treated B16 hosts showed several cachexia hallmarks, although less marked than untreated tumor bearers, while the small reduction of LLC mass was associated with significant prevention of cachexia. In C26 hosts, Sor significantly improved tumor-induced muscle wasting regardless of the ineffectiveness on tumor mass. Sor also reduced ERK and STAT3 hyperactivation in the C26 host muscle.

**Conclusions:** The adoption of anti-tumor treatment in experimental models currently used for the study of cancer cachexia increases the complexity of the models while supplying a more reliable tool for translational research.

3–38

### Radiotherapy-induced toxicity and impaired cardiac muscle function in head and neck cancer patients

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**Background:** In cancer patients, reduced skeletal muscle mass significantly contributes to increased chemotherapy-associated toxicity. Muscularity is reliably assessed through CT scanner-derived slices obtained at L3 level. However, this assessment technique has limitations, including impossibility to prescribe CT scan if no oncological decision has to be taken. We previously showed that cardiac muscle mass as measured by echocardiography reflects nutritional status and correlates with whole body lean body mass as measured by DXA. We

therefore aimed at investigating whether low cardiac muscle mass and function at baseline predicts the onset of toxicity during radiotherapy in head & neck (H&N) cancer patients.

**Materials & Methods:** Adult H&N cancer patients referring to our Department for radiotherapy were considered for the study. After providing written consent, patients' cardiac muscle mass ( $\text{g}/\text{m}^2$ ) was measured by echocardiography, using the Devereaux regression formula and normalized by body surface. Ejection fraction (%) was also measured. Then patients started radiotherapy, during which the possible onset of toxicities (i.e., skin, oral mucosa, esophageal, salivary glands, kidney) was clinically monitored, according to standard criteria. Data have been statistically analyzed and are reported as mean  $\pm$  SD.

**Results:** As of November 2013, 24 patients completed the study (M:F=16:8; age:  $58.6 \pm 14.0$  years). At baseline, mean body weight was  $72.4 \pm 16.0$  kg, BMI was  $24.9 \pm 3.8$  and cardiac muscle mass was  $99.4 \pm 25.3 \text{ g}/\text{m}^2$ . During radiotherapy, 8 patients developed at least grade 2–3 toxicity. Cardiac mass was not different among patients experiencing vs non-experiencing toxicity. Among patients with low ejection fraction ( $\leq 50\%$ ;  $n=6$ ), grade 2–3 toxicity was reported by 4 patients (66%), whereas by only 22% of those patients with baseline ejection fraction  $>50\%$  ( $n=18$ ) ( $p=0.134$ ).

**Conclusions:** Our study shows that low cardiac muscle function as easily assessed by echocardiography could be associated with the development of grade  $>2$  radiotherapy-induced toxicity in H&N cancer patients. If extension of the numerosity of the sample will yield to statistical relevance, this cardiac variable could be used to determine impaired muscularity.

3–39

### Renal function is impaired in cancer cachexia

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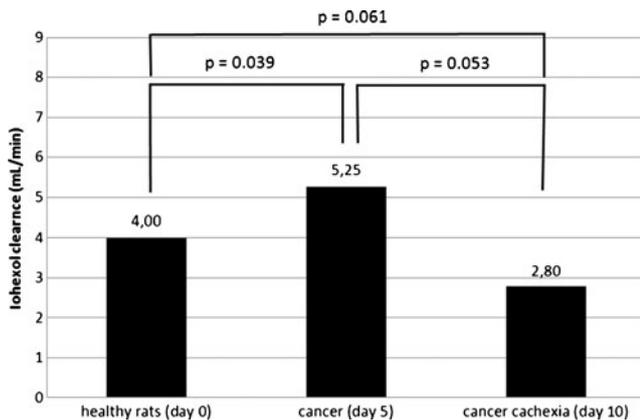
**Introduction:** In cancer cachexia, renal function may deteriorate over time but little is known on time course and mechanisms. We aimed to assess renal function in rats prior to and after induction of cancer cachexia.

**Methods:** Ten Wistar Han rats were included into the study. Renal function was measured with clearance of iohexol, a marker of glomerular filtration rate (GFR). Iohexol was administered intravenously and blood samples (dried blood spots) were taken 20 min, 30 min and 60 min after

application. Concentration of iohexol was measured and clearance of iohexol, which equals to GFR, was calculated. GFR was measured in rats at baseline, 5 days after tumor inoculation (cancer) and 10 days after tumor inoculation (cancer cachexia). Paired sample *t*-test was used to compare GFR.

**Results:** Seven rats completed the study. Renal function significantly increased from day 0 to day 5 (4.00 mL/min vs. 5.26 mL/min;  $p=0.039$ ) and decreased from day 5 to day 10 (5.26 mL/min to 2.80 mL/min;  $p=0.053$ ). The decrease between day 0 and day 10 was of borderline significance ( $p=0.061$ ).

**Figure 1:** Renal function in healthy rats, rats with cancer and rats with cancer cachexia



**Conclusion:** Renal function changes after cancer induction and deteriorates at stage of cancer cachexia.

3–40

### Autophagy contributes to muscle wasting in cancer cachexia

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**Background and Aims:** Cancer cachexia is a multifactorial syndrome characterized by anorexia, weight loss and muscle wasting that significantly impairs patients' quality of life, survival and tolerance to anti-neoplastic treatments. The mechanisms underlying muscle wasting are still unclear, although the ubiquitin-proteasome system has been involved in the degradation of bulk myofibrillar proteins. Recently also

autophagic degradation has been proposed to play a role in the onset of muscle wasting in cancer cachexia

Aim of this study has been to evaluate if autophagy is induced in the skeletal muscle of cancer patients.

**Methods:** Upon written informed consent, cancer patients and controls (patients with non-neoplastic diseases) were consecutively enrolled among those undergoing abdominal surgery. Biopsy specimens were obtained from rectus abdominis muscle during the initial phase of the operation and immediately frozen. The expression of autophagic markers has been evaluated using real-time PCR and western blotting. Statistical analysis was performed by using nonparametric tests.

**Results:** Beclin-1 protein levels were significantly increased in the skeletal muscle of cancer patients with body weight loss >5 %, suggesting an induction of the autophagic pathway. In addition, increased LC3B-II protein levels were observed, suggesting autophagosomes formation. Conversely, p62 protein levels were increased in all patients, independently of body weight loss. This result could reflect impaired autophagosome clearance, possibly due to exhaustion of the lysosomal degradative capacity. At the gene level, Beclin-1 and p-62 mRNAs were not modulated, while LC3B mRNA levels significantly increased in cancer patients with body weight loss >5 %.

**Conclusions:** Results obtained suggest that autophagy contributes to the complicated network that leads to muscle wasting in cancer patients. Further studies will be necessary to better understand the role of autophagy in the pathogenesis of cancer cachexia and to identify therapeutic approaches aimed to interfere with this proteolytic pathway.

Z. Aversa and F. Pin equally contributed.

3–41

### Psoas major muscle evaluated on CTscan is a useful prognostic factor in advanced cancer patients

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**Background and Aims:** We previously reported that the area of the psoas major muscle (APMM) obtained from the contour outline as measured by computed tomography (CT) is a quick and simple tool to estimate muscle depletion in advanced cancer patients. Here we evaluated the relationship between depletion of APMM and prognosis.

**Methods:** A retrospective analysis was performed in 20 cancer patients with colorectal malignancies (10 males; 66.9±11.2 years old) who underwent abdominal CT scans at initial

diagnosis in non-cachectic status and at follow-up in severe cachexia. Axial CT image at the top level of the iliac crest was obtained. APMM was measured by tracing the contour of the muscle using image analysis software. Decrease of APMM was expressed as percentage of initial CT image. Nutritional parameters body weight, albumin were also measured.

**Results:** Mean APMM decreased by  $71.3 \pm 12.4$  % (from  $16.7 \pm 5.5$  cm<sup>2</sup> to  $11.7 \pm 4.1$  cm<sup>2</sup>). Mean prognosis was  $31.8 \pm 14.1$  days (from date of last CT to date of death). There was a positive correlation ( $r=0.631$ ,  $p=0.003$ ) between prognosis and decrease of APMM, but not body weight and albumin.

**Conclusion:** A simple index, APMM obtained from routine axial CT imaging, is useful for evaluating muscle depletion and nutritional status in cachectic cancer patients, and may be useful as a prognosis factor.

3–42

#### A new genetic model for cancer cachexia - the ASV-B mouse: modulation by HIF-1 $\alpha$ depletion

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The ASV-B mouse has been widely studied as a transgenic hepatocellular carcinoma (HCC) model, in which the SV40 large T Antigen (SV40IT) is used as an oncogene. Male mice develop large tumours that display strong macro- and microscopical resemblance with human HCC. HIF-1 $\alpha$  is a known modulator of cancer growth and here we investigated the usefulness of the ASV-B mouse for cachexia research and the effects of hepatocellular depletion of HIF-1 $\alpha$ . Body weight and body composition of both mouse strains were monitored weekly during week 9–17 of life. Wild-type controls were assessed in week 17 only. The enzyme activities of the proteasome system, caspase 3 and caspase 6 were assessed in the heart and gastrocnemius muscle.

No difference was seen in body weight, fat mass and lean body mass were similar in ASV-B and ASV-B/HIF-1 $\alpha$  knock-out (KO) at baseline (week 9). Body weight of all strains was similar at week 17 (ASV-B:  $27.2 \pm 1.6$  g, KO:  $29.3 \pm 1.5$  g, wt control:  $29.3 \pm 1.0$  g). After removal of the liver the ASV-B and KO strains displayed a considerably reduced body weight ( $19.4 \pm 0.8$  g and  $20.9 \pm 0.8$  g, respectively) compared to wt controls ( $27.6 \pm 1.0$  g, both  $p < 0.05$ ). Lean body mass was reduced in ASV-B ( $14.0 \pm 0.6$  g) compared to baseline ( $19.6 \pm 0.7$  g,  $p < 0.01$ ) and WT controls ( $22.3 \pm 1.0$  g,  $p < 0.05$ ) after removal of the liver. Similarly, the KO strain's lean mass was reduced ( $16.3 \pm 0.7$  g) compared to baseline ( $20.7 \pm 1.4$  g,  $p < 0.05$ ) and WT controls ( $p < 0.05$ ). The reduction of fat mass was less pronounced, ASV-B (baseline:  $2.49 \pm 0.12$  g vs  $1.87 \pm 0.14$  g,  $p < 0.05$ ), KO (baseline:  $2.57 \pm 0.15$  g vs  $2.18 \pm 0.12$  g,  $p = 0.067$ ). Only in the ASV-B group fat mass was reduced compared to WT controls ( $2.82 \pm 0.23$  g,  $p < 0.05$ ). The activity of caspase 3 and 6 were not changed in the heart and M. gastrocnemius of the ASV-B and KO groups compared to WT controls. Proteasome activity was up-regulated to a similar extent in the hearts of ASV-B and KO mice compared to WT controls. The induction of proteasome activity was less pronounced in the M. gastrocnemius. In conclusion the ASV-B mouse can be used as a cachexia model, particularly if the wasting process should be independent of caspases (i.e. apoptosis) in myocytes. Hepatocellular depletion of HIF-1 $\alpha$  seems to have a fat mass protective effect that need to be studied further, but has no effect on lean mass or proteasome activity.

4–13

#### Preclinical investigation of the HDAC inhibitor AR-42 for the treatment of cancer-induced cachexia

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Cachexia is characterized by extreme loss of skeletal muscle mass and adipose tissue that cannot be reversed by nutritional support, and leads to pronounced weight loss and weakness that contributes significantly to morbidity and mortality. Cachexia occurs in more than 50 % of cancer patients, and is particularly prevalent in those suffering from pancreatic, gastric, or esophageal cancer. Cachectic cancer patients are often weak and fatigued, respond poorly to therapy, and have a lower tolerance to therapy and surgery. Thus, the development of effective therapies for cancer cachexia, which could provide tangible clinical benefits to patients, is clearly

warranted. AR-42 is a novel class I/IIb histone deacetylase (HDAC) inhibitor that was developed in our laboratory and is currently in Phase I/II trials in both hematological malignancies and solid tumors at The Ohio State University James Cancer Hospital. Here, we report the anti-cachectic activity of AR-42 in two murine models of cancer cachexia. In the colon-26(C-26) adenocarcinoma model, oral AR-42 attenuated cachexia-induced losses of skeletal muscle mass, adipose tissue, and body weight, with minimal effects on C-26 tumor growth, and prolonged survival time relative to mice treated with vehicle or other HDAC inhibitors (vorinostat and romidepsin). Metabolomic and gene expression analyses revealed that these anti-cachectic effects of AR-42 were associated with its ability to maintain metabolic and gene expression profiles in skeletal muscle comparable to those in non-cachectic muscle from tumor-free mice. Importantly, this AR-42-induced abrogation of cachexia and rescue of muscle weight was confirmed in the Lewis lung carcinoma (LLC) model of cancer cachexia. Together, these results support further evaluation of AR-42 as a potential treatment for cancer cachexia.

4–14

#### Complete reversal on muscle wasting in an animal model of cancer cachexia: additive effects of myostatin inhibition and beta-2 agonist treatment

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**Background and Aims:** Formoterol is a highly potent  $\beta_2$ -adrenoceptor-selective agonist which is a muscle growth promoter in many animal species, resulting in skeletal muscle hypertrophy. The inhibition of myostatin by soluble ActRIIB prevents and partially reverses skeletal muscle loss and prolongs the survival of tumor-bearing animals. The aim of the present investigation is to evaluate the effects of a combination of the soluble receptor antagonist of myostatin and the  $\beta_2$ -agonist formoterol in the cachectic Lewis lung carcinoma model (LLC).

**Methods:** The study presented involved mice bearing the LLC treated with either the beta-2 agonist formoterol, or the soluble ActRIIB or with the combination of both.

**Results:** The combination of formoterol and soluble ActRIIB is extremely effective in completely reversing muscle wasting associated with experimental cancer cachexia in mice. Muscle weights from tumour-bearing animals are completely recovered following treatment and this is also reflected in the measured grip strength. This combination increases food intake both in control and tumour-bearing animals. The double treatment also prolongs survival significantly without affecting the weight and growth of the primary tumour. However, it significantly reduces the number of metastasis. Concerning the mechanisms for the preservation of muscle mass during cachexia, the effects of formoterol and soluble ActRIIB seem to be additive, since formoterol reduces the rate of protein degradation (as measured in vitro as tyrosine release, using incubated isolated individual muscles) while soluble ActRIIB clearly only affects protein synthesis (as measured in vivo using tritiated phenylalanine). Formoterol also increases the rate of protein synthesis and this seems to be favoured by the presence of soluble ActRIIB.

**Conclusion:** Combining formoterol and the soluble ActRIIB seems to be a very promising treatment for experimental cancer cachexia. Further studies in humans are necessary and may lead to one of the most effective treatment for muscle wasting associated with cancer.

4–15

#### Rikkunshito, a Japanese Kampo medicine, ameliorates anorexia and muscle wasting via ghrelin secretion in mice with bleomycin-induced pulmonary fibrosis

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**Background and Aims:** Cachexia develops during the terminal stages of pulmonary fibrosis and chronic obstructive pulmonary disease, and its treatment is considered to be important in the improvement of patient outcome. Rikkunshito (RKT), a Japanese Kampo medicine, is reported to ameliorate anorexia by enhancing ghrelin secretion through serotonin 2B receptor (5-HT<sub>2B</sub>) antagonism (Gastroenterology 2008, 134:2004–2013). In this study, we examined the ameliorative effects of RKT on cachexia in mice with chronic respiratory disturbance. **Methods:** Bleomycin sulfate (BLM, 3.0 mg/kg) was endotracheally administered to 10-week-old male C57BL/6

mice. RKT (1.0 g/kg) was orally administered to the BLM mice once daily. The ghrelin receptor antagonist [(D-Lys3)-GHRP-6] or 5-HT2BR antagonist (SB215505) was intraperitoneally administered once daily. The body weight and food intake were measured daily and the skeletal muscle weight was measured on the day of autopsy. The plasma ghrelin levels were measured using ELISA. The effects of RKT were also investigated in ghrelin receptor-deficient mice.

**Results:** Along with the aggravation of pulmonary fibrosis, body weight and food intake constantly decreased in the BLM mice but this was ameliorated by RKT administration. The plasma ghrelin levels significantly increased in RKT administered mice compared with that in the pair-fed BLM mice. The decrease in muscle weight and area of muscle fibres in the BLM mice significantly improved following RKT administration. Enhanced expressions of atrogin-1 and Muscle RING-Finger Protein-1, which induce skeletal muscle atrophy, were suppressed by RKT administration. SB215505 administration also ameliorated anorexia and improved ghrelin secretion in the BLM mice. The beneficial effects of RKT were reversed by the concurrent administration of [(D-Lys3)-GHRP-6] and were lost in the ghrelin receptor-deficient mice.

**Conclusions:** These findings suggested that RKT may improve anorexia and cachexia by enhancing ghrelin secretion via 5-HT2BR antagonism.

#### 4–16

**A novel cachexia rat model with possible ghrelin resistance generated by implantation of a human gastric cancer-derived 85As2 cell line: a traditional Japanese medicine rikkunshito ameliorates cachexia symptoms by potentiation of ghrelin receptor-mediated signaling**

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**Background and Aims:** Cancer cachexia (CC) is a multifactorial syndrome characterized by anorexia, low fat-free mass (FFM) and weight loss. Gastric cancer patients have highest incidence of cachexia. Therefore, we established a novel rat CC model by implantation of the cachexia-inducible 85As2 cell

line, derived from mouse peritoneal dissemination with human gastric cancer cell line MKN-45, in order to examine the pathogenesis or potential therapeutics. With the model, we evaluated the effects of an orexygenic peptide ghrelin or a traditional Japanese medicine rikkunshito (RKT), effective for the treatment of gastrointestinal disorders, on the CC symptoms.

**Methods:** In the CC rat model with 85As2 subcutaneous inoculation, we evaluated the effects of single intraperitoneal ghrelin or oral RKT administration for 7 days after the development of CC. Further, the effects of RKT on ghrelin-induced receptor activity was measured with Ca<sup>2+</sup> imaging, Cellkey™ and receptor binding assays, using cells stably expressing ghrelin receptors.

**Results:** 85As2-inoculated rats showed significant weight loss, anorexia, low FFM (including loss of skeletal muscle). Ghrelin administration significantly increased food intake in non-tumor-bearing rats, but not in 85As2-induced CC rats. RKT administration significantly ameliorated anorexia and decreased FFM in the CC rats. RKT partly alleviated the diminished response to ghrelin. In cells expressing ghrelin receptors, RKT enhanced the ghrelin-induced increases in intracellular Ca<sup>2+</sup> concentrations ([Ca<sup>2+</sup>]<sub>i</sub>) and specific Gq protein-mediated signaling. We screened 43 compounds contained in RKT and finally found that atractylodin showed both marked increases in ghrelin receptor binding capacity and potentiated the ghrelin-induced increase in [Ca<sup>2+</sup>]<sub>i</sub> as RKT.

**Conclusions:** Our novel CC model showed ghrelin resistance. The ameliorative effects of CC symptoms by RKT may be due to enhanced ghrelin receptor activity. RKT as well as atractylodin may contribute to the development of novel therapeutics for CC patients.

#### 4–17

**A selective angiotensin-II receptor agonist for treatment of cancer cachexia**

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Cachexia is a severe complication of multiple separate illnesses and an area of significant unmet medical need. Angiotensin-II has been shown to be up-regulated in catabolic states and mediates its actions via AT1 and AT2 receptors. Angiotensin-II binds with equal affinity to both receptors, but because of the predominant expression of the AT1 receptor, angiotensin-II

predominantly elicits AT1 mediated responses. Recently, it has been described that the AT2 receptor acts in anti-proliferative, anti-inflammatory, anti-fibrotic and anti-apoptotic ways. These features are in contrast to what is usually associated with RAS activation, such as hypertension, inflammation, fibrosis and end-organ damage, all of which are mediated by the AT1 receptor. Using the Yoshida hepatoma model, the effect of a selective AT-2 agonist (C-21 at 0.2 or 1 mg/kg/d;  $n=15$  and  $n=10$ , respectively) on survival, body weight and body composition was tested in 200 g male Wistar rats vs placebo. At 0.2 mg/kg/day C-21 significantly improved survival vs placebo ( $n=44$ ): HR: 0.45 95%CI: 0.22–0.92,  $p=0.0275$ , whilst a higher dose C-21 was not effective. This is clearly due to overdosing the compound leading to toxic effects. Rats showed no difference in baseline body weight. Loss of body weight was attenuated by 0.2 mg/kg/d C-21 ( $-28\pm 10$  g) compared to untreated tumor-bearing animals ( $-50\pm 2$  g). Food intake and spontaneous activity were significantly improved compared to placebo (both  $p<0.05$ ). This indicates an improved quality of life. Heart weight was improved by C-21. The weights of the mixed fiber type muscle gastrocnemius, the fast fiber type EDL and the slow fiber type soleus were all higher compared to placebo (all  $p<0.01$ ). Interestingly, high dose also improved EDL and soleus weight (both  $p<0.05$ ). Both white and brown fat were improved by 0.2 mg/kg/d C-21 (both  $p<0.05$ ). The preservation of both muscle and fat mass as well as the improved quality of life and survival makes compound-21 an interesting compound for cancer cachexia.

#### 4–18

##### The angiotensin-II receptor 1 antagonists olmesartan and telmisartan have only limited effects in experimental cancer cachexia

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Cachexia is a severe complication of multiple separate illnesses and an area of significant unmet medical need. Angiotensin-II has been shown to be up-regulated in catechetic states. However, the ACE-inhibitor imidapril had no effect on survival in the Yoshida hepatoma model (Springer et al. Eur Heart J. 2013). Angiotensin-II predominantly elicits AT1 mediated responses, because of the predominant expression of the AT1 receptor. Activation of the renin-angiotensin system (RAS) is

usually associated with hypertension, inflammation, fibrosis and end-organ damage, all of which are mediated by the AT1 receptor. Using the Yoshida hepatoma model, the effect of the selective AT-1 antagonists olmesartan and telmisartan (both at 1 or 5 mg/kg/d) on survival, body weight and body composition was tested in 200 g male Wistar rats vs placebo. Both sartans had no significant beneficial effect on survival: 1 mg/kg/d olmesartan ( $n=12$ ; HR: 1.98; 95%CI: 0.82–4.79;  $p=0.13$ ), 5 mg/kg/d olmesartan ( $n=16$ ; HR: 2.34; 95%CI: 1.06–5.18;  $p=0.0357$ ), 1 mg/kg/d telmisartan ( $n=114$ ; HR: 0.62; 95%CI: 0.30–1.29;  $p=0.20$ ) and 5 mg/kg/d telmisartan ( $n=14$ ; HR: 4.76; 95%CI: 1.85–12.3;  $p=0.0012$ ); all vs placebo ( $n=44$ ). Rats showed no difference in baseline body weight. While weight loss was attenuated by olmesartan (1 mg/kg/d:  $-32\pm 5$  g; 5 mg/kg/d:  $-30\pm 6$  g vs placebo:  $-51\pm 2$  g, both  $p<0.05$ ), telmisartan had no effect on weight loss or body composition. Olmesartan (1 mg/kg/d:  $-17.3\pm 4.4$  g; 5 mg/kg/d:  $-9.2\pm 2.9$  g) and 5 mg/kg/d telmisartan ( $-26.4\pm 8.0$  g) reduced wasting of lean body mass compared to placebo ( $-33.1\pm 1.9$  g, all  $p<0.05$ ). Placebo-treated rats lost  $12.7\pm 0.5$  g fat mass, which was significantly reduced by 1 mg/kg/d ( $-7.3\pm 1$  g,  $p<0.001$ ) or 5 mg/kg/d olmesartan ( $-7.1\pm 0.8$  g,  $p<0.001$ ), while telmisartan had no effect on fat mass wasting. Taken together, olmesartan and telmisartan had very limited effects in the Yoshida hepatoma model and the reduction of wasting may be due to an earlier death of the animals.

#### 4–19

##### Beta-blocker for the treatment of cancer cachexia: are they all equal?

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We have previously shown that the beta-1 receptor selective beta-blocker (BB) bisoprolol reduces wasting and improves survival in the Yoshida hepatoma rat model (Springer et al. Eur Heart J. 2013). In this study the effects of several BB on survival were assessed. We used the beta-1 selective blockers metoprolol (50 or 100 mg/kg/d) and nebivolol (1 or 10 mg/kg/d), the non-selective beta-1/beta2 blockers bucindolol (0.3 or

3 mg/kg/d), carvedilol (1.2, 3 or 30 mg/kg/d) and espidolol (0.3 or 3 mg/kg/d), as well as the non-selective beta1/beta2/beta-3 blocker tertatolol (0.5 or 5 mg/kg/d). The doses for the individual BB were chosen according to publications on their use in chronic heart failure rat models. All treated groups  $n=10-25$ , placebo  $n=78$ . Change in body weight and body composition was also monitored. All hazard ratios (HR) are compared to placebo.

#### Beta-1 selective

50 mg/kg/d metoprolol: HR: 0.96 95%CI: 0.48–1.93  $p=0.91$   
 100 mg/kg/d metoprolol: HR: 0.65 95%CI: 0.32–1.29  $p=0.22$   
 1 mg/kg/d nebivolol: HR: 1.19 95%CI: 0.56–2.54  $p=0.66$   
 10 mg/kg/d nebivolol: HR: 24.24 95%CI: 8.53–68.88  
 $p<0.0001$

#### Beta-1/beta-2 non-selective

0.3 mg/kg/d bucindolol: HR: 0.50 95%CI: 0.22–1.13  $p=0.095$   
 3 mg/kg/d bucindolol: HR: 0.90 95%CI: 0.38–1.35  $p=0.80$   
 1.2 mg/kg/d carvedilol: HR: 5.42 95%CI: 1.62–18.17  $p=0.0061$   
 3 mg/kg/d carvedilol: HR: 2.48 95%CI: 0.95–6.21  $p=0.064$   
 30 mg/kg/d carvedilol: HR: 0.91 95%CI: 0.40–2.10  $p=0.82$   
 0.3 mg/kg/d espidolol: HR: 0.51 95%CI: 0.26–1.00  $p=0.051$   
 3 mg/kg/d espidolol: HR: 0.29 95%CI: 0.16–0.51  $p<0.0001$

#### Beta-1/beta-2/beta-3 non-selective

0.5 mg/kg/d tertatolol: HR: 0.70 95%CI: 0.35–1.41  $p=0.32$   
 5 mg/kg/d tertatolol: HR: 4.63 95%CI: 1.89–11.35  $p=0.0008$

Our results demonstrate that 3 mg/kg/d espidolol had a superior effect on survival in the Yoshida hepatoma model compared to all other BBs in the doses tested. Moreover, 3 mg/kg/d espidolol ( $-1\pm 13$  g) showed the greatest wasting reduction of all BBs. In conclusion, BBs have differential effects on survival in cancer cachexia and therefore more studies regarding their properties are merited.

## 4–20

### Febuxostat in a rat model of cancer cachexia

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**Background:** We have previously shown that the activity of the xanthine oxidase is induced in cancer cachexia and that its inhibition by allopurinol or oxypurinol improves survival and reduces wasting in the Yoshida hepatoma cancer cachexia model (Springer et al. Int J Cancer 2012). Here we tested the effects of the second generation xanthine oxidase inhibitor febuxostat at 5 mg/kg/d ( $n=16$ ) compared to placebo ( $n=41$ ) in the same model.

Wistar rats (approx. 200 g) were treated daily with febuxostat or placebo for 17 days. Weight change, quality of life and body composition were analysed. After sacrifice the proteasome activity in the gastrocnemius muscle was investigated. Muscle specific proteins involved in metabolism were analysed by Western blots.

**Results:** Treatment of the tumor-bearing rats with febuxostat led to a significantly improved survival compared to placebo (HR: 0.44, 95%CI: 0.21–0.91,  $p=0.0272$ ). Loss of body weight was reduced ( $-26.3\pm 12.4$  g) compared to placebo ( $-51.1\pm 1.9$  g,  $p=0.0041$ ). Wasting of lean mass was attenuated ( $-12.7\pm 10.8$  g) vs placebo ( $-31.9\pm 2.1$  g,  $p=0.0119$ ), while the effects on fat mass did not reach statistical significance ( $-10.1\pm 1.6$  and  $-12.5\pm 0.5$  for febuxostat and placebo, respectively,  $p=0.07$ ). No effect on locomotor activity and food intake were observed. While we did not see an effect of febuxostat on the proteasome activity at the end of the study, the pAkt/Akt ratio was improved by febuxostat ( $0.94\pm 0.08$ ) vs placebo ( $0.41\pm 0.05$ ,  $p=0.0004$ ) suggesting an increase in protein synthesis.

**Conclusion:** Febuxostat improved survival of tumor-bearing rats and attenuated cachexia progression.

## 4–21

### Pharmacokinetics of drugs in cachectic rats

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**Background and Aims:** Drug metabolism can be changed in cancer and cachexia. Midazolam and propranolol are extensively metabolized by liver, so their pharmacokinetics is a marker of liver metabolism. We aimed to determine changes in pharmacokinetics of midazolam and propranolol in rats with cancer and cancer cachexia in order to assess the influence of these two conditions on drug liver metabolism.

**Methods:** Eight male Wistar rats were included into the study. After a single oral dose of midazolam and propranolol,

multiple dried blood samples were obtained within 2 hours post drug application to obtain pharmacokinetic profiles of both drugs. After the first sampling (day 0), rats were injected with tumor cells. Drug application and sampling was repeated at cancer (day 5), and cancer cachexia (day 10) stage. The area under concentration time curve (AUC) of midazolam and propranolol was calculated for day 0, day 5 and day 10 and compared with paired sample *t*-test. Weight, fat mass and lean mass were measured on day 0 and on day 10 (after the removal of the tumor) and were correlated with AUC.

**Results:** AUC of midazolam decreased from day 0 to day 5 (5,179 to 1,307  $\mu\text{g}\cdot\text{min}/\text{L}$ ), but the difference was not significant. From day 5 to day 10 AUC of midazolam significantly increased (1,307 vs. 8,217  $\mu\text{g}\cdot\text{min}/\text{L}$ ,  $p=0.038$ ). Same trend was observed for propranolol (2,702 (day 0) vs. 569 (day 5) vs. 4,312  $\mu\text{g}\cdot\text{min}/\text{L}$  (day 10)), but the differences were not significant.

On day 0, AUC of midazolam and propranolol correlated with lean body mass ( $r=0.709$ ,  $p=0.049$  for midazolam;  $r=0.786$ ,  $p=0.021$  for propranolol). None of body composition parameters correlated with AUC on day 10.

**Conclusions:** Lower drug concentrations at cancer stage suggest liver enzyme induction whilst cancer cachexia leads to impaired liver metabolism and high midazolam and propranolol concentrations.

#### 4–22

##### Novel fish-derived peptides increasing glucose uptake in the skeletal muscle cells lower blood glucose in mice

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**Backgrounds and Aims:** Cachexia syndrome is characterized by insulin resistance, muscle atrophy, and anorexia. We therefore explored food-derived components, which ameliorated insulin resistance, and previously found that a fish protein improved glucose metabolism in diabetic mice fed a high-fat diet. In the present study, we searched for novel glucose metabolism-improving peptides derived from Alaska Pollack (AP) protein.

**Methods:** To screen for bioactive peptides lowering blood glucose, we performed intraperitoneal insulin tolerance test (IPITT) in mice. To measure glucose uptake, we also used differentiated skeletal muscle cell C2C12, a mouse myoblast cell line. The digest was fractionated using reverse-phase high-performance liquid chromatography.

**Results:** Tryptic digest of AP protein (100 mg/kg, i.p.) significantly lowered blood glucose levels in the IPITT. Two fractions from the digest exhibited blood glucose-lowering activity. Two candidate peptides derived from each fraction were identified using a protein sequencer and mass spectrometer. They were chemosynthesized and their bioactivities tested. A novel nonapeptide, corresponding to AP myosin (548–556), lowered blood glucose. It also enhanced 2-deoxyglucose (2-DG) uptake into C2C12. Based on the structure-activity relationships, the C-terminus was critical for glucose uptake activity. The C-terminal tripeptide was orally active and also lowered blood glucose levels in NSY mice, a type 2 diabetic model. In addition, another candidate did not change blood glucose levels itself; however, its fragment peptide, corresponding to AP actin (94–98), had blood glucose-lowering activity in the IPITT and increased 2-DG uptake into C2C12.

**Conclusions:** We identified two novel glucose metabolism-improving peptides derived from a fish protein.

#### 4–23

##### The mitochondria-localizing peptide Bendavia improves myocardial bioenergetics in heart failure and diabetes by targeting cardiolipin

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**Background:** Bendavia is a mitochondria-targeting peptide currently being tested in clinical trials for cardiovascular and renal disease. Our groups have previously shown that Bendavia reduces infarction, improves coronary “no-reflow”, and preserves mitochondrial energetics in models of acute coronary syndromes and heart failure. The objective of these studies was to determine Bendavia’s molecular mechanism of action in experimental models of heart failure and diabetes. Specifically, we tested the hypothesis that Bendavia’s mechanism involved targeting the mitochondrial phospholipid cardiolipin (CL).

**Methods:** For heart failure studies, LV tissue was obtained from 14 dogs with microembolization-induced heart failure randomized to 3 months therapy with subcutaneous injections of Bendavia (0.5 mg/kg once daily,  $n=7$ ) or saline (Control,  $n=7$ ). LV tissue from 7 normal dogs was used for comparison. Total CL and (18:2)<sub>4</sub>CL were measured using electrospray ionization mass spectroscopy and quantified in nmol/mg of non-collagen protein. For diabetes studies, LV mitochondria were obtained from 8 rats with streptozotocin-induced diabetes, or 8 diabetic rats treated with 1.5 mg/kg Bendavia, i.p for 10 days. Mitochondrial CL levels were assayed with HPLC-GC and expressed as a % of total mitochondrial CL. To confirm that Bendavia was targeting CL, we synthesized lipid vesicles of different phospholipid composition and examined the effects of fluorescent aldan-Bendavia

in these model membranes. Aladan undergoes a blue shift when it associates with a hydrophobic environment.

**Results:** In both heart failure and diabetes studies, daily treatment with Bendavia reversed the decrement in cardiac CL seen in disease. In heart failure studies, total CL was significantly decreased in LV myocardium of Control dogs compared to normal dogs ( $19.1 \pm 1.1$  vs.  $26.7 \pm 1.4$  nmol/mg,  $p < 0.05$ ) as was  $(18:2)_4\text{CL}$  ( $14.2 \pm 0.9$  vs.  $20.5 \pm 1.2$  nmol/mg,  $p < 0.05$ ). Long-term therapy with Bendavia significantly increased both total CL ( $23.6 \pm 1.1$  nmol/mg) and  $(18:2)_4\text{CL}$  ( $17.8 \pm 1.0$  nmol/mg) to near normal levels in LV myocardium of treated HF dogs compared to untreated HF Controls ( $p < 0.05$ ). In diabetic animals, 18:2 CL levels dropped from  $87.3 \pm 1.4$  to  $78.5 \pm 2.0$  % of total CL ( $P < 0.05$ ). Treatment with Bendavia restored 18:2 CL levels to  $86.3 \pm 1.5$  %, similar to healthy CL levels. Finally, our lipid vesicle studies indicated interaction of Bendavia with CL-enriched lipid vesicles. Peak aladan-Bendavia fluorescence shifted from 550 nm in phosphatidylethanolamine-enriched vesicles to 490 nm in CL-enriched vesicles. The effects of Bendavia were directly proportional to CL content in model membrane studies.

**Conclusions:** Across disease models, daily treatment with Bendavia normalized total CL and  $(18:2)_4\text{CL}$ . This peptide targets mitochondrial CL, and is a very promising therapy for the normalization of mitochondrial bioenergetics in heart failure, diabetes, and acute coronary syndromes.

## 5–10

### Why do patients decline participation in a low-risk, non-interventional cancer-associated weight loss study?

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**Background:** Fewer than 5 % of cancer patients participate in clinical research. Although this paltry rate has led to extensive research on such patient decision-making, previous studies have not sought verbatim comments in a real-time, comprehensive manner to understand patients' reasons for declining research participation.

**Patients and Methods:** The current study used a low-risk, non-interventional parent study on cancer-associated weight loss to understand patients' reasons for non-enrollment. If a patient declined, the clinical research assistant wrote down his name and verbatim reason for doing so to avoid approaching him again. These comments with accompanying demographic data on all patients are the subject of this report.

**Results:** Of 334 eligible patients, 51 (15 %) declined parent participation in this cancer-associated weight loss non-interventional trial; 3 themes emerged from these comments: 1) a repelling sense of too much institutional research; 2)

overwhelming health issues; and 3) a low likelihood of returning to the institution. In univariate and multivariate analyses, only age (older) and gender (female) were associated with non-enrollment. Interestingly, 41 patients with fatigue scores of 7 or worse and 26 with pain scores of 7 or worse did enroll; ironically, severe symptoms (fatigue and pain) and shorter survival were not associated with non-enrollment.

**Conclusions:** Patients declined research participation in this cancer-associated weight loss, non-interventional trial because they reported feeling overwhelmed by too much research and by their own health issues—but symptom severity (fatigue and pain) and survival were not associated with non-enrollment. Up front educational efforts might help cancer patients better prioritize their participation in research, and researchers should keep asking patients to participate, regardless of their baseline symptoms.

## 5–11

### Effects of acute infusion of magnesium on testosterone in older men

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**Background and Aims:** In the elderly, under-nutrition and the multiple hormonal dysregulation frequently coexist, determining an increased risk of mobility impairment and sarcopenia. Nutrition may influence a successful aging by modulating the hormonal anabolic milieu and physical performance. Magnesium, as Testosterone (T) does, affects skeletal muscle function and both factors also share similarities in age-related trajectories. Magnesium could exert a permissive role in increasing the serum concentration and the biological activity of T. However, data on this matter are limited in older population.

**Methods:** Pilot single-center, randomized, placebo-controlled, single-blind intervention study. 46 hospitalized elderly male subjects  $\geq 65$  years (21 in the treatment group), with magnesium serum levels  $< 2.5$  mg/dl, were randomly assigned to magnesium sulfate treatment (1 g/ml of ion  $\text{Mg}^{++}$  in 250 cc of normal saline solution) vs placebo (250 cc of saline solution). Serum creatinine, electrolytes, albumin, T, Insulin-like Growth Factor-1 (IGF-1), Sex Hormone Binding Globulin (SHBG) and C-reactive protein (CRP) concentrations were evaluated before and after treatment. Repeated-measures analysis of variance were used to examine and compare trends response data over time.

**Results:** As expected, magnesium sulfate administration induced a significant increase in serum magnesium levels ( $\Delta 1.28 \pm 0.61$ ) compared to placebo ( $\Delta -0.03 \pm 0.14$ ) ( $p < 0.001$ ). Total T levels remained substantially unchanged ( $\Delta 0.01 \pm 0.80$ ) in the

intervention group whereas a significantly decrease in the placebo group ( $\Delta -0.03 \pm 0.14$ ) was observed. This difference touched the statistical significance ( $p=0.1$ ). After adjustment for CRP levels at baseline the relationship between magnesium and total T was not affected. No changes in IGF-1 and SHBG concentrations after treatment were appreciated in both groups.

**Conclusions:** In this pilot study of male hospitalized elderly subjects, magnesium administration sulphate positively modulates total T levels that tend to decrease in placebo group. If confirmed in larger studies, these data may identify new strategies to counteract the catabolic milieu observed during accelerated aging.

5–12

### Disseminated intravascular coagulation in anorexia nervosa

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Anorexia nervosa (AN) is a mental health disorder typical for adolescent girls and young adults. It is potentially life-threatening syndrome often characterized by distorted body image and intense fear of gaining weight or becoming fat in spite of actual underweight. We report here a 36-year-old Japanese woman with AN accompanied by disseminated intravascular coagulation (DIC). She had been admitted to our department because of severe emaciation. In spite of our thorough management and prevention of refeeding syndrome, DIC developed 3 weeks after hospitalization. To treat her DIC with sepsis, we use anticoagulants, protease inhibitors, antithrombin, and platelet concentrate transfusion. We administered antimicrobial drugs and immunoglobulin to treat her bacterial infection. We started probiotic and prebiotic (synbiotics) treatment for bacterial translocation. We think that the prevention of sepsis due to bacterial translocation is an important aspect of care for patients with severe AN in addition to the prevention of refeeding syndrome.

5–13

### The implementation of dialectical integration psychotherapy on situational vomiting patient

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**Background and Aims:** To study the method and curative effect of Dialectical Integration Psychotherapy on situational vomiting. **Methods:** Through the implementation of Dialectical Integration Psychotherapy, such as build psychological models, clarify the target to efforts, lock the experimental subjects, to help a situational vomiting patient.

**Results:** Through three times of psychotherapy, a week later, the patient's physical symptoms have been eliminated. This situational vomiting patient was successfully cured.

**Conclusions:** Dialectical Integration Psychotherapy emphasizes the dialectical materialism, a unified methodology of various psychotherapy genres, is based on the fundamental theory of psychology, perspectives and methods, which make psychotherapy get rid of the shackles of various genres and play a role flexibility. This psychotherapy is feasible in situational vomiting.

5–14

### Functional outcomes following a short-term rehabilitation intervention in patients with cancer cachexia

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**Background and Aims:** Cachexia is an important problem in cancer management. This study aimed at determining whether cachexia is associated with rehabilitation outcomes in cancer patients.

**Methods:** We performed a retrospective analysis of clinical data of 48 cancer patients who received inpatient rehabilitation between August 2010 and August 2011 at Shizuoka Cancer Center Hospital and Keio University Hospital. We measured rehabilitation outcomes with the Eastern Cooperative Oncology Group-Performance Status (ECOG-PS), the Cancer Functional Assessment Set (cFAS) and the Functional Independence Measure (FIM). For the assessment of cachexia, we classified the participants into three groups according to the Glasgow Prognostic Score (GPS): patients with normal serum albumin ( $\geq 3.5$  g/dl) and normal CRP ( $\leq 1.0$  mg/dl) as having GPS of 0, those with low albumin ( $< 3.5$  g/dl) or elevated CRP ( $> 1.0$  mg/dl) as having GPS of 1, and both low albumin ( $< 3.5$  g/dl) and elevated CRP ( $> 1.0$  mg/dl) as having GPS of 2.

**Results:** This study included 15 females and 33 males (mean age, 71.2; range, 50–85 years; average rehabilitation duration, 15.8 days, 7–57 days). Eleven individuals (22.9 %) had normal GPS (score 0), while the remaining 37 participants (77.1 %) had

abnormal GPS (12 patients with score 1 and 25 with score 2). Although the ECOG-PS, the cFAS and the FIM increased significantly after the rehabilitation intervention in all patients with GPS score of 0 to 2, those at baseline were significantly lower in patients with GPS of 2 when compared with those with GPS of 0.

**Conclusions:** Cancer cachexia evaluated with the GPS was associated with rehabilitation outcomes in cancer patients. However, this study suggested that cancer patients might be able to obtain better functional outcomes following a short-term inpatient rehabilitation intervention regardless of the GPS score.

## 5–15

### **Enobosarm, a selective androgen receptor modulator (SARM), increases lean body mass (LBM) in advanced non-small cell lung cancer patients in two pivotal, international Phase 3 trials**

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**Background:** Cancer-induced muscle wasting begins early in the course of malignancy. Greater than 50 % of NSCLC patients have muscle wasting at diagnosis, increasing to >80 % prior to death. Muscle wasting is a selective and progressive cancer related symptom that is a consequence of a reduced rate of anabolic activity and increased catabolic activity. Enobosarm is a first in class nonsteroidal oral SARM. We report herein the top line results for two Phase 3 clinical trials conducted for the prevention and treatment of muscle wasting in patients with advanced NSCLC.

**Materials and Methods:** Six hundred forty-one patients, with Stage III or IV NSCLC, were randomized into one of two trials at initiation of first-line chemotherapy (platinum + taxane, G300504; platinum + non-taxane, G300505) plus add on, consisting of either enobosarm 3 mg or placebo for 5 months. Patients were males and postmenopausal females  $\geq 30$  y, and ECOG  $\leq 1$ . Coprimary endpoints (Day 84) are physical function response assessed by stair climb power (SCP) and lean body mass (LBM) as measured by DXA.

**Results:** Mixed model repeated measures (MMRM) slope analysis of coprimary endpoints:

	MMRM Slopes – G300504		<i>p</i> -value	MMRM Slopes – G300505		<i>p</i> -value
	Placebo ( <i>n</i> =161)	Enobosarm ( <i>n</i> =160)		Placebo ( <i>n</i> =161)	Enobosarm ( <i>n</i> =159)	
SCP (Day 84)	-0.0773	+0.0443	<b>0.0147</b>	-0.0245	-0.0305	0.8877
LBM (Day 84)	-0.011	+0.0049	<b>0.0002</b>	-0.0044	+0.0056	<b>0.0111</b>
SCP (Day 147)	-0.0794	-0.0191	<b>0.0492</b>	-0.0094	+0.0052	0.6138
LBM (Day 147)	-0.0104	+0.0014	<b>&lt;0.0001</b>	-0.0038	+0.0031	<b>0.0028</b>

The incidence of adverse events was similar between enobosarm and placebo subjects in both trials.

**Conclusions:** Overall, enobosarm was safe and well tolerated. Declines in both LBM and SCP were observed in the placebo group of both trials. In G300504, statistically and clinically meaningful differences between enobosarm and placebo were observed for SCP and LBM. In G300505, statistically and clinically meaningful differences between enobosarm and placebo were observed for LBM.

## 5–16

### **Long-term therapy with Bendavia (MTP-131), a novel mitochondria-targeting peptide, normalizes skeletal muscle fiber type composition in dogs with chronic heart failure**

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**Background:** Chronic heart failure (HF) is associated with a shift in skeletal muscle (SM) fiber type composition manifested by a decline in the number of aerobic, slow-twitch, fatigue-resistant type-1 fibers and an increase in the number of glycolytic, fast-twitch type-2 fibers. This fiber type shift may be responsible, in part, for the exercise intolerance that is characteristic of the HF state. Previous studies in rats with HF showed a decrease in SM citrate synthase activity and SM mitochondrial respiration without changes in SM capillary density and fiber bundle diameter. These findings are indicative of SM mitochondrial dysfunction in HF. Bendavia (MTP-131), a novel, first in class, mitochondria-targeting peptide, improves LV systolic function in dogs with chronic HF and has been shown to restore ATP synthesis in multiple organs including heart and kidney in other animal models of disease. In aging mice, Bendavia was also shown to improve SM mitochondrial ATP synthesis and phosphocreatine (PCr) to ATP ratio. This study tested the hypothesis that long-term therapy with Bendavia can reverse the maladaptive SM fiber type shift in

dogs with chronic HF possibly through restored mitochondrial oxidative capacity.

**Methods:** Studies were performed in triceps SM samples of 14 HF dogs produced by intracoronary microembolizations (LV ejection fraction ~30 %) and from 9 normal dogs. HF dogs were randomized to 3 months therapy with subcutaneous injections of Bendavia (0.5 mg/kg once daily,  $n=7$ ) or saline (Control,  $n=7$ ). SM type-1 and -2 fibers were differentiated histologically by myofibrillar adenosine triphosphatase staining. The proportion of type-1 and -2 fibers and the average cross-sectional area (CSA) of each fiber type was assessed in 5 randomly selected SM fields/dog each containing ~100 fibers.

**Results:** The proportion of SM type-1 fibers was lower and type-2 fibers higher in HF-Control compared to normal (table) leading to a significantly lower fiber type ratio. Treatment with Bendavia restored a near normal fiber type composition. There were no differences in fiber CSA among study groups (table).

**Conclusions:** Therapy with Bendavia reverses abnormalities of SM fiber type without influencing SM CSA. Reversal of this SM maladaptations following therapy with Bendavia can lead to improved exercise tolerance in HF.

#### Skeletal Muscle Fiber Type Composition

	Normal	HF-Control	HF-Bendavia
Type-1 SM Fibers (%)	32±5	23±4*	31±7†
Type-2 SM Fibers (%)	68±5	77±4*	69±7†
Type-1/Type-2 Ratio	0.47±0.04	0.30±0.07*	0.45±0.13†
Average CSA Type-1 Fibers ( $\mu\text{m}^2$ )	2996±176	2057±415	3058±354
Average CSA Type-2 Fibers ( $\mu\text{m}^2$ )	3445±240	3560±378	3495±258
CSA Type-1/CSA Type-2 Ratio	0.88±0.03	0.86±0.05	0.87±0.04

\* =  $p < 0.05$  vs. Normal; † =  $p < 0.05$  vs. HF-Control

5–17

#### Anamorelin HCl for the treatment of anorexia-cachexia in lung cancer: study design and baseline characteristics of patients in the phase III clinical trial ROMANA 2 (HT-ANAM-302)

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**Background:** Cancer anorexia-cachexia may develop in up to 80 % of advanced cancer patients, and is a

debilitating and life-threatening complication of underlying malignancy. Safe and effective treatments remain an unmet medical need. Anamorelin HCl is an orally administered ghrelin receptor agonist; it has significantly increased lean body mass (LBM) and body weight in cancer patients, and improved physical strength in Phase II studies. Anamorelin HCl is currently being evaluated in two Phase III trials enrolling patients with non-small cell lung cancer (NSCLC) and cachexia.

**Methods:** HT-ANAM-301 (NCT01387269) and HT-ANAM-302 (NCT01387282), also known as ROMANA 1 and ROMANA 2, are double-blind, placebo-controlled, randomized (2:1 anamorelin HCl vs. placebo) Phase III trials in patients with NSCLC cachexia (target of 477 patients per study). Patients receive once daily anamorelin HCl (100 mg) or placebo for 12 weeks. Eligible patients must have unresectable Stage III/IV NSCLC and cachexia (body weight loss >5 % within prior 6 months or BMI <20 kg/m<sup>2</sup>). Co-primary endpoints are change from baseline in LBM as measured by DXA scan and in muscle strength as measured by handgrip strength. Secondary endpoints include change in body weight, overall survival, and quality of life. Population pharmacokinetics is included in HT-ANAM-301. After 12 weeks of treatment, patients may continue in a separate 12-week safety extension study (HT-ANAM-303 [ROMANA 3] NCT01395914).

**Results:** Enrollment in ROMANA 2 completed in June 2013. Of the 495 randomized patients, preliminary data indicate that at baseline, 71 % had Stage IV cancer and 67 % were ECOG 1 performance status. All key baseline characteristics will be presented for ROMANA 2.

**Conclusions:** Anamorelin HCl is undergoing Phase III evaluation, where one trial has completed enrollment and the other is nearing completion. Efficacy and safety results are awaited.

5–18

#### Fast skeletal muscle troponin activators and their application to disease—clinical update

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Skeletal muscle contractility is driven by the sarcomere, the fundamental unit of skeletal muscle contraction. It is a highly ordered cytoskeletal structure composed of several key proteins. The first, skeletal muscle myosin, is the cytoskeletal motor protein that converts chemical energy into mechanical force through its interaction with a second protein, actin. A set of regulatory proteins, which includes tropomyosin and several types of troponin, make the actin-myosin interaction

dependent on changes in intracellular calcium levels. Fast skeletal muscle troponin activators slow the rate of calcium release from the regulatory troponin complex of fast skeletal muscle fibers, which sensitizes the sarcomere to calcium. The consequences for muscle function of this mechanism of action include an increase in muscle force at sub-maximal neuromuscular input, an increase in muscle power, and a decrease in muscle fatigability. The preclinical rationale for the application of fast skeletal muscle troponin activators to conditions marked by decreased skeletal muscle function will be presented as well as an update on their status in clinical development.

## 5–19

### Espindolol for the treatment and prevention of cachexia in patients with stage III/IV non-small cell lung cancer or colorectal cancer (ACT-ONE): randomised, double-blind, placebo-controlled, international multi-centre phase II study

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**Background:** Colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) have high incidences of cachexia (~28% and ~34%, respectively). There are no widely approved agents for the treatment or prevention of cachexia, which remains a major cause of morbidity and mortality in cancer patients.

**Rationale:** Neurohormonal overactivity is implicated in the genesis and progression of cachexia and  $\beta$ -blockers have been proposed as a potential therapy. Several pre-clinical and retrospective population based studies suggest that  $\beta$ -blockers may reduce metastasis, progression and mortality in patients with cancer. These effects may depend upon the receptor specificities of the  $\beta$ -blocker used and espidolol has been shown in preclinical studies using a rat cancer cachexia model to have superior effects upon cachectic signs (muscle and fat loss, physical activity, and food intake) and overall survival when compared to other  $\beta$ -blockers.

**Design:** This phase II study (EUDRACT 2010-022486-10) included patients with a confirmed diagnosis of stage III or IV NSCLC or CRC, who had received established first line chemotherapy (platinum-based for NSCLC or fluoropyrimidine-based for CRC) and who met a consensus definition of cachexia at screening (weight loss equivalent to >5% in previous 12 months or subjective weight loss and a BMI <20.0 kg/m<sup>2</sup>, with

associated symptoms and biomarkers). Patients with excessive weight loss ( $\geq 20\%$  in the previous 3 months or a BMI of less than 16 kg/m<sup>2</sup>), significant pre-existing cardiac disease or underlying factors other than cancer that could contribute to weight loss were excluded.

Patients were randomised 3:1:2 to high dose espidolol (HD = 10 mg bd) low dose espidolol (LD = 2.5 mg bd) or matching placebo (P) and treated for 16 weeks. Adverse events, vital signs, ECG and weight were recorded at every visit. Physical function tests [hand grip strength (HGS), six minute walking test (SMWT), stair climbing power (SCP), and short physical performance battery test (SPPBT)] were performed every 4 weeks and DEXA scans were obtained at baseline 8 and 16 weeks.

**Results:** 87 patients were recruited at 17 centres as summarised in the table below. Espindolol was well tolerated in the majority of patients with one patient withdrawing due to adverse events in each group (7.14% LD, 2.38% HD and 3.23% P). Dyspnoea occurred more frequently in HD vs P (8/42 vs 1/31). For the primary endpoint in the ITT population, the slope of weight change (Kg per 4 weeks) in the HD group demonstrated a positive slope of 0.42 (CI 0.20, 0.64) compared to a negative slope of -0.37 (CI -0.62, -0.11) in the P group ( $p < 0.0001$ ). Lean mass change at day 112 (Kg [IQR]) was +1.54 [0.90/2.89] and +0.54 [-0.44/1.67] for HD vs P and the corresponding fat mass change was +0.50 [-0.58/1.58] and -0.75 [-2.78/0.23]. Physical function tests were all numerically improved in HD vs P, but this was only statistically significant for HGS (-0.63 vs -3.38;  $p = 0.0012$ ).

**Conclusion:** This study provides encouraging information on the tolerability, efficacy and appropriate dosing of espidolol in the treatment and prevention of cachexia in patients with non-small cell lung cancer and colorectal cancer in stage III/IV. Data from this trial support the further investigation of espidolol for the treatment of cancer cachexia.

**Table**

Baseline characteristics of the ITT population	Low Dose 2.5 mg bd N=14	High Dose 10 mg bd N=42	Placebo N=31
Age (mean $\pm$ SD, years)	56.12 $\pm$ 12.22	59.29 $\pm$ 10.15	55.92 $\pm$ 11.00
Gender (% female/% male)	28.6 % /71.4 %	31.0 % /69.0 %	40.0 % /60.0 %
BMI (mean $\pm$ SD, kg/m <sup>2</sup> )	21.50 $\pm$ 4.21	20.12 $\pm$ 3.82	20.65 $\pm$ 2.80
Disease (% CRC/% NSCL)	50.0 % /50.0 %	28.6 % /71.4 %	32.3 % /67.7 %
Stage (% IIIA/ % IIIB/% IV)	7.1 %/7.1 % /85.7 %	7.1 %/4.8 % /88.1 %	6.5 %/29.0 % /64.5 %
ECOG (% 0/% 1/% 2)	28.6 %/ 71.4 % /0 %	35.7 %/ 54.8 % /9.5 %	45.2 %/ 38.7 % /16.1 %
Time from diagnosis (mean $\pm$ SD, years)	1.59 $\pm$ 1.48	0.95 $\pm$ 1.00	1.00 $\pm$ 1.33

### The suppression of plasma ghrelin levels is mediated by the activation of peripheral alpha2-adrenergic receptor in urocortin1-induced anorexia

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**Background and Aims:** CRF is increased in the state of cachexia and known to induce anorexia and weight loss. Intracerebroventricular injection of CRF-related peptide urocortin1 (ICV UCN) causes anorexia via reduction of gastric ghrelin secretion. We elucidated the role of the autonomic nervous system in abnormal ghrelin secretion in UCN-induced anorexia using c-fos expression as a marker of neuronal activation in brain, and surgical and pharmacological approaches.

**Methods:** Adult male rats were subjected to ICV UCN (300 pmol/rat), and the brain was processed for studying expression of c-fos by *in situ* hybridization and Fos by immunohistochemistry. Adrenergic receptor (AR) antagonists were intraperipherally or intracerebroventricularly injected, and rikkunshito (RKT), which has certain components with a potential to act as alpha2-AR antagonists, was orally administered to examine the effects on plasma acyl ghrelin levels and food intake.

**Results:** ICV UCN increases c-fos mRNA and Fos expression in the paraventricular nucleus of the hypothalamus, locus coeruleus, and others, which are known to influence sympathetic outflow. In contrast, the dorsal motor nucleus involved in gastric vagal outflow did not exhibit Fos expression. Subdiaphragmatic vagotomy did not influence UCN-induced decrease in plasma ghrelin levels. Decreased ghrelin levels and food intake were prevented by the alpha-AR antagonist. The selective alpha2-AR antagonist and RKT prevented UCN-induced inhibition of ghrelin secretion and food intake, whereas the selective alpha1-AR antagonist had no effect. In contrast, it was not modified by the ICV alpha2-AR antagonist. The food intake restoration was abolished by the coadministration of a ghrelin receptor antagonist.

**Conclusions:** In conclusion, the suppression of acyl ghrelin secretion by ICV UCN is mediated by the activation of the sympathetic efferent nerve, while the vagus does not play a role; the sympathetic component involves peripheral alpha2-AR, unlike central alpha2-AR or peripheral alpha-AR; and peripheral alpha2-AR antagonism may improve anorexia.

### Association between nesfatin-1 and anorexia nervosa

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**Background and Aims:** Anorexia nervosa (AN) is a serious disorder affecting adolescents and young adults, and it decreases the quality of life of affected individuals for prolonged periods. Despite various treatments, AN continues to be a refractory disease because of its unknown pathogenesis. The mechanisms underlying persistent anorexia are largely unknown, but some studies have suggested that the decrease in food intake in AN is because of changes in feeding regulatory peptides such as ghrelin. Although Nesfatin-1 is a recently identified satiety peptide and is involved in the physiological regulation of feeding behavior and body weight by suppressing food intake and peristalsis, few reports have described the relationship between AN and nesfatin-1. Therefore, we examined the association between nesfatin-1 and AN.

**Methods:** Fifteen women participated in this study: seven patients with AN and eight age-matched healthy controls. We examined plasma nesfatin-1, acyl ghrelin, and des-acyl ghrelin in all subjects.

**Results:** The plasma nesfatin-1 levels were significantly lower in AN group than in control group, and they positively correlated with BMI in the entire study population. Plasma acyl ghrelin and des-acyl ghrelin levels were significantly higher in AN group than in control group.

**Conclusions:** Our study indicates that plasma nesfatin-1 levels are regulated by nutrition status and response to starvation.

### Intestinal permeability during anorexia nervosa: an experimental approach in activity-based anorexia model in mice

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**Background and Aims:** Anorexia nervosa is a severe eating disorder, which pathophysiology involves complex interaction of biological, environmental and social factors. Gut-brain axis signaling is involved in regulation of feeding behavior

suggesting that gut barrier can be altered in anorexia nervosa. Thus, in this work, we aimed to evaluate the intestinal permeability in the activity-based anorexia (ABA) model in mice.

**Methods:** Male C57Bl/6 mice were placed in cages with activity wheel (ABA) or not (CT). After 5 days of acclimatization, ABA and CT mice had a progressive limited access to food from 6 h/d at day 6 to 3 h/d at day 9 and until the end of experiment. A control pair-fed group (PF) compared with ABA was also performed. Body weight and food intake were daily recorded as the wheel activity. Jejunal and colonic permeability was assessed in Ussing chambers by FITC-dextran fluxes and tight junction proteins by immunoblot and immunostaining.

**Results:** On day 17, body weight decrease was lower in the ABA and PF than in CT ( $23.6 \pm 1.6$  and  $24.7 \pm 0.7$  vs.  $16.5 \pm 1.2$ ;  $p < 0.05$ ). Food intake was also decreased in ABA ( $2.0 \pm 0.18$  vs.  $3.0 \pm 0.14$ ;  $p < 0.001$ ). Thickness of colonic muscularis layer was decreased in ABA as compared to CT ( $p < 0.05$ ). Colonic permeability was increased in ABA and PF as compared to CT ( $p < 0.05$ ). Expression of claudin-1 was decreased in ABA group vs. CT ( $p < 0.05$ ), whereas occludin expression remained unaffected. The structure of tight junctions was impaired in all groups. In the jejunum, paracellular permeability was not affected.

**Conclusion:** In the ABA mouse model of anorexia nervosa associating physical activity, reduced food intake and loss of body weight, colonic permeability was increased suggesting its contribution to altered gut-brain axis signaling in anorexia.

## 6–13

### Association between anti-aging hormone klotho, anorexia nervosa and obesity

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**Background and Aims:** Aging is associated with an increased risk for metabolic disorders and with becoming overweight and obese. Caloric reduction is the only non-pharmacologic intervention that has protective effects against aging. Aging and morbidity are thus associated with the body mass index (BMI). The klotho gene, which encodes a single-pass transmembrane protein expressed primarily in renal tubes, has been identified as a systemic anti-aging hormone. Adiponectin is a protein hormone produced almost exclusively in adipose tissue. Because adiponectin has a strong association with metabolic dysfunction and is a biomarker of

metabolic syndrome, we hypothesized that there would be a relationship between klotho and adiponectin levels. To verify the role of klotho in human metabolism in this study, we therefore examined the association between plasma klotho levels and BMI, and we compared the levels of adiponectin and its isoforms in participants on the opposite ends of the body weight continuum: AN and obesity.

**Methods:** We examined plasma klotho as well as adiponectin and its isoform levels in comparison in 11 obese patients, 12 AN patients, and 11 control participants.

**Results:** Plasma klotho levels were markedly lower in the obesity and AN groups than in the control group. Moreover, plasma klotho levels increased significantly after the recovery of BMI in AN patients. Total and high-molecular-weight adiponectin levels were significantly decreased only in obesity. There was no relationship between klotho and total adiponectin levels or klotho and respective adiponectin isoform levels in the entire study population.

**Conclusions:** These results suggest that klotho may reflect normal nutritional state, and that the decrease of klotho in AN and obesity may underlie the deteriorating processes of these disorders.

## 6–14

### Novel beef as a protein source for patients with masticatory disturbance

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**Background and Aims:** Nutritional intervention on protein intake is necessary for elderly or cancer patients during an early stage to prevent or delay the onset of hypoproteinemia, sarcopenia or cachexia. However, most of the patients have structural or functional disorders in oral cavity, which cause difficulty in eating. Thus, we modified physical properties of meat by Homogeneous Enzyme Permeation (HEP) method to provide meat as a protein source for the patients with inadequate oral intake. This study was conducted to determine nutritional value and ease of ingestion of HEP-treated beef (HEPB).

**Methods:** HEPB was compared with normally cooked beef (NB) and commercial food products for people with mastication difficulty in terms of nutritional value. HEPB and NB were examined mastication frequency for induction of first swallowing and firmness before and after mastication. Samples obtained before and after mastication were also tested for static viscoelastic properties and compression stress, as well as disintegrability in water.

**Results:** The protein content in HEPB was approximately 75 % as much as that in NB, but was 1.7 times higher than those in commercial products. Compared with NB, HEPB was shown to require less than 1/3 the number of mastications for induction of first swallowing, and to have about 1/15 the firmness. HEP treatment reduced the viscous modulus to less than 1/20 of that of NB. The stress increased abruptly with compression on post-mastication samples of NB, but increased less on HEPB. The disintegration test revealed little residue from HEPB.

**Conclusions:** HEPB was shown to have higher protein content than commercial products, sufficient softness, good deformability and quick water dispersibility, while retaining the same shape as NB.

## 6–15

### Association between body weight, intelligence quotient and cognitive functions in anorexia nervosa

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**Background and Aims:** Anorexia nervosa (AN) is a serious disorder affecting adolescents and young adults that decreases quality of life over a long period. Despite various treatments, AN continues to be a refractory disease because of its unknown pathogenesis. AN treatment difficulties usually increase in proportion to the severity of emaciation. The difficulties of patients with severe AN include impaired cognition and increased memory loss. The aim of this study more comprehensively investigated the overall IQ scores and cognitive functions of patients with AN who were more severely emaciated than the participants studied in previous research and compared them before and after weight gain.

**Methods:** We administered the Wechsler Adult Intelligence Scale (WAIS-III), Third Edition and the Eating Disorder Inventory–II (EDI-II) in 14 AN patients and 10 control participants.

**Results:** In the AN group, overall IQ scores showed borderline intelligence (e.g., full-scale IQ 75.86  $\pm$  1.79,  $P < 0.01$ ); the scores were significantly lower than those in the comparison group. There were negative correlations between lower IQs and higher Eating Disorder Inventory–II scores. After the weight restoration, the IQ scores of subjects with AN with regard to the visuospatial scales were significantly higher than before ( $P < 0.01$ ); however, the auditory cognitive scores were unchanged.

**Conclusions:** Low IQ scores could be connected to the psychological and behavioral traits in patients with AN. Therefore, the WAIS-III should be administered to determine the intelligence quotient and cognitive functions of patients with AN-R.

## 6–16

### Correlation between protein intake and sarcopenia in older adults with hip fracture

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**Background and Aims:** An adequate intake of dietary proteins is necessary for muscle trophism homeostasis. The aim of the present study was to determine protein and energy intake as well as the prevalence of sarcopenia among older patients admitted to the Orthopedic and Trauma Surgery ward for traumatic hip fracture. We investigated the association between daily protein intake and muscle mass.

**Methods:** This is an observational study involving individuals aged  $> 65$  years with hip fracture. Muscle mass was estimated by bioimpedance analysis (BIA) within 24 h from admission. Daily protein and energy intake was collected via three-day dietary record. Dietary intake data were coded and energy and macronutrient intakes calculated using a food-calculation system (MetaDieta, Italy).

**Results:** Among 62 patients (mean age  $84.6 \pm 7.6$  years, 84 % women), sarcopenia was present in 11 (7 %); men were more likely to be sarcopenic than women (40 % vs. 5.8 %,  $p < 0.001$ ). The mean baseline habitual protein intake was  $0.8 \pm 0.27$  g/kg body weight/day, without significant difference between males and females. More than 70 % of subjects showed a protein intake below 1.0 g/kg body weight/day. Patients with the lower protein intake showed the higher prevalence of sarcopenia relative to those reporting the higher intake (11.8 % vs. 8.2 %,  $p < 0.001$ ). A positive correlation between daily protein intake, leucine intake and skeletal muscle mass was observed, which reached the statistical significance in men.

**Conclusions:** A low intake of protein and essential amino acids is associated with sarcopenia in older patients with hip fracture. An increase in protein intake should be recommended to older adults, especially in men.

## 6–17

### Gender differences in the mechanisms of action of rikkunshito on the improvement in food intake after exposure to a novel environmental stress in aged mice

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**Background and Aim:** In an aging society, late-life anxiety disorders increase following bereavement and isolation from society. Stress and depression are also considered to be involved in the onset and progression of functional dyspepsia. Rikkunshito, a Kampo medicine, is known to alleviate loss of appetite via ghrelin signaling; however, gender differences in its mechanisms of action remain unknown. This study examined the effect of rikkunshito on food intake of aged male or female mice after exposure to a novel environmental stress to determine the distinct gender-specific mechanisms of action of rikkunshito.

**Methods:** Aged male and female mice (age 79–80 weeks) were housed in groups and acclimated to their environment. They were fasted for 24 h and moved to isolation cages (1 per cage) for exposure to a novel environmental stress. We measured food intake, plasma ghrelin levels, and expression of appetite-related genes in the hypothalamus after isolation. Rikkunshito was orally administered at a dose of 1,000 mg/kg at the onset of isolation.

**Results:** Aged male mice exhibited a significant decrease in food intake after stress exposure. However, food intake was not decreased to the same extent in aged female mice. Plasma acylated ghrelin levels were significantly decreased in aged male mice after stress exposure, but aged female mice showed no such difference. Administration of rikkunshito significantly increased food intake after isolation in both aged male and female mice. Furthermore, administration of rikkunshito tended to increase plasma acylated ghrelin levels and suppressed the decrease in the hypothalamic expression of neuropeptide Y mRNA in aged male mice; however, no effect was observed in aged female mice.

**Conclusion:** Rikkunshito suppressed the decrease in food intake during stress in aged male mice via an increase in ghrelin signaling. However, it may operate by a different mechanism in aged female mice.

6–18

### Dietary treatment of weight loss in patients with advanced cancer and cachexia: a systematic literature review

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**Background:** Patients with progressive cancer often suffer from weight loss. This might be caused by inadequate energy intake alone occurring in patients having secondary nutrition impact symptoms, or be a consequence of cancer cachexia, having both inadequate intake and metabolic and endocrine abnormalities. Basic guidelines for dietary treatment of weight loss in patients with incurable cancer are not available. The aim in this review is to assess the effect of dietary counseling on weight loss and energy intake in patients with advanced incurable cancer and investigate whether it is more effective to give dietary counseling for patients in the stage of pre-cachexia or cachexia than in refractory cachexia.

**Methods:** A systematic literature search in of PubMed, Embase and the Cochrane Central register of controlled trials database was performed using both text words and MeSH/EMTREE terms, applying classical standard methodology of abstract and paper selection, data extraction based on predefined criteria, handsearch and interpretation of a research team.

**Results:** Five trials were retrieved, of which three were randomized. Two out of five studies showed less weight loss in patients treated with dietary counseling (+1 % weight gain vs. –1.5 % weight loss,  $p=0.03$  and 1.4 kg vs. –2 kg,  $p<0.05$ ), two studies presented positive effect on energy intake (92 % of total caloric need vs. 73 %,  $p<0.01$  and 1,865±317 kcal vs. 1,556±497 kcal, ns). Two out of five studies were small ( $N<50$ ) and population and effect sizes poorly described in most of the trials.

**Conclusion:** Based on this systematic literature review it is not possible to conclude that dietary advice given to all advanced cancer patients is of benefit in improving weight or energy balance. Reason are that populations have not been defined clearly with regards to starvation and cachexia phases, and the methodology of interventions, duration of interventions and defined outcomes has been too heterogeneous to allow any conclusion. These findings can also underline the correctness of the international cachexia definition that states that cachexia cannot be treated with nutrition alone. Additionally, it was not possible to describe a difference in the effectiveness between the interventions considering the different cachexia stages. Nevertheless, nutrition remains an essential part of a multimodal cachexia treatment as it is not considered possible to increase or stabilise weight if nutritional needs are not met.

6–19

### Branched-chain amino acids prevent cardiac cachexia in rats

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**Background and Aim:** Heart failure (HF) is associated with abnormal cardiac and systemic energy metabolism. Particularly, cachexia is a common complication and is associated with poor prognosis in patients with HF. Branched-chain amino acids (BCAA) reportedly increase the average life-span of mice, associated with increased mitochondrial biogenesis in heart and skeletal muscles. The aim of the study is to examine the effect of BCAA on rats with cardiac cachexia.

**Methods:** Dahl salt-sensitive (DS) rats fed a high-salt (HS) diet from 6 weeks of age were used as a model of cardiac cachexia. BCAA (1.5 mg/g body weight/day) were administered in drinking water from 11 weeks of age. DS rats fed a low-salt (LS) diet and tap water as a control (LS-C,  $n=8$ ), DS rats fed a LS diet and BCAA (LS-BCAA,  $n=8$ ), DS rats fed a HS diet and tap water (HS-C,  $n=30$ ), and DS rats fed a HS diet and BCAA (HS-BCAA,  $n=30$ ) were used. Survival and body weight were monitored, and animals were sacrificed at 21 weeks of age and analyzed.

**Results:** BCAA improved the survival of rats with HF (HS-BCAA 57 % and HS-C 37 %,  $p=0.030$ ). The body weight was decreased in HS-C compared with LS-C (HS-C  $364\pm 11$  g and LS-C  $448\pm 6$  g,  $p<0.001$ ), and BCAA prevented the body weight loss (HS-BCAA  $393\pm 6$  g and HS-C  $364\pm 11$  g,  $p=0.037$ ). The expression of genes related to mitochondrial function was changed in heart and skeletal muscles of rats with HF, and BCAA modified the change.

**Conclusion:** BCAA preserved the body weight and the cardiac function, and prolonged survival in a rat model of HF.

6–20

### Krill Oil supplementation improves blood lipids status in cachectic advanced cancer patient

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**Background:** Cancer patients with cachexia symptoms often show a lipid profile disorder, consisting in severe

hypertriglyceridemia due to increased circulating free fatty acids and variable total cholesterol levels, decreased activity of lipoprotein lipase and increased lipolysis, which is correlated with proinflammatory cytokines levels, particularly IL-6 and TNF $\alpha$ . Recent evidence further confirms impairment of adipose tissue in chronic inflammatory condition like cancer cachexia. The aim of this study was to assess the effect of nutritional supplementation with a high title of Omega 3 (EPA and DHA) by Krill Oil on main circulating parameters of blood lipid profile and IL-6 and TNF $\alpha$  blood levels.

**Methods:** From 2011 to date, 45 IV stage cachectic patients (M/F 29/16; age range 55–85 y) with cancer at different sites were enrolled. 21 healthy subjects were studied as controls. All patients had high blood tryglicerides, low total cholesterol levels, high levels of blood IL-6 and TNF $\alpha$  compared to controls. Patients received 3 capsules (3 g/day) of Krill Oil, extract from Krill, a crustacean, which contains Omega-3 polyunsaturated fatty acids mainly in the phosphatidylcholine form, of which 98 % are EPA and DHA (Superba Krill Oil, Aker Biomarine, Norway). Treatment duration was 8 weeks.

**Results:** A significant reduction of tryglicerides blood levels ( $113\pm 57.6$  vs  $149.53\pm 89.26$  mg/dl) and a significant increase of HDL cholesterol ( $59.1\pm 14.2$  vs  $41.6\pm 17.5$  mg/dl) and total cholesterol ( $161\pm 39.9$  vs  $134.6\pm 39.1$  mg/dl) was observed after treatment. Cytokines Il-6 and TNF $\alpha$  blood levels were also decreased at the end of treatment ( $26.1\pm 32.2$  vs  $20.5\pm 18.5$  pg/ml for Il-6 and  $30.0\pm 9.3$  vs  $26.2\pm 19.6$  pg/ml for TNF-alpha).

**Conclusions:** Nutritional supplementation with krill oil resulted in an improved blood lipid profile,, probably modulating different metabolic and inflammatory pathways. .. Krill Oil could be useful in multi-targeted, combined pharmaco-nutritional approaches to treat cancer-cachexia. Further phase III clinical studies are warranted.

6–21

### Refractory reasons and strategies to cure anorexia nervosa

**Jiang Bo Li<sup>1</sup>, JianZhong Li<sup>2</sup>, Ai Guo Huang<sup>2</sup>, Xiao Bin Liu<sup>3</sup>, Xin Chen<sup>3</sup>, Wie Rong<sup>1</sup>, Cui Zhen Zhu<sup>1</sup>, Gui Gui<sup>4</sup>**

<sup>1</sup>Clinical Psychiatry, Wannan Medical College Wuhu Second People Hospital, <sup>2</sup>College of Psychology, Nanjing University of Chinese Medicine, <sup>3</sup>Shenzhen city public security bureau Rehabilitation hospital, <sup>4</sup>Wuhan University, People's hospital Anorexia nervosa (AN) is a very intractable disease. If AN who can not get effective treatment, some patients may be died. So it is very necessary to analysis the refractory reasons of the AN and put up some countermeasures for individualized treatment according to specific cases. The author has treated four patients of AN in the clinic in recent 2 years, and have analyzed deeply to refractory reasons of AN.

Summarize the reason of the AN refractory. First, many patients without or defective insight for AN. So Many patients to treatment requirement is not urgent for AN and someone even has negative resistance. Contrary to the doctor's instructions in the treatment, it is easy to lose heart for treatment. Second, they have intractable obsessive and fear. Third, have a stubborn, distort, similar to delusional belief and stubborn around these beliefs to actions. Fourth, often have impulse want to vomit. Fifth, easy to take drastic action (Such as overeating or Refusing to eat). In the last, patients put more attention on the symptoms, so note that is narrow, they ignore others' advice and his thin body seems to be invisible, his life under threat not invisible. In view of the refractory factors, the authors take drugs and psychological therapy to treat AN, series of specific measures in the psychological treatment, this paper will detail report.

6–22

#### Orally active delta-agonist peptide derived from a green leaf protein stimulates food intake in aged mice with ghrelin resistance

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**Background and Aims:** There is a decline in food intake with aging, even in healthy old people. It is known that decreased food intake, sarcopenia with hypodynamia, and inflammation often induce malnutrition in elderly people. In the current study, we investigated the mechanism underlying anorexia of aging in mice. We also tested responsiveness to exogenously administered orexigenic peptides in aged mice.

**Methods:** Male C57BL/6N mice (2, 5, 15 and 27-month-old) were used. Each mouse was individually housed with free access to food pellets and water. Food intake and body weight were measured. The hypothalamic mRNA expression of genes associated with regulation of food intake was measured by RT-PCR. To test effect of ghrelin on food intake in aged mice, it was administered intraperitoneally. We also investigated effect of rubiscolin-6 which is a  $\alpha$  opioid agonist hexapeptide derived from RuBisco, a major green leaf protein, on food intake.

**Results:** Food intake and body weight of male 27-month-old C57BL/6N mice were lower than those of 15-month-old mice. Epididymal and mesenteric fat mass, and blood glucose, triglyceride and leptin levels were also decreased. However, the hypothalamic mRNA expression of neuropeptide Y (NPY) and agouti-related protein (AgRP), endogenous orexigenic peptides, was increased, possibly by starvation signals transferred from the periphery.

Ghrelin did not increase food intake after intraperitoneal administration to aged mice, suggesting that ghrelin resistance occurs. In contrast, orally administered rubiscolin-6, stimulated food intake even in aged mice. We previously found that rubiscolin-6 stimulates food intake via activation of  $\alpha$  opioid receptor followed by the prostaglandin (PG) D2-NPY system. Thus, we hypothesized that the NPY system downstream of PGD2 but not ghrelin is not impaired in aged mice.

**Conclusion:** We found that rubiscolin-6 stimulated food intake in aged mice with ghrelin resistance.

6–23

#### Impact of malnutrition and reduced body mass index on prognosis in Japanese patients with heart failure

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**Background and Aims:** Many factors, including aging, disuse phenomenon, chronic inflammation and malnutrition, affect reduced body mass index (BMI) in patients with heart failure. This study investigated what percentage of Japanese heart failure patients with low BMI had malnutrition. The influence of malnutrition and BMI on prognosis was also assessed.

**Methods:** This prospective analysis included 52 consecutive patients with heart failure admitted to our hospital between 26th December 2011 and 5th February 2013. On admission, nutritional status was assessed according to the Mini Nutritional Assessment (MNA). The patients were divided into the L (BMI  $\geq 22$  kg/m<sup>2</sup>) or S (BMI  $< 22$  kg/m<sup>2</sup>) group. The correlation between MNA and BMI was investigated; a logistic analysis was also performed to identify nutrition-related risk factors.

**Results:** The mean age was 71.1  $\pm$  14.7 years old. The correlation was found between BMI and MNA score ( $r = 0.31$ ,  $p = 0.027$ ). MNA score was significantly higher in the L (21.9  $\pm$  0.8) than S (18.3  $\pm$  1.0,  $p = 0.009$ ) group. In the L group, the MNA categories showed 16.1 % patients poor, 51.2 % patients at risk, and 32.3 % patients good nutritional status,

whereas 33.3 % patients poor, 52.4 % patients at risk, and 14.3 % patients good nutritional status in the S group. The logistic analysis demonstrated the event of risk as follows: odds ratio 0.84, 95 % confidence interval 0.67–1.00,  $p=0.05$  in the L group; odds ratio 0.81, 95 % confidence interval 0.60–1.01,  $p=0.06$  in the S group.

**Conclusions:** Many patients with heart failure were malnourished or at risk of malnutrition in this study. Various factors influence reduced BMI in heart failure. This study result demonstrated that malnutrition was closely associated with lower BMI and the independent predictor of heart failure, suggesting the importance of nutritional management. Nutritional screening and improvement are indispensable particularly in Japanese patients with heart failure because of their lower BMI than patients in Western countries.

6–24

### Rikkunshito improves cisplatin-induced ano-rexia

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**Background and Aims:** Chemotherapy with an anticancer agent generally leads to gastrointestinal tract disorders, such as vomiting and anorexia; however, the mechanism that leads to these adverse reactions remains unclear. Rikkunshito, a Japanese Kampo medicine, is known to alleviate such adverse reactions. In this study, we attempted to identify the mechanisms.

**Methods:** We investigated the decrease in plasma ghrelin levels and food intake caused by cisplatin and serotonin (5-HT) agonists. In addition, the suppressing effects of rikkunshito and 5-HT antagonists on cisplatin-induced decreases of ghrelin levels and/or food intake were investigated, and inhibitory effects of the components of rikkunshito were determined using receptor-binding assays with 5-HT2B and 5-HT2C receptors. Furthermore, we determined whether rikkunshito plays a role in the degradation of peripheral ghrelin.

**Results:** Cisplatin, 5-HT2B agonist, and 5-HT2C agonist markedly decreased the plasma acylated-ghrelin levels,

although 5-HT3 and 5-HT4 agonists had no effect on these levels. 5-HT2B or 5-HT2C antagonists suppressed the cisplatin-induced decrease in plasma acylated-ghrelin levels and food intake. Rikkunshito also suppressed the cisplatin-induced decrease in the plasma acylated-ghrelin levels and food intake. The suppressive effect of rikkunshito was blocked by the co-administration of a ghrelin antagonist. Certain components of rikkunshito, such as 3,3',4',5,6,7,8-heptamethoxyflavone, hesperidin, and isoliquiritigenin, showed a 5-HT2B antagonistic effect in vitro, and oral administration of these components suppressed the cisplatin-induced decrease in the plasma acylated-ghrelin levels. Furthermore, rikkunshito enhanced the acylated- to desacyl-ghrelin (A/D) ratio in cisplatin-treated rats. Several components of rikkunshito demonstrated inhibitory activity against ghrelin-deacylating enzymes. In addition, the administration of 10-gingerol, a component of rikkunshito, may inhibit ghrelin deacylation in rats.

**Conclusions:** The cisplatin-induced decrease in the plasma acylated-ghrelin levels and food intake are mediated by 5-HT2B/2C receptors and suppressed by the flavonoids in rikkunshito. Furthermore, rikkunshito also inhibited ghrelin deacylation and possibly helped maintain plasma ghrelin levels.

6–25

### Vitamin D and endothelial vasodilation in older individuals: data from the PIVUS study

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**Background and Aims:** Vitamin D in addition to its traditional effects on bone health appears to play a role in a wide range of extra-skeletal activities. In particular, Vitamin D by modulating endothelial function could influence vascular biology and physical performance. However, the relationship between vitamin D levels and indexes of vascular function has never been addressed in older individuals. To investigate the relationship between vitamin D and endothelial function, we used a large community-based sample.

**Methods and Results:** 428 women and 424 men aged 70 yrs from the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) Study, with data on vascular function and 25-OH Vitamin D were evaluated. We assessed endothelium-dependent vasodilation (EDV) by invasive forearm technique with acetylcholine, endothelium-independent vasodilation

(EIDV) by sodium nitroprussiate, flow-mediated vasodilation (FMD) and the pulse wave analysis (reflection index, RI), while Vitamin D levels were measured by chemiluminescence. Multivariate regression models adjusted for BMI (Model 1) and for multiple confounders (CRP, fasting insulin, HDL-cholesterol, smoking, sex hormones, hypertension, diabetes, cardiovascular medications and diseases, statin usage, calcium intake, PTH, physical exercise, renal and liver function) (Model 2) were used. The two models were performed separately for men and women because of 2 reasons: the different trajectory of vitamin D in men and women and

the positive significant relationship between interaction term sex\*Vitamin D and EIDV. In women, but not in men, vitamin D levels were positively associated with EIDV in both model 1 ( $\beta \pm SE = 1.41 \pm 0.54$ ,  $p = 0.001$ ), and model 2 ( $\beta \pm SE = 1.2 \pm 0.62$ ,  $p < .001$ ). No significant relationship was found between vitamin D levels and EDV, FMD and RI in both sexes. **Conclusions:** In older women, but not in men, vitamin D is positively and independently associated with EIDV. Further studies are needed to evaluate the role of vitamin D in endothelium-derived diseases in older population and the potential different role in the two sexes.